Heilbrigðisvísindasvið

Identification of quality and environmental hotspots during pelagic fishmeal and fish oil production

Exploring process changes toward higher-value products and promoting positive environmental impacts by choice of energy sources

Guðrún Svana Hilmarsdóttir

Thesis for the degree of Philosophiae Doctor

March 2022

HÁSKÓLI ÍSLANDS

Identification of quality and environmental hotspots during pelagic fishmeal and fish oil production

Exploring process changes toward higher-value products and promoting positive environmental impacts by choice of energy sources

Guðrún Svana Hilmarsdóttir

Thesis for the degree of Philosophiae Doctor

Supervisors and doctoral committee:

Professor María Guðjónsdóttir, University of Iceland and Matís
Assistant professor Ólafur Ögmundarson, University of Iceland
Professor Emeritus Sigurjón Arason, University of Iceland and Matís

March 2022

Meginárifaþættir gæða og umhverfisáhrifa við framleiðslu fiskmjöls og lýsis úr uppsjávarfiski

Ferlagreining í átt að framleiðslu hágæða afurða og umhverfisvænni framleiðslu með tilliti til orkugjafa

Guðrún Svana Hilmarsdóttir

Ritgerð til doktorsgráðu

Leiðbeinendur og doktorsnefnd:

Dr. María Guðjónsdóttir, prófessor við Háskóla Íslands og Matís
Dr. Ólafur Ögmundarson, lektor við Háskóla Íslands
Sigurjón Arason, prófessor emeritus við Háskóla Íslands og yfirverkfræðingur hjá Matís

Mars 2022

ISBN: 978-9935-9665-0-6

Printing by Háskólaprent

Reykjavik, Iceland 2022

Ágrip

Í dag eru uppsjávartegundir, hliðarstraumar frá vinnslustærri fisktegunda og annar meðafli uppistaðan í því hráefni sem unnið er í fiskmjöl og lýsi. Fiskmjöl og lýsi eru helst notuð sem fóður í fiskeldi, en þar sem verð á fiskmjöli hefur lækkað síðasta áratuginn og hlutur fiskmjöls hefur minnkað í fiskeldisfóðri, þarf að finna nýjar leiðir til að nýta hráefnið á hagkvæman hátt. Mikið hefur verið lagt í að bæta kælingu á hráefni uppsjávarveiðiskipa sem skila sér í betri gæðum þegar aflinn kemur í vinnsluna. Þessi þróun hefur þó ekki skilað sér í fiskmjöls- og lýsisvinnsluna, sem hefur lítið breyst síðan á sjöunda áratugnum.

Þessi rannsókn varpar ljósi á núverandi ástand á hráefninu og þeim gæðabreytingum sem eiga sér stað í hverju vinnsluþrepi í fiskmjöls- og lýsisframleiðslunni (Grein I-II), ásamt því að meta áhrif breytilegs hitastigs í sjóðara á gæði (Grein I), kannar hvernig framleiðsluferlið höndlar mismunandi hráefni (Grein II), rannsakar eiginleika hliðarstrauma m.t.t. próteingæða (Grein III), kannar umhverfisáhrif á framleiðslu fiskmjöls og lýsis úr loðnu með mismunandi orkugjöfum (Grein IV) og kannar hvort hægt sé að nota nærinnrauða litrófsgreiningu (NIR) til gæðamælinga og ferlastýringar í fiskmjöls- og lýsisframleiðslunni (Grein V). Greinarnar leggja grunninn að bættum fiskmjöls- og lýsisferlum og auka þekkingu á vinnsluferlunum sem mun nýtast við þróun þeirra næstu árin.

Þessi rannsókn sýndi jafnframt fram á að fituinnihald í fiskmjöli sem framleitt er í hefðbundnu fiskmjöls- og lýsisframleiðsluferli er breytilegt, en það mældist á bilinu frá 2% upp í 20%. Þröskuldur á fituinnihaldi fyrir hágæða fiskprótein miðast við fituinnhald innan við 0,5%, sem gefur til kynna að róttækar breytingar í vinnsluferlinu þurfa að eiga sér stað ef varan á að uppfylla kröfur til manneldis. Innihald himnufitu í fiskmjöli mældist hátt, sem bendir til þess að aðskilnaður fitunnar frá þurrefninu sé ekki nægur. Hins vegar benti magn frírra fitusýra til þess að niðurbrot af hráefninu var talsvert. Lækkun á hitastigi í sjóðara var rannsökuð til að skoða hvort fita skilaði sér betur frá þurrefninu, og fengust meiri gæði fiskmjöls við 85°C samanborið við 90° og 95°C (Grein I). Bent er á að bæta þurfi niðurbrot hráefnisins í fyrstu skrefum fiskmjöls og lýsisvinnslunnar, auk þess sem gufunin skilaði ekki eins lágu vatnsinnihaldi og búist var við (Grein I-II).

Fitu- og próteingæði voru rannsökuð í mismunandi hliðarstraumum og í hverju vinnsluþrepi, og niðurstöður þessara rannsókna leggja grunn að vöruþróun á nýjum afurðum og endurhönnun á vinnsluferlinum. Leysanleiki próteina í saltlausn (SSP) og styrkleiki líffrænna amín-sambanda minnkuðu í gegnum framleiðsluna, sem hafði áhrif

á gæði fiskmjölsins, og benda til niðurbrots, líklega vegna mikils hita í vinnslunni (Grein III). Trímetýlamín (TMA) fylgdi vökvahlið vinnslunnar og jókst í gegnum framleiðsluna, en gagnstæð þróun sást í styrki dímetýlamíns (DMA). Þessi þróun gæti skýrst af flóknum niðurbrotsferlum trímetýlamínoxíð (TMAO) yfir í TMA og DMA, auk mikils hitaálags i vinnslunni.

Bættir vinnsluferlar, með lækkun hitastigs í sjóðara (Grein 1), sýndu fram á betri gæði fiskmjöls. Það gaf tilefni til að kanna hvort slík lækkun hitastigs skilaði mögulegum umhverfislegum ávinningi. Til þess að meta slíkan ávinning varlífsferilsgreiningu beitt, en aðerðin er stöðluð aðferðafræði. Lífsferilsgreining metur umhverfisáhrif umfram losun gróðurhúsalofttegunda og í þessari greiningu voru greind umhverfisáhrifin sem hljótast frá veiðum til og með vinnslu fiskmjölsins. Áhersla var lögð á að skoða áhrif mismunandi orkugjafa til vinnsluhlutans (fiskmjölsframleiðslunnar). Um orkufrekan iðnað er að ræða og því mikilvægt að kanna mismun á umhverfisáhrifum fiskmjölsframleiðslu knúinni með endurnýjanlegri orku annars vegar, sem og orku framleiddri með brennslu jarðefnaeldsneytis hins vegar. Það var gert til að sýna fram á mögulegan umhverfislegan ávinning af því ef fiskmjöl er framleitt með endurnýjanlegri orku. Til viðbótar, voru umhverfisáhrif af fiskmjölframleiðslu á Íslandi reiknuð miðað við mismunandi orkugjafa fyrir vinnsluárið 2018. Niðurstöður lífsferilgreiningarinnar sýndu að þrátt fyrir að lækkun hitastigs í sjóðara hafi einungis numið um 5°C, þá skilar slík lækkun minnkuðum umhverfisáhrifum sama hver orkugjafinn er. Það sýnir því að þróun vinnsluferla fiskmjöls, með áherslu á lækkun hitastigs vinnsluferla, gefur umtalsverðan ávinning við minnkun umhverfisáhrifa af fiskmjölsframleiðslu (Grein IV). Áhersla framtíðarþróunar vinnsluferla í fiskmjölsframleiðslu ætti því að vera á enn frekari lækkun hitastigs í vinnsluferlum, án þess þó að minnka gæði vörunnar. Auk þess leiddi lífsferilsgreiningin í ljós að veiðarnar og vinnslan höfðu mest umhverfisáhrif (Grein IV). Áhrif mismunandi orkugjafa voru skilgreind út frá umhverfislegum áhrifum, sem kemur sér vel þegar skipta á út eða endurbæta vinnsluskref í vinnslunni. Auk þess voru talsverð umhverfisleg áhrif frá þeim hreinsiefnum sem notuð eru í vinnslunni, og er ráðlagt að skipta þeim út fyrir umhverfisvænni efni eða takmarka notkun þeirra.

Þar sem vinnsluafköstin eru mikil, og endurhönnun eða gæðaeftirlit ferla á sér stað er mikill fjárhagslegur og umhverfislegur sparnaður fólginn í hraðari mælingum og eftirliti á gæðaþáttum í vinnslunni. Nútíma framleiðslukröfur gera ráð fyrir miklum áreiðanleika mælinga og einsleitari afurðum en áður. Litrófsgreiningar hafa verið nýttar í fjölbreyttum tilgangi við mati á framleiðsluferlum, þar á meðal nærinnrauð litrófsgreining (NIR) sem gæðaeftirlit á lokaafurðum í fiskmjöls- og lýsisframleiðslu. Í rannsókninni var NIR litrófsgreining notuð til að spá fyrir um breytingar á efnainnihald vinnslustrauma í gegnum vinnsluna alla. Spálíkön fyrir vatns- og fituinnihald, þurrefni,

fosfólípíð, mettaðar fitusýrur, einómettaðar fitusýrur og fjölómettaðar fitusýrur, dókósahexaensýru (DHA) og eíkósapentaensýru (EPA) voru búin til með góðum árangri. Nýting á NIR litrófsgreiningu flýtir þannig greiningu mælinga umtalsvert og minnkar notkun á leysum og öðrum búnaði, og skilar sér í auðveldari stýringu vinnsluferilsins.

Lykilorð:

Fiskmjöl, lýsi, hitameðhöndlun, fitugæði, próteingæði, lífsferilsgreining (LCA), nærinnrauð litrófsgreining (NIR), massabókhald, orkuflæði.

Abstract

Today, small pelagic species, side-streams from the production of larger fishes intended for human consumption, and other by-catch are currently processed into fishmeal and fish oil. Fishmeal and fish oil are presently mainly used as feed, primarily for aquaculture, but fishmeal prices have declined over the last decade. Great effort towards improving handling on board the fishing vessels has resulted in higher quality of the catch reaching the harbor. Meanwhile, the fishmeal and fish oil production processes have remained similar since the 1960s. Hence, this thesis aims to shed light on the current state of the raw material and the quality changes occurring during each operational step of the production (Paper I-II), to assess the effect of cooking temperature on the fishmeal quality (Paper I), investigates how the production process handles different raw materials (Paper II), investigates promising protein rich side-streams for the production of higher value products (Paper III), investigates the environmental impacts of producing 1 tonne of fishmeal and fish oil (Paper IV), and investigates if Near infrared spectroscopy (NIR) can be used as a monitoring tool during the fishmeal and fish oil production. The papers, I-V, also lay a foundation for redesigning purposes of the currently operated fishmeal and fish oil factories.

The study demonstrates that the traditional fishmeal and fish oil production processes currently returns high-lipid fishmeal from raw material ranging between 2% to 20% lipids. The lipid content should be less than 0.5% fat content to classify as high-quality fishmeal, indicating that extracting lipids from the raw material is inefficient, and drastic changes need to happen if the products are intended for human consumption. Although a lower cooking temperature is proposed for higher quality fishmeal (Paper I), attention towards higher lipid separation during the initial production steps is suggested for a more effective breakdown of the raw materials. Further improvements suggest adjusting the evaporation step, as the concentrate was relatively high in water content compared to the other solid streams entering the drying steps (press cake, sludge, and concentrate) (Paper I-II).

Protein quality was investigated in promising side-streams, where salt soluble proteins content (SSP) decreased throughout the production, affecting the solubility of the fishmeal (Paper III). The same trend was observed in biogenic amines, indicating protein decomposition, possibly due to extensive heat treatment. The press-cake (solid stream) had the lowest amount of volatile nitrogen compounds during production from both the fatty and lean raw material. Trimethylamine (TMA) followed the liquid stream, increasing throughout the production, and reaching the highest values in the fishmeal, while the opposite trend was observed in Dimethylamine (DMA) concentrations. These trends could be the result of

trimethylamine oxide degradation into TMA and DMA due to high thermal exposure during processing.

The effects of changing the cooking temperature on the fishmeal quality and processing efficiency were investigated, both on a quality basis and the effect on the environment. Lowering the cooking temperature by 5°C resulted in higher lipid quality fishmeal (Paper II), and overall lower environmental impacts (Paper IV). Furthermore, the environmental impacts were studied with environmental hot-spot analysis, assessed with the Life Cycle Assessment methodology in a cradle-to-gate study, based on the functional unit "*the production of 1 tonne of capelin fishmeal including fish oil from cradle to factory gate, produced in Iceland in 2018*. The assessment showed that the raw material acquisition was the highest environmental hotspot, contributing the most to almost all impact categories, followed by the production process. The assessed processing steps contributing the least to the assessed environmental impacts were packaging and back-up power, which effects were negligible, but cleaning agents (assessed with waste) were the highest contributors in many impact categories. The focus of the study included the effect of different energy sources for operating the fishmeal production, on the overall environmental impacts, and changes in the environmental hotspots (Paper IV). Furthermore, Paper IV identified optimization potentials for lower energy consumption, e.g. using purse seiner compared to trawling, during the raw material acquisition. Identified steps with potential for lower environmental impacts of fishmeal and fish oil production lie within the drying and evaporation steps, and includes exchanging the current cleaning agents for more eco-friendly cleaning agents or limiting their use altogether. The environmental impacts of cleaning and waste were independent of the assessed energy sources for the processing.

In processes, such as fishmeal and fish oil production processes, with high throughput, and process redesigning, and quality monitoring are ongoing there is great financial and environmental gain to achieve by applying fast and robust monitoring techniques. Modern demands further require both higher precision in quality monitoring and higher product consistency than before. Spectroscopic analytical techniques have been used for a wide variety of quality assurance applications, including the use of near infrared spectroscopy to evaluate fishmeal and oil end-product quality. In the current study were NIR spectroscopy used to assess chemical composition of the streams throughout the whole process. Prediction models based on NIR data were successful in predicting water- and lipid content, fat-free dry matter, phospholipids, along with the fatty acid classes (SFA, MUFA, PUFA), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) with acceptable precision. The application of NIR spectroscopy during the fishmeal and

fish oil production provides faster analysis and minimizes the use of solvents, chemicals and instruments, which is reflected in easier process control and monitoring.

Increasing the quality of the raw material and reducing the environmental impacts requires excessive measurements, emphasizing the need for robust measuring methods to secure quicker and less costly analysis, e.g., the lipid quality and the separation from the water and FFDM. Assessing changes aimed to lower the environmental impact may not only reduce the energy cost but encourage sustainable practice and responsible production, as demonstrated in this thesis.

Keywords:

Fishmeal, fish oil, process optimization, heat treatment, lipid quality, protein quality, Life Cycle Assessment (LCA), mass balance, energy flow, near infrared spectroscopy

Acknowledgments

This study was carried out at the Faculty of Food Science and Nutrition of the University of Iceland and Matís ohf (Icelandic Food and Biotech R&D) in collaboration with Síldarvinnslan hf. The study was financially supported by the AVS (The Added Value of Seafood) fund of the Ministry of Fisheries and Agriculture in Iceland (grant number: R18 031-18), the Rannís Technology Development Fund (Product development from flexible fish processing, no. 198883-0611 and BIOZOOSTAIN no. 2021267-0611), ERA-NET BlueBio Cofund Call for supporting the BIOZOOSTAIN project, as well as the University of Iceland research fund.

I would like to give sincere thanks to my supervisors, Prof. María Guðjónsdóttir, Prof. Sigurjón Arason, and Dr. Ólafur Ögmundarson, for their endless support, excellent guidance, and solution-minded mindset. It has been an honor being your apprentice and student. I am very grateful for your insight and guidance in helping me grow into the scientist I am today. I know that this is not the end of our journey together, and I look forward to our future projects and discussion over coffee or meat soup in the east. Sincere thanks go to all my co-workers at the University of Iceland and Matís for their support. Special thanks to Sindri and Hafþór for their reply to my endless questions and experiments at the SVN facility, including guidance, help, and solution aimed mindset. Thanks to Heimir for his advice, assistance, and friendly atmosphere, which goes as well for the rest of the crew, Eiríkur, Þröstur, Þór, Magni, and Hjörtur, for constant help and additions to the pilot plant. Huge thanks to my co-worker and friend, Cecilé, during the setup of the pilot plant we investigated shortly. Furthermore, sincere thanks to Dr. Stefán and his family for the help he gave during these long sampling nights and weeks during the whole run of my Ph.D. I am grateful to both Dr. Hildur and Dr. Stefán for your guidance, support, and friendship. I look forward to our fish-processing-oriented discussion in the future and acknowledge that I could not imagine my fishy life without you! Lastly, I would like to thank my family, Hjalti and Emma Kristín, for their endless support, love, and patience. Thank you for helping me see the surface during difficult moments, and life is indeed better with you by my side, being there and making every day better than the previous one with your presence.

This thesis, I dedicate to my parents, Kristín and Hilmar, for teaching me to seek education that I find interesting and can give me joy, but this road was not always easy to find. As I finally found it, this Ph.D. is a thank you for their contribution to my education throughout the years, for their endless support, bad jokes, and hypnotic chemistry talk.

Contents

List of abbreviations

1D = 1st derivative

2D = 2nd derivative

DHA = Docosahexaenoic acid (22:6*n–3*)

EPA = Eicosapentaenoic acid (20:5*n–3*)

FAC = Fatty acid composition

FAME = Fatty acid methyl ester

FFA = Free fatty acids

GC = Gas chromatography

LCA = Life cycle assessment

MUFA = Monounsaturated fatty acids

MSC = Multiplicative scatter correction

NIR = Near infrared spectroscopy

n = Sample replicates

PCA = Principal component analysis

PUFA = Polyunsaturated fatty acids

PL = Phospholipid

PLS = Partial least square regression

R^2 = Regression coefficient

RMSE = Root mean square error

RMSEC = Root mean square error of calibration

RMSEP = Root mean square error of prediction

SFA = Saturated fatty acids

TBA = Thiobarbituric acid value

TVN / TVB-N = Total volatile nitrogen or total volatile basic nitrogen

TMA = Trimethylamine

DMA = Dimethylamine

List of figures

List of tables

List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-V [as needed]):

I. Hilmarsdottir, G. S., Ögmundarson, Ó., Arason, S., Gudjónsdóttir, M. (2021). Efficiency of fishmeal and fish oil processing of different pelagic fish species: Identification of processing steps for potential optimization towards protein production for human consumption. *Journal of Food Processing and Preservation*, 45(4). https://doi.org/10.1111/jfpp.15294.

II. Hilmarsdóttir, G.S., Ögmundarson, Ó., Arason, S., Gudjónsdóttir, M. (2020). The effects of varying heat treatments during pelagic fishmeal production. *Processes*, 8(9), 1142. https://doi.org/10.3390/pr8091142

III. Nguyen, H.T., Hilmarsdóttir, G.S., Sveinsdóttir, H.I., Tómasson, T., Arason, S., Gudjónsdóttir, M. (2022). Changes in protein and non-protein nitrogen compounds during fish meal processing – identifications of unoptimized processing steps. *Processes*, 10, 621. https://doi.org/10.3390/pr10040621

IV. Hilmarsdóttir, G.S., Ögmundarson, Ó. (2022). Arason, S., Gudjónsdóttir, M. Identification of environmental hotspots in fishmeal and fish oil production towards the optimization of energy-related processes. *Journal of Cleaner Production 343*, 130880. https://doi.org/10.1016/j.jclepro.2022.130880

V. Gudjónsdóttir, M., Hilmarsdóttir, G.S., Ögmundarson, Ó., Arason, S. (2022). Near infrared spectroscopy and chemometrics for effective online quality monitoring and control during pelagic fishmeal and oil production. Being prepared to be submitted for publication to *Food Engineering.*

All papers are reprinted by kind permission of the publishers

Declaration of contribution

I. The author and the co-authors designed the experiment and wrote the manuscript in collaboration. The author collected, measured, and analyzed the data, wrote the original draft, led the revisions, and prepared the manuscript for publication.

II. The author and the co-authors designed the experiment and wrote the manuscript in collaboration. The author collected, measured, and analyzed the data, wrote the original draft, led the revisions, and prepared the manuscript for publication.

III. The author and co-authors designed the experiment and wrote the manuscript in collaboration. The author collected the samples, created the mass balance, and took part in revisions and preparation of the manuscript for publication.

IV. The author and co-authors designed the experiment and wrote the manuscript in collaboration. The author collected, measured, and analyzed the data, performed the analysis in SimaPro along with the co-authors, wrote the original draft, led the revisions, and prepared the manuscript for publication.

V. The author and co-authors designed the experiment and wrote the manuscript in collaboration. The author collected and measured the data. The author and co-authors analyzed the data in cooperation, and the author took part in the preparation of the manuscript for publication.

1 Introduction

For the last six decades, direct human consumption of pelagic fish species increased from 67% to 88% (Figure 1) (FAO, 1997, 2020). As the world's population is growing and marine raw materials are not foreseen to increase significantly, there is a need to produce more food for human consumption requiring better utilization of underutilized raw materials, including within the fishmeal and fish oil sector (FAO, 2020). The total fish amount intended for fishmeal and fish oil production globally in 2018 accounted for 18 million tonnes, excluding discards which summed up to over 9 million tonnes (FAO, 2020). However, this number might increase due to the increasing utilization of discards, e.g., fishmeal production, as predictions have foreseen a 6% increase in fishmeal production from 2018 to 2030 and a 5% increase in fish oil production (FAO, 2020). However, as 34% of the marine fish stocks in the world were classified as being overfished in 2017, fishmeal production from captured target species (e.g., anchoveta, capelin) are expected to decline by 18% by 2030 (FAO, 2020).

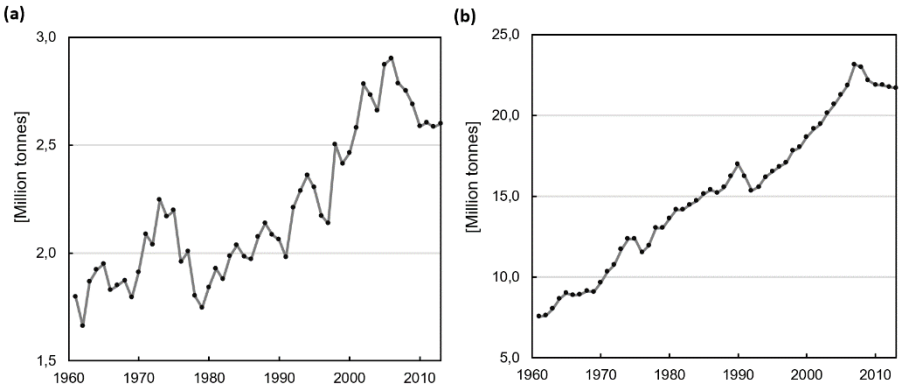


Figure 1: Pelagic fish for direct human consumption in the European Union (a) and the world (b) from 1961-2013. Data from Food and Agriculture Organization of the United Nations (1997).

Over the last ten years, fishmeal and fish oil prices of Peruvian fishmeal (65% protein) have dropped by 11% (Index Mundi, 2022), which is largely related to supply and price variations, along with an increasing demand from the aquafeed industry (FAO, 2020). However, primarily due to price, the proportion of fishmeal incorporated into feed is decreasing, and studies indicate that up to 90% of fishmeal used in aquaculture could be replaced by other animal by-products (Galkanda-Arachchige et al., 2020). Furthermore, due to the non-sustainability of the fisheries, wild stocks are forced to cover the increasing demand for fishmeal (Naylor et al., 2009). Reduced

prices for fishmeal for aquafeed and simultaneously increased demand for high-value proteins calls for changes in part of the primary fishmeal markets from aquafeed to start feed, or to human consumption. To ensure this market shift, the fishmeal needs to have low lipid content and high protein content, as prices primarily depend on these factors (Index Mundi, 2022). The traditional fishmeal and fish oil processes have remained relatively similar throughout the last seven decades (Bimbo & Crowther, 1992; Einarsson et al., 2019; FAO, 1986; Hall, 2010; Oterhals & Vogt, 2013). Since many fishmeal and fish oil production processes focus mainly on the high throughput of the production plants rather than the quality of product, the fishmeal generally ends up with a high lipid content (Einarsson et al., 2019; FAO, 1986). Due to the focus on mass throughput during fishmeal processing, little or no attention has been towards effective water and lipid separation during processing or detailed classifications of the raw materials according to species or specific fish parts, particularly with higher utilization of cut-offs and side-steams during the fishmeal and fish oil production. Furthermore, overlapping of the catching season of the different target species causes high variation of the raw material. The heterogeneity of the raw materials could lead to variable quality and condition of the raw materials, making them inadequate for high-value product production (Thorkelsson et al., 2009).

During the last decades, catching strategies and optimized handling of pelagic fish species on-board have improved substantially (Bao et al., 2007; Margeirsson et al., 2010). Raw materials of higher quality are thus being brought ashore than before. Hence, improving the fishmeal and fish oil processes by redesigning the fishmeal and fish oil production processes towards the production of higher-value products, and utilizing wild stocks more sustainably, could maximize the production revenue while adding to the growing need for food intended for humans. Assessing the environmental impacts of those changes would prioritize each operating step and shed light on the environmental impacts for an optimized or redesigned processing. Furthermore, assessing the correlation between the environmental impacts and both the raw material acquisition and the process gives a holistic view of the whole production and puts into perspective the effects of each assessed life cycle stage.

Producing higher-value products more sustainably encourages responsible fisheries, and the need to utilize the whole catch to its maximum potential. As the raw materials vary, different challenges may emerge during the production as the raw materials range from cut-offs to whole fish in several species. Therefore, further relative information was provided on these topics, along with lipid and quality parameters, the assessment of near infrared spectroscopy as a quality monitoring tool, and the environmental impacts of fishmeal and fish oil production

2 Literature review

2.1 Raw materials for fishmeal and fish oil production

The primary raw materials processed into fishmeal and fish oil are by-catch, cut-offs, and small pelagic species (FAO, 2018; Thorkelsson et al., 2009). Iceland's most caught pelagic species are Atlantic herring (*Clupea harengus*) and capelin (*Mallotus villosus*). However, in recent years, the catching of blue whiting (*Micromesistius poutassou*) and Atlantic mackerel (*Scomber scombrus*) have increased (ICES, 2011; *Statistics Iceland*, 2020b) due to changes in their feeding opportunities further north due to warming oceans (ICES, 2018). The catching season of each species depends on seasonal variation, and which species are in the Icelandic jurisdiction at a given time (Statistics Iceland, 2019).

Capelin is often caught in the spring around Iceland and is spawning from December to March (Vilhjálmsson, 2002) (Figure 2). The chemical composition of caplin varies from ~4% lipid content during spring to 15-20% lipid content in autumn (Vilhjálmsson, 2002). Capelin is mainly produced into fishmeal and fish oil used for feed production due to its small size, which is 13-20 cm on average. Norway is considered the largest buyer of capelin fishmeal for salmon feed (Hilmarsson et al., 2015). Few other products are currently produced from capelin, although during spring processing, capelin eggs are often harvested and sold as capelin caviar (Hilmarsson et al., 2015)

Figure 2: Capelin (*Mallotus villosus***)** drawn by Jón Baldur Hlíðberg (www.fauna.is).

Blue whiting is found in the North Atlantic, mainly around the southern part of Greenland, Iceland, Norway, Faroe Islands, and Britain's west coast but can stretch down to the northern part of Africa and south-east of Canada (ICES, 2011). Blue whiting migrates in the summer after spawning to the Faroe Islands, Norway, and east of Iceland, before returning to spawning areas in January/February (ICES, 2011). The species migrated first into Icelandic waters in 1995 (Statistics Iceland, 2020a). The fish is relatively small, with an average length of around 30-40 cm (Hilmarsson et al., 2015) (Figure 3).

Products include skinless fillets for the frozen laminated blocks for fish fingers or other portioned production, and mince from skinless fillets used for surimi production (FAO, 2001b). However, since 2015 >93% of the blue whiting caught in Icelandic waters is processed into fishmeal and fish oil (Statistics Iceland, 2020a). Most of the blue whiting is frozen at sea or inland, if not processed into fishmeal and fish oil (Statistics Iceland, 2020a)(Hilmarsson et al., 2015).

Figure 3: Blue Whiting (Micromesistius poutassou) drawn by Jón Baldur Hlíðberg (www.fauna.is).

Herring is a pelagic species found in the North Atlantic and is on average around 30 cm and 500 gr heavy (Hilmarsson et al., 2015) (Figure 4). Its most important fishing grounds are the North Sea, the Baltic Sea, and the coastal waters near Britain, Norway, Iceland, and Canada (Stroud, 2001). Herring can variate in lipid content, from 9-26%, with the highest lipid content in the autumn, and lowest in the spring due to spawning (Henderson & Almatar, 1989).

Most of the herring is processed before being sold, where it is commonly smoked, canned, salted, marinated, or canned for pet food (Stroud, 2001). However, the herring side-streams are currently considered unsuitable for human food and are thus generally processed into fishmeal and fish oil (Stroud, 2001).

Figure 4: Herring (*Clupea harengus*) drawn by Jón Baldur Hlíðberg (www.fauna.is).

The Atlantic mackerel is a pelagic fish, around 40 cm long and 600 gr. (Hilmarsson et al., 2015) (Figure 5), and has been caught around Iceland since 2006, from 4 kilo tonnes (kt) on average in 2006 to 160 kt annually from 2011 (ICES, 2018). Since 2011, the fishing ground has moved from the north and northeast side of Iceland to the south and west of Iceland (ICES, 2018).

Today, the Atlantic mackerel is either frozen whole or headed and gutted, where the remaining material, including guts, heads, viscera, bones etc. accounts for 26-32% (Eysteinsson et al., 2020; Sveinsdóttir et al., 2020). The gut can be high in

zooplankton, mainly the enzyme-rich copepod *C. finmarchicus,* which induces protein and lipid degradation of the fish muscle (Eysteinsson et al., 2018). These side-streams are generally collected, blended with other by-catch, and into fishmeal and fish oil.

Figure 5: Atlantic mackerel (Scomber scombrus) drawn by Jón Baldur Hlíðberg (www.fauna.is).

The chemical composition and physical properties of the raw materials processed into fishmeal and fish oil depend highly on the season (Statistics Iceland, 2019), and thus also in value. When comparing the pelagic species caught in Icelandic waters in 2018, the mackerel was the most valuable, with a market value of 0.55 EUR/kg, followed by the Atlantic and Scandian herring combined, with a value of 0.37 EUR/kg, capelin with a value of 0.32 EUR/kg, and finally 0.22 EUR/kg for blue whiting (Statistics Iceland, 2019). Moreover, due to their small size, capelin and blue whiting are usually processed directly into fishmeal, while herring and mackerel are primarily caught for human consumption, explaining the difference in value partially.

When processing side-streams from fillets or whole fish intended for human consumption, such as from herring and mackerel, the raw materials are often highly heterogeneous. The raw material can include more than one species, by-catch, heads, intestines, stomach content, and damaged whole fish, which are all blended and collected over time. When processing capelin and blue whiting, the raw materials are highly heterogeneous as well, but the chemical composition is more predictable as the ratio between the viscera, head, and muscle is relatively similar in all batches. Moreover, as most fishmeal and fish oil factories are designed to produce large quantities, collecting the appropriate amount of raw materials prior to the process initiation can cause delays. Hence, the raw material must often wait several days in storage tanks until they are full, and enough materials have been collected before the process is initiated. This delay increases the risk of degradation of the raw materials due to microbial, enzymatic, and oxidative processes (Ocaño-Higuera et al., 2011; Standal et al., 2018).

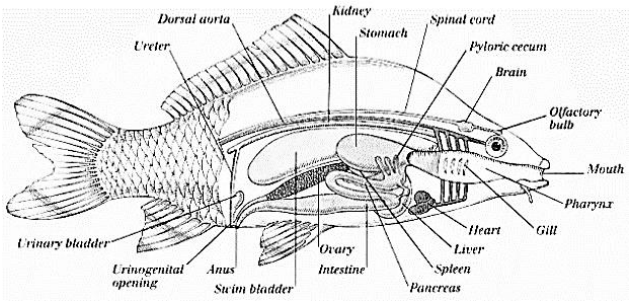


Figure 6: Different parts of a fish can make the production of fishmeal and fish oil difficult, as the diversity of the fish protein is high, and the different parts of the fish are not sorted ("Fish Anatomy," 1913).

Although the side-streams endure a delay on land, the catch is at a high quality until reaching the harbor, as chilling protocols and handling have improved substantially on-board in the last decades (Bao et al., 2007; Margeirsson et al., 2010). There is therefore clear room for improvement in the raw material collection for the fishmeal and oil production.

Several processing challenges have been encountered, mainly when processing mackerel, as the mackerel caught in Icelandic waters has generally been feeding heavily on *Calanus finmarchicus* (Eysteinsson et al., 2018; Prokopchuk & Sentyabov, 2006). *C. finmarchicus* is a copepod with high enzymatic activity, which can have immediate degradative effects on the pelagic raw material if not appropriately treated (Eysteinsson et al., 2018; Prokopchuk & Sentyabov, 2006). As a response, the leading processing companies tend to head and gut the mackerel to remove the *C. finmarchicus* from the process, and thus prolong the shelf life of the mackerel. However, with increasing discards, which are high in enzymatic activity and of various origins, the production of fishmeal and fish oil from side-streams results in a more challenging production. The high variation in the raw materials (Thorkelsson et al., 2009) emphasizes the importance of quality description and classification of the raw material upon arrival to shore from the vessels.

2.2 Traditional fishmeal and fish oil production process

Traditional fishmeal and fish oil processing was developed in the 1940s, and became more prominent and established in the 1960s (Kose, 2010).

During traditional fishmeal and fish oil processing (Figure 7), the raw material enters a ***cooking*** step where a cooking temperature from 95-100°C for 15-20 min is common practice (FAO, 2018). Before cooking, a ***pre-heating*** step can be added for

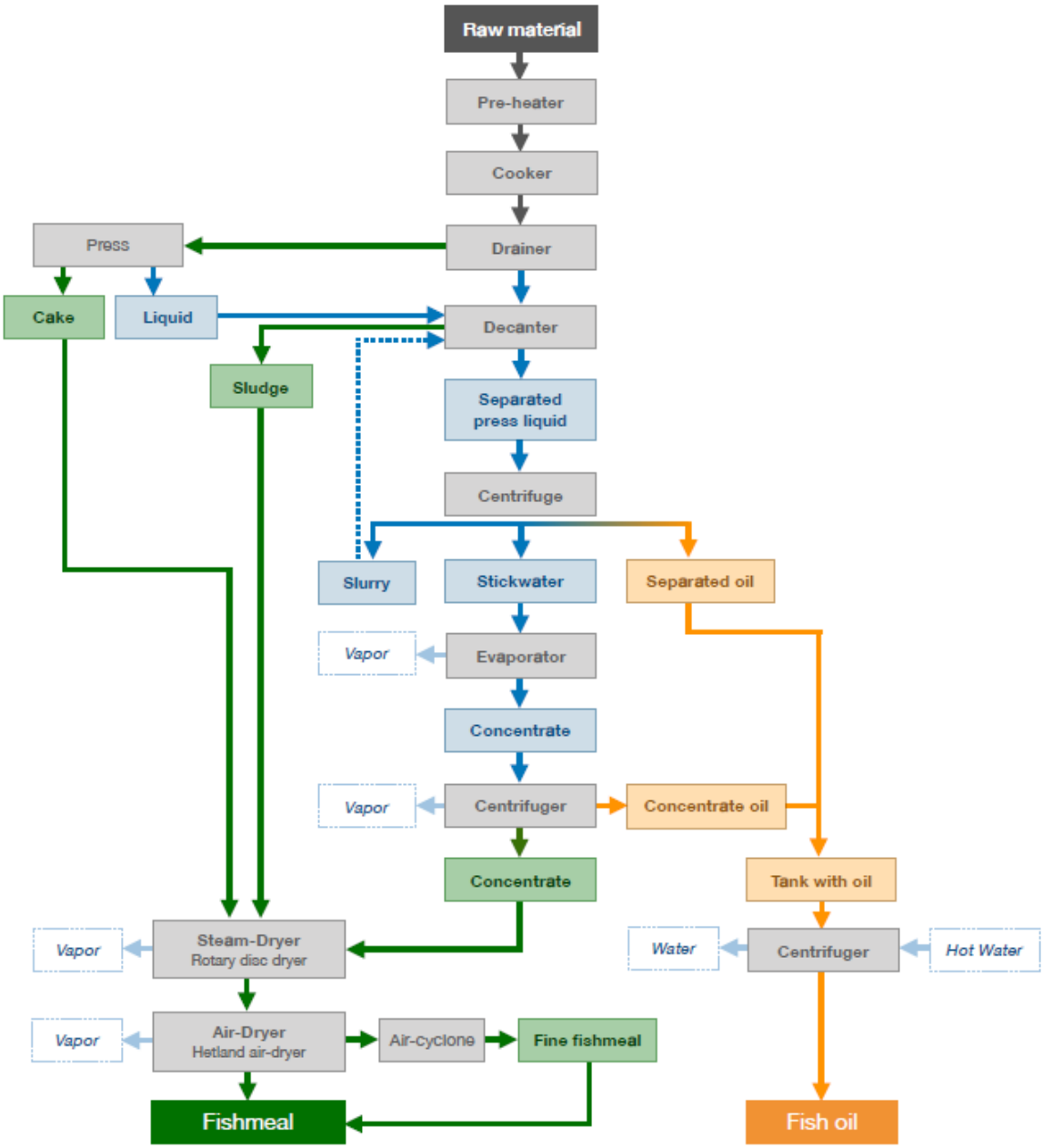


Figure 7: Traditional production of fishmeal and fish oil. Solid streams are identified with green color, liquid streams with blue color, and oil streams with yellow color. Machinery is identified with grey color.

energy-saving, heating the raw material to approximately 55°C for 20 min. Excessive energy from other processing steps is often used during the pre-heating step. Next, the cooked material is ***drained,*** followed by a ***press*** for water and oil removal.

The liquid stream is pumped to a decanter to recover the solid particles before oil recovery via a ***centrifuge***. The liquid stream is then concentrated by ***evaporation***, and the ***concentrate*** is dried along with the ***press cake*** and ***sludge***, forming the commercial ***fishmeal*** (Figure 7)***.*** The oil streams from the centrifuges were blended and washed with hot water to create the final fish oil.

Several opportunities for optimizing or redesigning the traditional fishmeal and fish oil processes towards producing protein powder for human consumption are promising as the pelagic fish raw materials are an excellent source of minerals, proteins, and lipids (Jayathilakan et al., 2012; Luten, 2009). However, if intended for human consumption, hygienic processing conditions must be improved to ensure minimal degradation in the raw material (Einarsson et al., 2019; Windsor, 2001). Degradation processes, such as lipid-oxidation induced rancidity could lead to further accumulative toxic effects, lower the nutritional value, and form unacceptable flavors and odors (Einarsson et al., 2019; Windsor, 2001).

2.2.1 Heating effects on fishmeal and fish oil quality

Traditional fishmeal and fish oil processes (Figure 7) include several heating and drying steps, which require high energy (Smarason et al., 2017). The primary purpose of heating and cooking during fishmeal processing is to break down and degrade the raw material, to ease extraction and separation of the lipids from the raw material into the liquid stream, resulting in protein-rich fishmeal with a low lipid content (FAO, 1986). Heat is known to affect different parts of the muscle at different temperatures (Hastings et al., 1985), highlighting the importance of applying an optimal temperature for an efficient break-down of the raw material during cooking. Appropriate cooking temperatures during fishmeal and fish oil production have been discussed in earlier studies (Einarsson et al., 2019; Nygaard, 2010), where 95-100°C for 15-20 minutes is the most common practice (FAO, 1986). However, fishmeal produced at a temperature below 90°C has been shown to have a lower content of lipids and FFAs, as well as higher digestibility (Opstvedt et al., 2003), possibly due to lower degradation of the proteins through non-enzymic browning (Maillard reactions) (Hall, 2010). Furthermore, high-temperature exposure of fishmeal produced with the outlet meal temperature at about 100°C showed a significant reduction in digestibility compared to fishmeal produced at the outlet meal temperature about 70°C researched in-vitro with mink (*Mustela vison*, L) as a model animal (Opstvedt et al., 2003).

Prior to cooking, the raw material is often pre-heated by using an excess heat source from the processing, such as from the steam dryer or evaporator. This energy recycling can lead to substantial energy savings (Einarsson et al., 2019). The temperature during pre-heating is often 55°C for 20 minutes, which increases the enzymatic activity of the raw material (Einarsson et al., 2019). The increased enzymatic activity can lead to hydrolysis of proteins into unwanted peptides and amino acids (Thorkelsson et al., 2009), which can further transform into biogenic amines with off-flavors as ammonia (Toldrá & Reig, 2011). Furthermore, increased enzymatic hydrolyzation can lead to the formation of free fatty acids (FFAs) from triacylglycerides (TAGs) and phospholipids (PLs) (Ackman, 1967; Arason, 1994). Correlations between off-flavors and FFA formation have been reported (Refsgaard et al., 2000), and heating has been linked to the formation of off-odors and off-flavors during initiation of oxidative reactions (Toldrá & Reig, 2011). This initiation consists of free radical formation being catalyzed, by enzymes, metallic cations, light, moisture, or heat (Toldrá & Reig, 2011), which all can affect the quality during processing. Furthermore, autolysis can also degrade proteins to low molecular weight peptides and free amino acids, which is one of the problems arising in ungutted fish during heavy feeding periods (Thorkelsson et al., 2009). Hydrolysis can also affect the efficiency of the press by lowering the viscosity of the press cake and press liquid due to a higher separation between solids and water (Einarsson et al., 2019). Furthermore, hydrolyzation can decrease the variation of solid particles in the stream, lowering the viscosity (Hall, 2010)

Generally, at high temperatures, health-promoting ingredients such as polyunsaturated fatty acids (PUFAs) are likely to be degraded during fishmeal and fish oil production (Fellows, 1988; Fournier et al., 2006; Jacobsen, 2015). However, as high temperatures cause accelerated oil deterioration to free fatty acids (Fellows, 1988), traditional Hetland air-dryers, in which the temperature may go up to 450°C, can highly affect the raw material. Hence, heating and drying techniques need to be optimized to maintain high lipid quality throughout the process for a high-quality end-product. In addition to high heat, fishmeal and fish oil factories are not closed systems, and hence oxygen is continuously introduced to the system during processing. Thus, as PUFA are highly susceptible to lipid oxidation, undesirable fishy and rancid off-flavors may form (Jacobsen, 2010) during processing. Hence, adjusting the air-drying temperature or choosing alternative drying methods might result in a more attractive color of the fishmeal. Furthermore, significant color changes were observed at temperatures above 60°C when drying sardines due to lipid oxidation (Tarhouni et al., 2019). Therefore, adjusting the temperature during drying can result in a higher quality product.

Different drying techniques require various durations and temperatures, which affect the quality of the fishmeal. Traditionally, the drying is carried out in two steps due to energy saving. A rotary disc steam-dryer lowers the water content to approximately 40% water, followed by an air-dryer, which reduces the content further down to 5-10% water. However, the drying steps combined take around 40-45 minutes, where the steam in the steam dryer is at 95°C for 30 minutes, and the air input at the air dryer is 450°C for 10-15 minutes (middle of the air-dryer is at 150°C).

2.3 Quantitative lipid and protein quality attributes in fishmeal and fish oil production

Common methods for characterization of the raw materials include analysis of the proximate composition, salt soluble proteins (SSP), the production of volatile and biogenic amines, thiobarbituric acid value (TBA), anisidine values (AV), free fatty acids (FFA), and more. High protein content is of special interest when producing fishmeal. However, the proteins can decompose into amines and ammonia, affecting the protein recovery (Einarsson et al., 2019; Keller, 1990), which occurs if the raw material is not fresh when produced into fishmeal and fish oil (EFSA, 2010).

The freshness of the raw material entering the fishmeal and fish oil factory is often assessed by measuring total volatile nitrogen (TVN) or total volatile basic nitrogen (TVB-N). Nitrogen bases, such as trimethylamine (TMA) and dimethylamine (DMA), along with ammonia, are formed during the spoilage of fish (EFSA, 2010). The freshness criterion in the current Hygiene Regulations for whole fish fit for human consumption is 60 mg TVB-N/100 g fresh weight, although no scientific evidence lies behind that criterion (EFSA, 2010). Other components often used in the industry to assess the quality of the fishmeal include biogenic amines such as cadaverine, putrescine, tyramine, and histamine, which combined levels cannot exceed 2000 ppm. Furthermore, are additional threshold values set to histamine content, or 500-1000 mg histamine/kg food (Einarsson et al., 2019; Pike & Hardy, 1997). The only fishmeal fulfilling the recommended criteria is produced from fresh raw materials, while moderately fresh or stale raw materials rarely reach acceptable criteria (Einarsson et al., 2019; Pike & Hardy, 1997).

Although fishmeal and fish oil buyers emphasize the importance of the above quality parameters, attributes such as salt soluble proteins (SSP) can give valuable information on protein degradation effects during processing. However, the analysis of SSP has not gained as important status since fishmeal and fish oil are generally not used for human consumption. However, protein solubility is an essential function in food applications (Thorkelsson et al., 2009) and is widely used as an index for protein denaturation (Xiong, 1997). When denaturation occurs, hydrophobic amino acid side

chains increase at the surface, and the apolar groups interact, forming protein aggregates (Xiong, 1997). Furthermore, heating has been reported to decrease protein solubility in SSP extracted from Pacific oysters (*Crassostrea gigas*), inhibiting digestibility (*in vitro*) and negatively affecting proteolysis (Zhang et al., 2020). Hence, measuring SSP in protein-rich processing streams could explain how the fishmeal and fish oil production processes affect protein solubility, degradation and overall protein quality.

Although many quality attributes can characterize the fishmeal, one of the most critical factors is its lipid content, since it is used in industry to categorize fishmeals into quality types or classes (Einarsson et al., 2019; FAO, 1986). Fishmeal produced under hygienic conditions and with no lipid content limit is defined by The Food and Agriculture Organization (FAO) of the United Nations as ***fish protein concentrate (FPC) Type-C*** (Einarsson et al., 2019; FAO, 1986). ***FPC Type-B*** allows up to 3 g lipid/100 g sample, and having a fishy flavor and odor after production due to rancidity after storage, and ***FPC Type-A*** should contain <0.75 g lipid/100 g sample and have no flavor and no odor (Einarsson et al., 2019; FAO, 1986). Hence, more effective separation of the lipids from the solid streams would benefit fishmeal processing companies as the simultaneously obtained higher yield of fish oil would result in a higher yield of low-lipid fishmeal. However, the lipids remaining in the fishmeal are likely to be dominated by phospholipids (PLs), as the PLs form the lipid bilayer of the cell membrane (Alberts et al., 2002). Hence, it could be challenging to access and separate those lipids from the solid streams compared to triglycerides, which are stored in the fish raw material as fat deposits (Huss, 1995).

During fishmeal processing and storage, free fatty acids (FFA) can form due to enzymatic hydrolysis of triglycerides, which is accelerated at the pre-heating stage at 40-50°C (Einarsson et al., 2019). Correlations between off-flavors and FFA levels have been found (Refsgaard et al., 2000), indicating the importance of minimizing autolysis. Autolysis can degrade proteins to low molecular weight peptides and free amino acids, which remain one of the problems in ungutted fish during heavy feeding periods (Thorkelsson et al., 2009).

Observing the fishmeal and fish oil production process on a lipid quality basis can identify potential challenging processing steps or hot spots in the overall process and identify occurrences leading to fishmeal production with a high lipid content production. Hence, in the current study, the primary focus was set on investigating the process on a lipid basis to achieve effective lipid separation from the process. The detailed lipid analysis was then followed up by analyzing changes in the protein quality during processing, including crude protein content, SSP and amines (both biogenic and TVB-N), and further quality attributes of the final fishmeal and fish oil.

2.4 Environmental impacts of fishmeal and fish oil production

The fisheries and aquaculture sector promotes environmental, economic, and social sustainability along the value chain (FAO, 2020). Life-cycle assessment (LCA) can be applied to determine the environmental impacts of the production and provides a quantitative assessment perspective of sustainable solutions within the value chain (Hauschild et al., 2018).

The environmental impacts of a production are described in different impact categories, such as global warming, fossil resource scarcity, and terrestrial ecotoxicity, which represent the lifetime of the system or the product investigated. The impact categories covered by a LCA should reflect a comprehensive set of environmental issues that relate to the system or product under study, along with the goal and scope of the investigation (Hauschild et al., 2018). Hence, evaluating fishmeal and fish oil production with LCA can give a quantitative estimate of environmental impacts.

Environmental impacts of fishmeal production (Samuel-Fitwi et al., 2013) and fishmeal factories (Fréon et al., 2017) for feed applications have been studied, but information on effects of the raw material acquisition, the production, and cleaning and waste are missing from literature. Moreover, as the fishmeal and fish oil production processes have remained similar since the 1960s, the production process needs optimization. The effect of each processing step was investigated on an environmental impact basis. Hence, decision-makers can access all information to take an enlightened decision on which processing steps to prioritize for optimization both from a quality and environmental impact aspect. The driving force in such process adjustments generally aims to increase product quality, although higher quality does not always go hand in hand with environmental gain. However, as the fishmeal and fish oil production processes are energy-intensive (Fréon et al., 2017; Smárason et al., 2017), relatively minor adjustments within the energy usage of the production process could result in a significant environmental gain, including higher product quality. Therefore, LCA was applied in the current study to estimate the environmental impacts of the current production process. The LCA further identified hot spots within the process with high environmental effects, which was included in the discussion of which processing steps should be prioritized during the required process optimization, leaning towards higher product quality, cleaner production, and environmental sustainability.

Earlier assessments of the environmental impacts of fishmeal factories applying midpoint evaluation showed that the highest values in most of the impact categories originated from running the fishmeal factory, compared to the effects of maintenance and construction (Fréon et al., 2017). Furthermore, 87% of prime

fishmeal facilities processing Peruvian anchoveta ran on heavy fuel oil (or natural gas used during heating) during processing in 2008-2012 (Fréon et al., 2017), highlighting the importance of choosing the energy sources used during production wisely. However, the environmental impacts have also been studied in aquaculture feed (Boissy et al., 2011; Samuel-Fitwi et al., 2013).

Fishmeal and fish oil products are mostly used in aquaculture and are considered the most nutritious and digestible ingredients for farmed fish feed (FAO, 2020). However, studies show that exchanging the fishmeal for other animal by-products up to 90% of the feed did not affect fish growth and had minor effects on amino and fatty acid requirements (Galkanda-Arachchige et al., 2020), where examples of total fishmeal replacement has resulted with equal growth performance, feed conversion and survival for salmon (Davidson et al., 2016). Moreover, Samuel-Fitwi et al. (2013) reported a 58% to 59% reduction in global warming by substituting the fishmeal with 50% rapeseed- or soybean meal concentrate, where the standard feed was 65% fishmeal, compared to soybean- or rapeseed meal with 32.5% soybean/rapeseed meal and 32.5% fishmeal. However, studies have shown that substituting fishmeal for vegetable originating meals can have a negative impact on the fatty acid composition of the farmed fish species, including the omega-3 fatty acids (Huy et al., 2021; Torrecillas et al., 2017).

2.5 Near infrared spectroscopy and quality detection in fishmeal

Near infrared (NIR) spectroscopy is a valuable method to monitor various physicochemical properties during seafood production (Cozzolino & Murray, 2012; Oehlenschläger, 2014). NIR spectroscopy builds on covalent bonds absorption in the near infrared spectrum, at wavelengths ranging from 700-2500 nm, which are descriptive for the physical and chemical environment of the studied molecules. Furthermore, as the technique is fast, robust, and sample non-destructive, NIR spectroscopy is highly suitable for monitoring various physical and chemical properties in industrial implementations (Guðjónsdóttir, 2011). Moreover, applying NIR monitoring during processing allows fast decision-making and redirection of processing ways in agreement with the characteristics of the raw material and processing streams. Due to its online application features, the production processes can thus be easily altered if problems arise during the fishmeal and fish oil production.

NIR has been used for diverse fishmeal quality assessment, such in distinguishing between low-value Chinese fishmeal and high-value Peruvian fishmeal samples, with respect to differences in absorbance related to their water-, lipid-, and protein content (Shen et al., 2017).

Predicting moisture, ash, oil, TVN, and NaCl content in the final fishmeal products has also shown promise in several studies (Cozzolino et al., 2002; Fontaine et al., 2001; Lv et al., 2013; Masoum et al., 2011). Furthermore, the fishmeal content in other feed compounds can be determined and quantified in other compound feeds using NIR with high precision models (R^2>0.95) (Lv et al., 2013) Indirect quality assessments of calcium, copper, and phosphorus have also been predicted using NIR, but a prediction of minerals is important as fishmeal is currently used in animal feed (Masoum et al., 2011). Furthermore, (Cozzolino et al. (2002) predicted crude protein content with an acceptable prediction (R^2>0.85), which could be useful monitoring under industrial conditions. Further studies have been conducted predicting heat damage on fishmeal quality by NIR (Cozzolino et al., 2009). All of the essential amino acids, except histidine and phenylalanine have been predicted using NIR in wide applications, indicating pea-, soy-, rapeseed-, sunflower-, meat-, poultry- and fishmeal (Fontaine et al., 2001). However, as the explained variance for the linear crude protein regression (RSQ_{CP}) in the study by Fontaine et al. (2001) had a wide range from 0.4-0.8(Fontaine et al., 2001)determining those amino acids with NIR spectroscopy should be taken with care.

All above mentioned studies applied NIR spectroscopy to assess the quality characteristics of the final fishmeal. Furthermore, NIR assessments of fishmeal quality are generally considered easy and inexpensive and are already currently widely adopted by the industry (Cozzolino et al., 2002). However, according to the best knowledge of the research team, no references are found in the literature on the use of NIR to monitor fishmeal and oil quality ***during processing***. Therefore, emphasis was put on applying NIR for process analytics and monitoring in the current study.

Fish oils have also been studied with NIR and PLS regression models to monitor hydrolytic and oxidative changes during the production and storage of fish oils (Cozzolino et al., 2005). The FFA and moisture predictions were adequately determined by NIR, with coefficients of determination in the calibration set R^2>0.94, and coefficients of correlation in the validation set r >0.80 (Cozzolino et al., 2005). In that study the peroxide value and anisidine values were not considered adequately determined. However, the industry calls for more specific chemical information on the nutritional qualities of the oil, including their fatty acid profiles (Cozzolino et al., 2005). This led to the investigation of the correlations between the NIR reflectance and the changes in fatty acid compositions during the fishmeal and oil processing in the current study.

3 Research questions and objectives

The main objective of this study was to investigate the traditional fishmeal and fish oil production processes and the effect of each processing step on the processing streams and products, with a focus on lipid and protein quality. Moreover, the study investigated the environmental impacts of producing 1000 kg of capelin fishmeal and accompanied fish oil during standard procedures compared with reduced cooking temperature and according to choice of fuel source. The final objective was to investigate whether NIR spectroscopy could be used to effectively monitor the quality of the streams during processing.

The detailed objectives of the study were thus to:

- Evaluate the current quality and effectiveness of traditional fishmeal and fish oil processes (Paper I-III).
- Assess traditional fishmeal and fish oil production quality and effectiveness while processing different pelagic fish species (Paper I).
- Evaluate the effect of changing the temperature in the cooker during fishmeal and fish oil production (Paper II).
- Evaluate the protein quality changes in the protein-rich production streams during traditional fishmeal and fish oil production (Paper III).
- Assess the change in the environmental impacts of the fishmeal and fish oil production process by decreasing the temperature in the cooker from 90 to 85°C (Paper IV)
- Investigate the environmental impacts of using heavy fuel oil, hydropower or a combination of both during the production of fishmeal and fish oil (Paper IV)
- Evaluate the robustness of using NIR spectroscopy to assess chemical quality changes during fishmeal and fish oil processing (Paper V)

4 Materials and methods

4.1 Experimental design

The study was divided into five scientific papers, aiming to investigate the current quality and effectiveness of traditional fishmeal and fish oil production processes, and to identify where the process could be improved towards increased quality, yield, and sustainability. Moreover, the study investigated how raw material variations and changes in cooking temperatures affect production and impact the environment. A study overview, including short titles on Papers I-IV, is described in Figure 8.

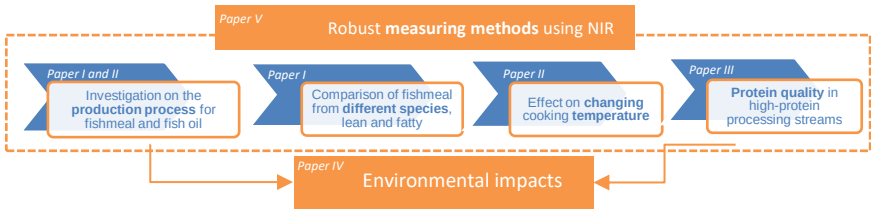


Figure 8: Flowchart of the study design.

A flowsheet of the studied fishmeal and fish oil production process can be seen in Figure 8 and details regarding the raw material processed into fishmeal and fish oil in Table 1.

4.1.1 Experimental design for Papers I-III

The objectives of Paper I-II were to study the current product quality and effectiveness of a traditional fishmeal and fish oil production process. Paper I focused on the lipid quality of each processing step during the production of both fatty and lean species, comparing the processing of capelin (*Mallotus villosus*), a mackerel (*Scomber scombrus*) and herring blend (*Clupea harengus*), and of blue whiting (*Micromesistius poutassou*). Paper I and II describe a detailed investigation of the effect of each processing step on the raw material during capelin and mackerel/herring blend production. However, blue whiting samples were only collected at key processing locations, which were defined as the solid streams entering the drying steps (press cake, sludge, and latter concentrate), and characterization of the raw material and end products (Figure 7). Details of the raw materials used in the study can be seen in Table 1.

Table 1 Details of the raw material used in the fishmeal and fish oil production studied in Paper I-V. The Table adjusted from Table 1 in Paper I.

Name in text	Blue whiting	Capelin	Mackerel/herring blend
	whole	*whole*	*cut-offs*
Species and by-catch	**100% *Blue whiting*** (*Micromesistius poutassou*)	**100% *Capelin*** (*Mallotus villosus*)	58% ***Atlantic mackerel*** (*Scomber scombrus*) 37% ***Atlantic herring*** (*Clupea harengus*) 4.5% ***Blue whiting*** (*Micromesistius poutassou*) <0.5% By-catch
Catching date	30.04.19	28.02.18	03.09.17 - 07.09.17
Dates fishmeal processed	02.05.19 - 03.05.19	01.03.18 - 02.03.18	07.09.17 - 08.09.17
Catching grounds (of Iceland)	South of Faroe Island (60°North 7°West)	320 (south), 616 (northeast)*	400, 511, 512, 553-555, 600 (southeast)*
Fishing equipment	Midwater trawling	Purse seine	Midwater trawling
Total catch	2,170 tonnes	2,450 tonnes	884 tonnes
Temperature at landing	2.1°C (one trawler)	4±1.5°C	3±1.5°C
Sample analysis time	Up to 3 months	Up to 7 months	Up to 6 months

Abbreviations: TVN (total volatile nitrogen), *Location around Iceland

The objective of Paper II was to investigate quality changes affected by changing the cooking temperatures in the cooker, from the traditional 90°C to either 85°C or 95°C, where the separation between lipids and fat free dry matter (FFDM) was of particular interest. As the production input was 1200 tonnes per day during the operation time, more drastic temperature changes could not be made during production, since the tested fishmeal, and fish oils are sold commercially. However, the literature lacks clear evidence on the effects of temperature changes on the quality and effectiveness of fishmeal production during cooking. Hence, the impacts of changing the cooking temperature were investigated during processing of a mackerel/herring blend. The result from Paper II indicated a higher lipid quality with decreased cooking temperatures. However, the focus was set on protein quality in the following paper to get a holistic view of the fishmeal and fish oil production.

Paper III thus focused on protein quality of protein-rich side streams during the production in critical processing steps (raw material, press cake, sludge, and latter concentrate) as those processing streams are later combined to form the final fishmeal after drying. Moreover, the samples taken from these key processing steps were shown in Paper I-II to have very different chemical compositions. Therefore, the protein quality was investigated and categorized in the before mentioned key processing streams in Paper III, along with the raw materials and end products.

4.1.2 Design for Paper IV

The objective of Paper IV was to investigate the environmental impacts of traditional fishmeal and fish oil production, including the environmental impacts of lowering the cooking temperature by 5°C, from 90°C to 85°C. This decrease in temperature resulted in higher lipid quality, as described in Paper II. The life-cycle assessment was based on the functional unit of "*1000 kg capelin fishmeal produced, with the simultaneous production of fish oil, produced in a plant run on hydropower in Iceland in 2018*". Mass balances and energy flow were calculated based on the obtained chemical, mass, and energy analyses from the standard process (90°C) and the reduced temperature process (85°C) presented in Paper II. Data on the annual, overall fishmeal and fish oil production data for 2018, chemicals used for cleaning, and energy usage used for the analysis were obtained from the company's green accounting reports obtained from the Environmental Agency of Iceland (Síldarvinnslan, 2018). Furthermore, the mass balance and energy flow calculations were aligned and fitted with the production and energy numbers provided in these reports. The mass balances and LCA calculations were performed in close collaboration with Síldarvinnslan.

4.1.3 Experimental design for Paper V

The objective of Paper V was to investigate the potential application of near infrared (NIR) spectroscopy, a fast and non-destructive measuring technique, to assess chemical quality changes in the fishmeal and oil during processing. Using NIR to measure and analyze samples and predict lipid quality would be beneficial for future changes in the production, as the technique is generally robust, results and feedback are quick, and samples do not require sample preparation prior to analysis. The samples used in Paper II were thus also analyzed for NIR reflectance, and partial least square (PLS) models were built to assess the predictability of the chemical composition of the samples throughout the processing.

4.2 Analytical methods

Various analytical methods were used in the papers to evaluate the performance of the fishmeal and fish oil processing, which included the water and lipid quality of the samples, evaluation of the robustness of quality monitoring with NIR spectroscopy, and the overall environmental impacts of the fishmeal and fish oil production.

The focus was set on lipid quality and the separation between lipids and fat-free dry matter in the production streams. The separation of lipids has proven to be challenging, although its implications are vast. A high lipid content lowers the value and stability of the fishmeal due to a higher risk of lipid oxidation, and the oxidative

stability decreases with increasing unsaturated fatty acids (Mozuraityte et al., 2016; Tengku-Rozaina & Birch, 2013). Detailed knowledge regarding the effect of each processing step on the lipid separation is deficient in literature, and hence lipid quality-oriented methods were prioritized before protein quality-oriented methods in the current study.

Lipids can be stored in the raw material as fat depots, expressed as triacylglycerides (TAGs), consisting of glycerol and three fatty acids (Huss, 1995). However, lipids in the cell membrane are mostly phospholipids (PLs) and are both hydrophobic and hydrophilic (Alberts et al., 2002), making them difficult to extract. Hence, separating the PLs from the solid streams is more complex than separating the TAGs from the solid streams. Thus, measuring the PLs throughout the production could give an overview of where processing steps should be altered. Otherwise, the majority of the PLs are likely to follow the solid material to the dryers and not be extracted out, leaving the fishmeal high in lipid content, as the PLs undergo no significant chemical hydrolysis if processed traditionally (de Koning, 2002; de Koning et al., 1986).

However, as excessive water, heat and oxygen are present during fishmeal, and fish oil production, lipid oxidation and hydrolyzation seem inevitable as lipid oxidation is induced by light, temperature, and oxygen (Jacobsen, 2015). Hence, lipid hydrolysis as assessed by the formation of free fatty acids (FFA) was measured to identify adverse effects during the fishmeal and fish oil production processes. Furthermore, correlations between off-flavors and FFA levels have been found (Refsgaard et al., 2000). During hydrolysis, PLs and TAGs were expressed as FFAs. The composition of the fatty acids was analyzed to investigate the nutritional value of each processing step; quantifying and locating any loss in fatty acids would hence be possible.

4.2.1 Water and lipid quality

Water content was measured according to the ISO 6496 method (ISO, 1999) except for water in oil samples, where titration by an 851 Titrando instrument (Metrohm, Herisau, Switzerland) was used. Lipid extraction, according to Bligh and Dyer (1959), was used for analyzing total lipid content, phospholipids (Stewart, 1980), free fatty acids (Lowry and Tinsley, 1976; Bernárdez et al. 2005), and fatty acid composition (AOCS, 1998; Dang et al., 2017, 2018; Romotowska et al., 2016, 2017).

In the current study, FFA and PL content might be underestimated as the standard curve of FFA was prepared with oleic acid, and the PL standard curve for the PLs assessment was made with phosphatidylethanolamine. As FFAs are amphiphilic, they can dissociate in aqueous systems, form micelles, and are dispersed into the water as emulsion particles during lipid extraction (Frankel, 2012), and thus often

underestimating the FFA concentration of the sample. Since oleic acid was the only fatty acid measured during the FFA assessment, the risk of underestimating the free fatty acids in the sample is quite high. In the same way, the PLs content is potentially also underestimated as phosphatidylcholine was the only PL measured, as it is the most abundant phospholipid class in the cell membrane (Alberts et al., 2002). Other classes such as phosphatidylethanolamines, phosphatidylserines, and sphingomyelins were not measured. Around 5-10% of the peaks remained unknown and unassigned during the fatty acid composition assessment.

4.2.2 Protein quality

Crude protein content was measured according to ISO 5983-2 (ISO, 2009), and a method described by Kelleher & Hultin (1991) was followed to investigate salt soluble protein (SSP). Biogenic amines were measured using the method described by Olajos (2015) and total volatile basic nitrogen (TVB-N), trimethylamine (TMA) and dimethylamine (DMA), were measured by the method described by Malle & Poumeyrol (1989). Protein degradation could hence be assessed as non-protein nitrogen compounds, including biogenic amines, TVB-N, TMA, and DMA, as they contribute to both taste and smell (EFSA, 2010; Pérez-Villarreal et al., 2008). TMA formation is produced mainly by bacterial degradation and DMA through endogenous enzymatical pathways. However, TVB-N represents the sum of ammonia, DMA, TMA and other volatile basic nitrogenous compounds in the samples (Pérez-Villarreal et al., 2008; Toldrá & Reig, 2011). Hence, the deduction of the TVB-N from the total crude protein content indicates how much of the protein is denatured. Along with DMA, formaldehyde is produced and increases protein denaturation (researched in cod) and decreases solubility (Xiong, 1997; Zayas, 1997b). Furthermore, protein denaturation could increase due to interactions between the formaldehyde and the side chain groups, resulting in protein aggregation through non-covalent interactions (Xiong, 1997; Zayas, 1997b), while protein aggregation due to exposure of hydrophobic residues can happen during thermal treatment (Kristinsson & Rasco, 2000). Solubility was studied by measuring salt soluble protein content (SSP), but solubility is the main characteristic of proteins used in liquid foods and beverages (Zayas, 1997b).

4.2.3 Life cycle assessment

A life-cycle assessment (LCA) was performed to study the effect and impacts on the environment of the entire process. The advantages in applying this method are involved with the flexibility in the evaluation and how detailed it is (Gregory et al., 2009). The disadvantages mainly include the extensive, thorough knowledge needed to conduct and interpret the results and value judgment on environmental priorities

for an effective application (Gregory et al., 2009). Collecting data can be problematic, as companies do not necessarily report all incidents or occurrences (intentionally or unintentionally) (Hauschild et al., 2018). However, as the focus was set on energy and power consumption during the LCA in Paper IV, assessing such challenging factors was at a minimum.

The study follows a standardized LCA methodology, as presented by the ISO 14044 and ISO 14040 standards and described in detail by Hauschild et al. (2018). The LCA was performed in SimaPro (PRé Sustainability, Amersfoort, Netherlands) with inventory from "Ecoinvent 3.6". Data comparison was performed in Microsoft Office Excel (Microsoft Inc., Redmond, WA, USA).

4.2.4 NIR assessment

The effectiveness and prediction precision using near infrared (NIR) spectroscopy to monitor several chemical quality parameters was assessed and compared to the traditional analytical methods described in the next chapter. The benefits of applying this fast, non-destructive analytical technique for both time and cost-efficiency were also discussed. Although it is not a standardized method during fishmeal processing yet, NIR has been used in earlier studies to predict the composition of both the raw materials and final products in real-time with good precision and accuracy. Moreover, risk of human error would be minimized during sample measurements with NIR spectroscopy. However, studies on the robustness and precision to predict and monitor quality during fishmeal and oil processing was lacking. The study set up for paper V tried to fill that knowledge gap.

The near infrared (NIR) measurements were done by a Bruker Multi-Purpose Analyzer system with a fiber probe (Bruker Optics, Rheinstetten, Germany). The wavelength ranged from 800 to 2500 nm, and five scans were used to build an average spectrum for each sample. All analyses were then performed in triplicate. Partial least square (PLS) prediction models using the spectral data and chemical assessment results in Paper II were built and validated to predict the chemical composition of the samples during processing using the Unscrambler® software (Version 10.5.1, CAMO ASA, Trondheim, Norway). Two of the sampling replicates were used for the calibration building, while the third sample replicates were used to form an independent validation matrix.

4.2.5 Statistical analysis

Data summaries and handling, figures, and tables were generated in Microsoft Office Excel 16 (Microsoft Inc., Redmond, WA, USA). Analysis of variances (ANOVA) and Tukey's HSD tests was performed in RStudio (RStudio Inc., Boston, MA, USA) to assess differences between samples and treatments. The significance level was set $p<0.05$

for all statistical tests and models, and the results were shown as mean values ± standard deviation (SD) from three replicates for each sample.

5 Results and discussions

A traditional fishmeal and fish oil process was analyzed to investigate how the different processing steps affected the quality of the raw materials. Firstly, the production was analyzed on a lipid quality basis (Paper I-II), where the effects of processing different species (Paper I) and applying different cooking temperatures (Paper II) were investigated. Thereafter, the mass balance (Papers III and IV) was calculated and estimated, followed by an evaluation of the protein quality of the protein-rich production streams (solid side-streams), and the benefits of processing them individually (Paper III).

The main results of the study are presented in the following chapter and in more detail in Papers I – V.

5.1 Water and lipid separation and quality (Paper I-II)

Similar trends were observed in the water and lipid content during the processing of capelin (C) and the mackerel/herring blend (MHB) (Figure 9). In both species, the ***draining step*** required optimization as the water content of the cooking step and draining did not differ significantly, indicating that the water removal during draining was ineffective. The draining needs to sieve the solid particles more effectively, as higher FFDM in the protein-rich processing streams (such as the press cake) would also lower the energy usage during pressing and the drying steps. Moreover, with more effective draining, a lower proportion of FFDM would enter the liquid streams, benefiting the whole process. This applies particularly to the evaporation, as viscosity changes of the liquid streams can affect the degree and efficiency of evaporation dramatically (Einarsson et al., 2019).

The ***press*** worked adequately as the water content significantly decreased in the press cake in all species (C and MHB in Figure 9, blue whiting (BW) in Paper I). However, as the press and drained liquids were mixed in the decanter, emulsions might immerse due to the oil still being a part of the liquid streams. The water content of the ***sludge*** variated highly between species, and the lipid content was generally lower than in the press cake. However, the sludge had a higher variation in lipid content when processing fattier species. The third solid stream entering the drying steps was the ***latter concentrate***, which had the highest water content, or

around 80% of the sample, and had the lowest lipid content of the three solid streams entering the steam dryer. The water content is usually 50-70% after evaporation (Einarsson et al., 2019; Hall, 2010) which was not the case in the current study, and no significant changes were observed in water content during the concentration step. Hence, the evaporation process requires optimization. Moreover, as the three solid streams entering the ***dryers*** differed widely in water content, from 50% to 80% water, the drying might burn some of the materials, in addition to exposing the lipids to additional heat and oxygen. Simultaneously, some material might be too wet for the following air-drying step. Hence, if the three streams are instead dried individually, the drying time and temperature of the streams could be optimized depending on the water content of each side-stream entering the dryers, possibly saving energy while simultaneously increasing the quality of the resulting products. Similar trends in water reduction during drying were noticed between species, although the water content from C was around 50% after stream drying, while the steam dried MHB material was close to 40% in water content (Figure 9).

The water content of the fishmeal was 4.6-6.4% in all species studied, which is considered relatively low. However, according to FAOs Feed and Feed Ingredient standards, no minimum water content is defined (FAO, 2001a). As fishmeal prices depend on the protein content, the water content is often adjusted with more extensive drying but simultaneously risking material burning and other heat-induced degradation. Another attribute affecting the price of fishmeal is the lipid content. The lipid content was relatively high in the fishmeal of all species. The BW fishmeal resulted in a lipid content slightly below 10%, classifying it as a Type B fish protein concentrate (FPC), while the lipid content of the MHB and C was >10%, resulting in a Type C FPCs classification. The fishmeal products were thus too high in lipid content to be considered for human consumption in all species under the current production conditions. Furthermore, their current odor and color did not help make them an attractive food option.

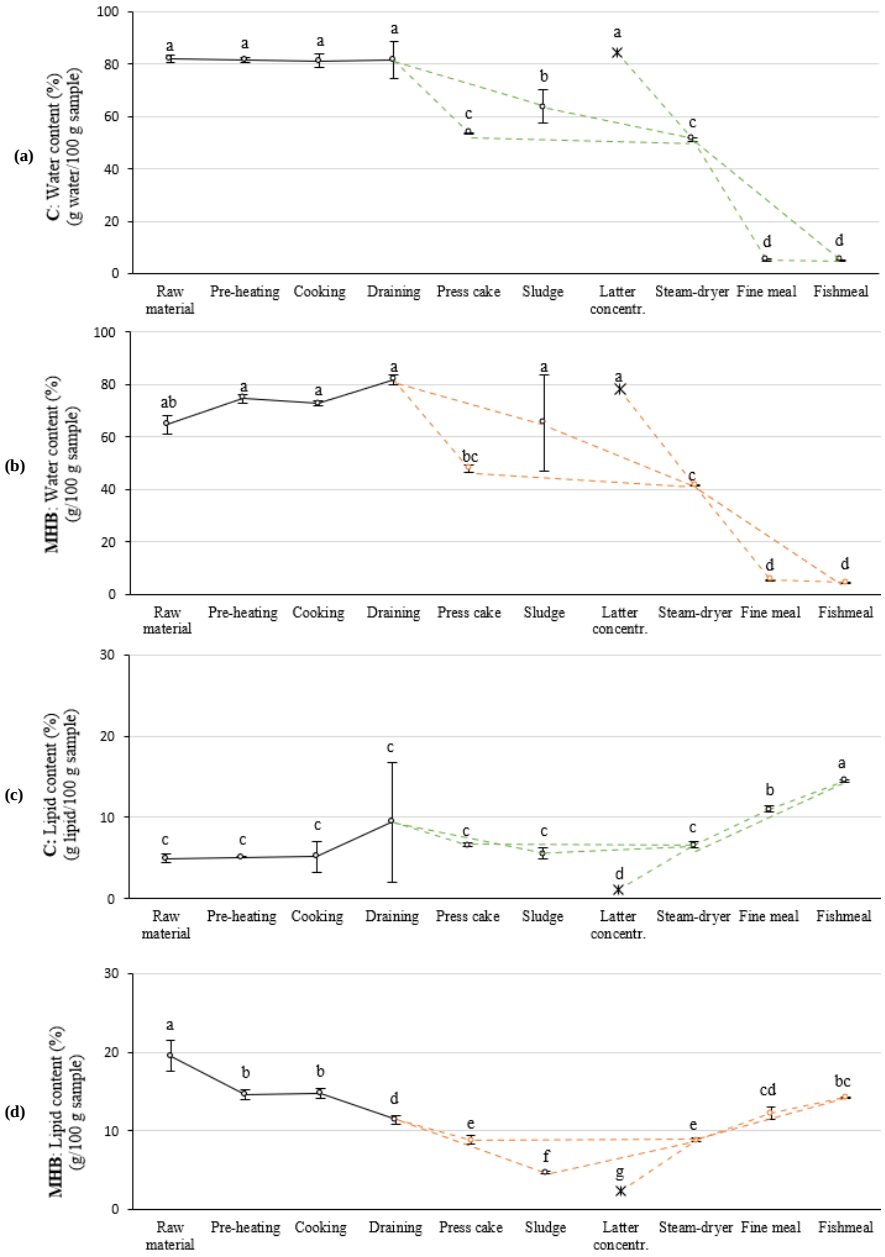


Figure 9: Water (subfigure a and c) and lipid (subfigure b and d) content changes during fishmeal and fish oil production of capelin (C, presented with green color), and a mackerel/herring blend (MHB, presented with orange color). Dashed lines indicate where more than one stream was connected between the processing steps, and an unbroken line where only one stream was connected. Letters indicate significant difference, where $p<0.05$.

The lipid content of the press cakes, sludge, and the latter concentrates differed between the species (C, MHB, BW). The highest concentration of lipids entering the drying steps came from the press cake in the fattiest raw material (MHB), while the highest amount of lipids originated from the latter concentrate in the leanest raw material (BW). The TAGs seem to aggregate in the separated press liquid before lipid extraction in the following step, depending on the lipid content of the fish species processed. The separation between the lipids and the solids could be more efficient with more optimized processing steps aiming for oil extraction. Moreover, as the solid streams are blended prior to drying, despite their different chemical composition, the lipid composition of the fishmeal did not significantly differ between the studied species, although the raw material variated between 2-20% in lipid content.

In both the C and MHB, free fatty acids (FFA) (Figure 10) increased drastically in the slurry and stickwater, possibly due to the recirculation of the slurry. This could introduce additional heat, oxygen, and water to the process, but they are all known factors to induce lipid oxidation (Jacobsen, 2015). When looking at the FFA concentrations on a lipid basis, the lipids in the stickwater were 25±15% (MHB) and 12±12% (C) FFAs, and the slurry 19±8% (MHB) and 14±10% FFAs, indicating that in such variated raw materials, the recirculation most likely introduced higher water, heat, and oxygen to the processing. FFA protein binding is known to be the driving force in lipid hydrolysis (Ackman, 1967) and is involved in the formation of secondary oxidation products, which have no beneficial health effects (Tena et al., 2018). Hence, low FFA is a crucial factor for in improving the processes towards producing products for human consumption. Recirculation could be minimized with a more effective way of breaking down the protein-lipid bonds during the initial processing steps. Moreover, if the streams are processed individually, bypassing problematic streams would be possible. This could for instance benefit the capelin fishmeal production, where high FFA values originated from the capelin sludge. Interestingly, FAO has not specified a maximum value for FFAs in fishmeal intended for feed (FAO, 2001a). However, fishmeal buyers might have their own individual preference of FFA limits.

As FFAs are among the products of hydrolyzation of phospholipids (PLs) (Ackman, 1967), it is suggested that PLs should decrease during hydrolyzation while FFAs increase. This was mainly observed during the drying steps (Figure 10). Moreover, high temperatures can cause increased deterioration of the lipids to FFA (Fellows, 1988), including long-chain PUFA degradation at temperatures 180°C-220°C (Fournier et al., 2006), which can explain the loss in PLs during drying, primarily in the air-dryer. During capelin processing, both the relatively high PLs in the latter concentrate and the high FFAs in the sludge could indicate that the processing of capelin was challenging. The reason could be higher viscosity of the C sludge, lowering the oil

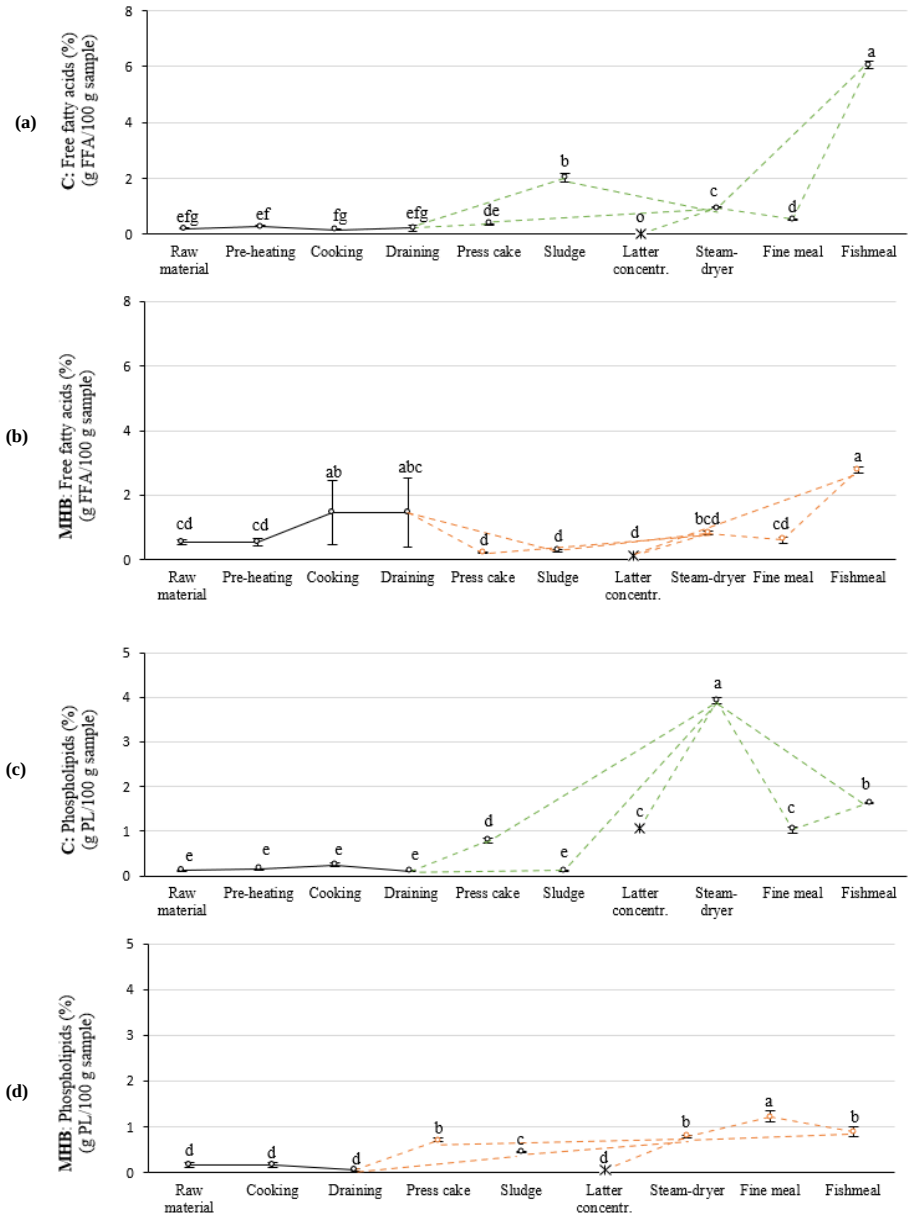


Figure 10: Free fatty acid (FFA, subfigure a and c) and phospholipid (PL, subfigure b and d) content changes during fishmeal and fish oil production, of capelin (C, presented with green color), and a mackerel/herring blend (MHB, presented with orange color). Dashed lines indicate where more than one stream was connected between the processing steps, and an unbroken line where only one stream was connected. Subscript letters indicate significant difference, where $p<0.05$

recovery from the liquid stream processing. Interestingly, the FFAs and the PLs differed significantly between the fine meal and fishmeal in both C and MHB. This questions the effects of particle size on composition and reactivity, as the fine meal samples were collected from the air duct after the air-dryer, which in theory should contain a similar chemical composition as the final fishmeal.

The fatty acid composition (FAC) was dominated by monounsaturated fatty acids (MUFAs) in all species (Paper I), in most of the processing steps. During MHB fishmeal production, most of the SFAs and MUFAs were extracted in the press-cake and the sludge, while PUFAs were highest in the concentrate. In contrast, C production showed the highest PUFAs in the sludge and press cake, while MUFAs were highest in the concentrate. Hence, to obtain the highest concentrations of PUFA, processing the C press cake and C sludge or MHB concentrate separately is advised. However, these results indicate that the mechanical process is not separating lipids from all classes effectively, and an additional oil extraction step is needed before the press. If extracted successfully, the fish oil would yield a higher PUFA yield, MUFAs, and SFAs. However, the fish oil differed significantly in composition between species. The average PUFA content in the MHB fish oil was twice the amount of PUFAs in the C fish oil. The MHB raw material is thus more suitable for process development towards the production of high PUFA oils. With more lipids extracted from the protein-rich solid streams, the increased fish oil yield would also benefit this production.

The PUFAs, omega-3 PUFAs, and EPAs (Table 3, Paper I) tend to follow the solid stream of the fishmeal and fish oil production, ending up with higher PUFAs in the fishmeal compared to the fish oil. As fishmeal is mainly used as feed for aquaculture, high PUFAs are of interest, as feed with high levels of omega-3 and omega-6 FAs are digested up to 6% better than feed with high rendered animal fats and SFA (Bureau et al., 2002). Hence, the production process could produce different products, depending on the end consumer, and side-streams with additional oil extraction steps could suit the pet food industry or human consumption, while other parts of the production could suit fishmeal production.

As a result of the lipid quality and water content investigations presented in Paper I-II, the following recommendations are suggested:

- Separate collection and processing of the fine meal and the fishmeal
- Exchange and/or optimization of the cooking process for a more effective breakdown of the raw materials. The potential use of enzymes should be investigated.
- Optimization of the draining for higher water removal from solid streams
- Adjusting the evaporation for more effective water removal

- Drying the press-cake, sludge, and the concentrate separately and adapting the individual drying times depending on the water content of each stream
- The effects of the processing are species-specific, and the process require adaptation and optimization towards the processing of each raw material.

With a more effective breakdown of the raw materials, the lipid and water separation from the solid streams should increase. Moreover, extracting the oil and PUFAs more effectively from the production process, the final oil could result in higher PUFA levels, with the improved quality and potential higher health benefits that go with them. Furthermore, with enhanced lipid separation from the fishmeal, the shelf-life of the fishmeal may thus also be prolonged (Mozuraityte et al., 2016; Shahidi & Zhong, 2010) as omega-3 PUFAs are highly susceptible to lipid oxidation (Jacobsen, 2010).

5.1.1 The effect of different cooking temperatures (Paper II) and different cookers on fishmeal quality

The choice of temperatures during cooking has been questioned in earlier studies, and most studies investigate the effects of cooking temperature ranging from 75-100°C (Einarsson et al., 2019; FAO, 1986; Nygaard, 2010). Heat-induced muscle denaturation and degradation are highly dependent on the chosen heat treatment (Fernandez-Segovia et al., 2003), highlighting the importance of using the correct temperature during cooking. Traditionally, the most common practice has been cooking at 95-100°C for 15-20 min (Einarsson et al., 2019; FAO, 1986), although temperatures down to 75°C for 25 min have been suggested (Nygaard, 2010). Hence, the lipid separation from fat-free dry matter was compared at three different cooking temperatures, or 85°C, 90°C, and 95°C in the current study.

Analysis of the MHB fishmeals showed that using a cooking temperature of 85°C resulted in fishmeal with the lowest water, lipid, FFA, and PL content. Furthermore, the PL contents were lower at 85°C and 95°C in the fishmeal, compared to 90°C, and higher at the same temperatures in the fish oil, indicating a better separation at 85°C and 95°C compared to 90°C. The fish oil had the lowest FFAs at 95°C and highest PLs at 90°C, but the difference was relatively small, which did not justify applying temperatures above 85°C.

Thus, as the water and lipid content of the fishmeal was lower at 85°C compared to 95°C, lowering the cooking temperature to 85°C can be recommended. Future research with decreasing the temperature is suggested, with or without enzymes. The purpose of the cooking step would shift towards lipid separation and homogenous solution, rather than aiming for raw material breakdown and high hygiene. Furthermore, other steps of the production process would aim for higher hygiene.

5.2 Mass and energy balances (Paper III-IV)

Investigations of the mass and energy flow throughout the fishmeal and fish oil production were performed for the capelin (C), mackerel/herring blend (MHB), and blue whiting (BW) raw materials (Figure 11).

The mass balances show that in leaner species (C and BW), higher amounts of fat-free dry matter (FFDM) enter the liquid streams already after the draining step (Figure 11). Hence, most of the FFDM in the liquid stream could be water-soluble or low kDa particles that the sieve cannot catch. Furthermore, the separated press liquid can include high lipid content depending on the raw materials processed (MHB was 15%, C 6%, and BW 1%) as the lipids have not yet been extracted from the process. Moreover, as the lipids in the separated press liquid are the main processing stream contributing to the final quantity of oil extracted from the process, a less harsh extraction process is recommended as the fish oils had lost all PLs in the final oil (<0.04 g PL/100 g lipid). Furthermore, the PLs are more susceptible to lipid oxidation compared to TAGs due to their close location to prooxidants in the aqueous phase or the liquid stream (EFSA, 2010), indicating hydrolyzation of the PLs during the production.

The first centrifugation remains a problematic step during processing, as the slurry is recirculated back to the decanter, although the main mass exits as sludge with a decreased water content. Evaporation of the stickwater was also ineffective, returning the concentrate with higher water content than expected. Evaporation of stickwater in a study by Hall (2010) showed similar tendencies and was assumed to relate to the high viscosity and stickiness of the material, which increased with lower water content. The viscosity of the processing streams must therefore be taken into consideration during process optimization.

5.2.1 Effectiveness of the concentration and drying steps

The press cake, sludge, and concentrate are blended prior to drying. The size of each stream (quantity) contributes differently to the final composition of the final fishmeal in each species (Table 2).

The majority of the FFDM and lipids in the fishmeal originated from the press cake (44-54% and 41-69%, respectively), indicating that the press worked adequately in liquid removal for both water and lipids (Table 2). This also highlights that improved lipid separation prior to pressing needs to be achieved if a high protein product with a low lipid content is to be produced. Furthermore, a high proportion of lipids were added to the fishmeal through the sludge (contributing to 18-38% of the lipids in the

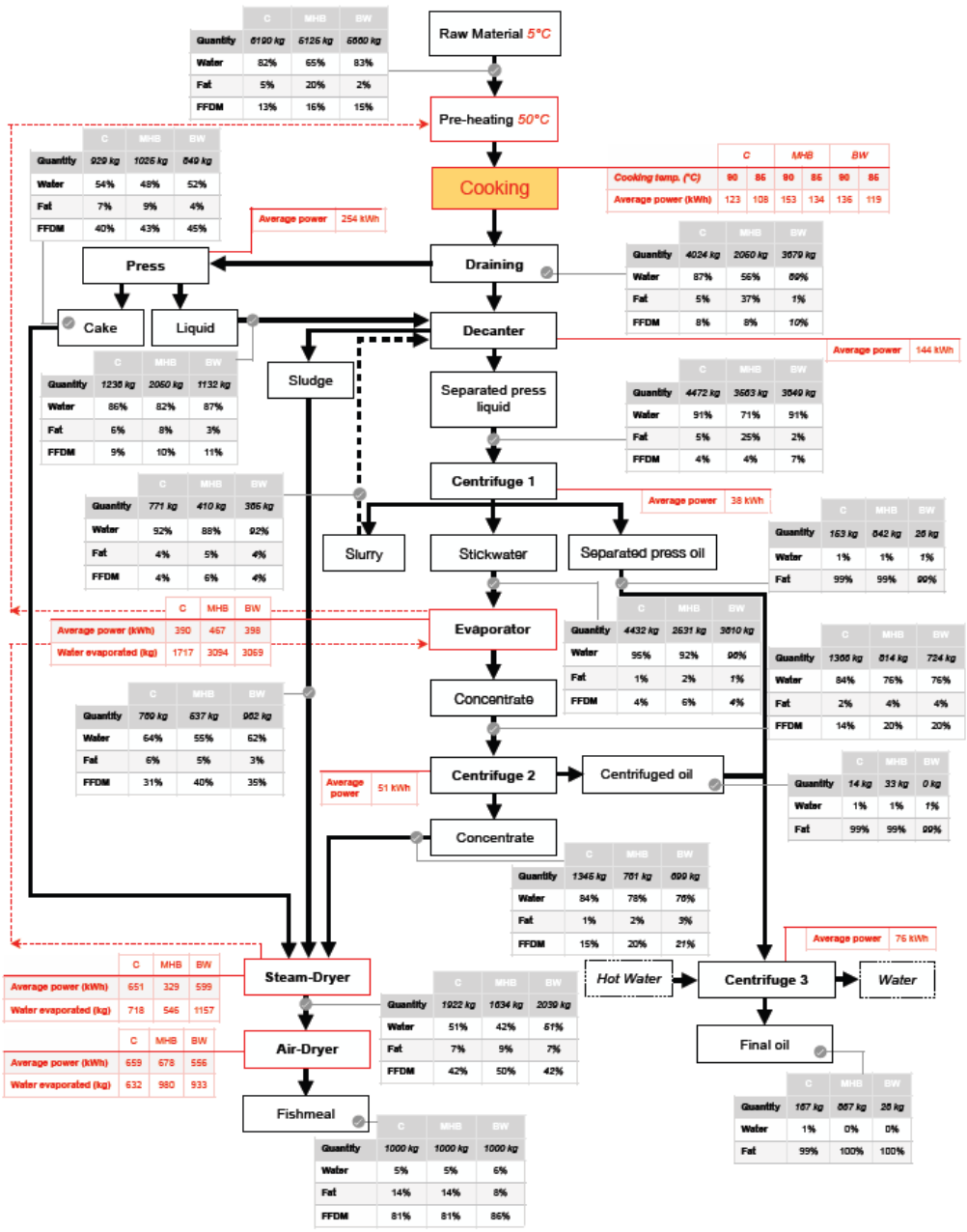


Figure 11: Mass balance (grey color) and energy flow (red color) from a traditional fishmeal and fish oil production process, with the functional unit "production of 1 tonne of capelin fishmeal including fish oil, produced by hydropower in Iceland in 2018". The quantity of each stream was calculated from water-, fat-, and fat-free dry matter (FFDM) measurements throughout processing. Water-, and lipid content was measured first-hand and presented in detail in Papers I and II . Calculated data is shown with italic letters.

fishmeal), identifying that mixing the streams is not beneficial to achieve a low lipid content fishmeal.

The concentrate resulted in the lowest contribution of lipids to the fishmeal, which was not surprising as the concentrate had passed two centrifuges aimed at extracting the lipids from the lipid stream during the production process (Figure 7, 11). However, the concentrate contained high water content (highest contributor in C and MHB), making the dryer efficiency substantially lower for these species. Furthermore, the high-water content makes the material more likely to stick to the metal plates in the steam-dryer, possibly burning part of the raw material. These findings support even further that the streams should not be mixed but rather processed individually. Optimization of the drying times and temperatures would also allow products of higher quality, potentially for human consumption.

The press cake and the sludge were the highest contributors to both PLs and FFAs in the fishmeal. Furthermore, high lipid content and high FFDM seemed to go hand in hand, indicating that the lipids were not appropriately separated from the FFDM. This suggests that the PL and FFA primarily followed the solid phase (dry matter-rich streams) during the fishmeal processing.

Table 2: Proportional contributions of ingredients (water, lipid, FFDM, PL, and FFA) from processing streams entering the dryers to the final chemical composition of the resulting fishmeal products from capelin (C), a mackerel/herring blend (MHB), and blue whiting (BW), respectively. Values represent the average percentage of water/lipids/FFDM/phospholipids/free fatty acids in the fishmeal originating from the press cake, sludge, and concentrate, respectively.

Proportional ingredient contribution to fishmeal composition [%]						
Capelin	**Quantity [kg]**	**Water**	**Lipids**	**FFDM**	**PL**	**FFA**
Press cake	929	23%	**52%**	**46%**	**51%**	**58%**
Sludge	789	24%	36%	30%	45%	38%
Concentrate	1345	**53%**	12%	24%	4%	4%
Blue whiting						
Press cake	849	27%	**41%**	**44%**	**51%**	**39%**
Sludge	962	**38%**	34%	39%	36%	34%
Concentrate	699	35%	25%	17%	13%	27%
Mackerel/herring blend						
Press cake	1025	35%	**69%**	**54%**	43%	22%
Sludge	537	21%	18%	27%	**53%**	**61%**
Concentrate	781	**44%**	13%	19%	4%	17%

To obtain a high concentration of PLs, the press cake and sludge are promising raw materials for further product development, possibly for pet food production.

However, the best solution would be a more efficient lipid extraction, adding higher value to all streams, including the fish oil.

5.3 Protein quality changes during fishmeal processing (Paper III)

The protein quality changes occurring during fishmeal processing were investigated by measuring protein content, salt soluble protein (SSP), biogenic amines (BA), enzymatic degradation (dimethylamine content, DMA), and microbial spoilage (TVB-N and TMA) during the processing of the BW and MHB. This assessment was important since the first quality indicator fishmeal customers tend to look at is the protein content, followed by the biogenic amines (mainly cadaverine and histamine) and TVB-N content. However, references in literature on how these parameters change during fishmeal processing are scarce.

Of the biogenic amines (BA) analyzed (tyramine, putrescine, cadaverine, and histamine), cadaverine was the most abundant BA in all sampled processing steps in both BW and MHB. Histamine was detected above threshold values in the BW raw material, BW press liquid, and BW separated press liquid, and in all sampling locations studied during the MHB processing. However, no histamine was observed in the BW fishmeal. The obtained higher BA values in the MHB were not surprising as histamine levels are expected to be higher during the processing of industrial side-streams containing high amounts of guts, gills, and heads (Köse et al., 2003). These high histamine values in the raw material indicate ineffective cooling and handling prior to processing, as the formation of BAs in seafood primarily depends on the time and temperature conditions from catch until processing (Visciano et al., 2020). The histamine mainly followed the liquid streams throughout the production and was expected not to be destroyed by further processing (Köse et al., 2003), which was not the case in the current study as all the BAs decreased during processing by 76% and 86% during BW and MHB processing, respectively. Although histamine is heat-resistant to a certain degree (Ienistea, 1971; Köse et al., 2003), extensive heat treatment ending with 450°C for 15 min in the air dryer could decompose parts of the histamine into other compounds. Moreover, several microorganisms can break histamine and other BAs down during processing, including in canned and sterile tuna flesh (Arnold & Brown, 1978; Köse et al., 2003). Higher BA amounts were obtained in the press liquid than press cake (in the BW), and higher BA in the separated press liquid than the sludge (BW and MHB). Mixing the liquid streams back into the fishmeal processing, as is currently done, could thus cause problems in BA accumulation during processing, and should be avoided, at least for histamine-rich species such as mackerel. However, the effect of BA levels of other species, such as BW, should not be neglected since high levels of individual BAs, including cadaverine, can cause problems when adapting the processes towards human consumption.

The TVB-N, TMA, and DMA values showed similar trends during the BW and MHB fishmeal processes, where the press-cake had the lowest values of the volatile nitrogen compounds. Interestingly, the press-liquid showed higher TMA levels in the BW production, indicating higher microbial activity in the BW than in the MHB samples. This trend was seen throughout processing, showing increased TMA levels throughout the BW liquid streams, which finally increased the TMA values in the fishmeal when the concentrate was blended with the press cake and sludge. However, the opposite was observed in DMA formation as the highest DMA values were obtained in the fishmeal in both species studied. This proportional increase in DMA concentrations during drying is probably due to the water removal, rather than an increase in enzymatical activity. Furthermore, as formaldehyde is formed alongside DMA during enzymatic degradation of TMAO (Xiong, 1997; Zayas, 1997a), possible ring formation between the proteins and the formaldehyde may occur, which would regulate the function of the material, e.g., inhibit proteolysis or alter folding kinetics of the raw material (Kamps et al., 2019) during processing. Hence, formaldehyde formation could affect not only the quality of the raw materials but the mass flow, energy use, and overall efficiency of the production.

Salt soluble protein (SSP) levels were high in the raw material and decreased immediately after cooking, resulting in low SSP values in streams after pressing (press cake and press liquid) and in the separated press liquid. However, the SSP content increased slightly in the fishmeal, possibly due to the water removal during drying. The decrease in SSP after the cooking indicated that most of the proteins were already denatured. This is in agreement with Hastings et al. (1985), who showed that at 87°C, most of the myofibrillar and sarcoplasmic proteins, collagen, and other cod muscle proteins were already denatured. However, protein solubility is considered the first functional characteristic when testing new protein ingredients and is the main characteristic of proteins used in liquid foods and beverages since high solubility in the product expands the proteins applications (Zayas, 1997b). Hence, for future development of the protein stream, solubility is an essential function that limits the applications towards human consumption and should be considered further.

5.4 Environmental impacts of fishmeal and fish oil production (Paper IV)

The drivers behind changes in the fishmeal processing were related to quality issues. Therefore, a positive correlation between increased quality and decrease in temperature brought up the question if the decrease in temperature would reduce the environmental impacts of the fishmeal and fish oil production, and to which extent? To address these questions, a Life Cycle Assessment was conducted, based on the functional unit of 1000 kg of fishmeal produced, in a cradle-to-gate study. Also, due to that fishmeal and fish oil is produced worldwide and not only in Iceland but with very similar technology and processes, the Life Cycle Assessment included three different scenarios, each representing different energy sources. The energy source scenarios included the use of hydropower (*Base Scenario 0*), heavy fuel (*Scenario 1*), and a combination of both energy sources (*Scenario 2*) to represent the Icelandic energy mix for the total fishmeal and fish oil production in Iceland, in the reference year 2018. The base case, *Scenario 0*, ran on 100% hydropower and was calculated and assessed from first-hand data from the Síldarvinnslan (SVN) fishmeal and fish oil factory in Neskaupstaður in Iceland. The same mass and energy balances were used for Scenarios 1 and 2, but scenario 1 assessed the use of heavy fuel oil to represent energy usage in most of the fishmeal and fish oil factories in Europe (EUfishmeal, 2019). *Scenario 2* assessed a combination of hydropower (75.4%) and heavy fuel (24.6%) oil use, which represented the average overall energy use in fishmeal and fish oil factories in Iceland in 2018 (FIF, 2019). All scenarios included an assessment of the potential reduction of environmental impacts due to lower cooking temperatures.

Paper IV includes a complete LCA, but to address the question on optimization potential, a hotspot analysis was included in the study to identify the most contributing life cycle stages assessed within the system boundary shown in Figure 12, and the different energy source during the processing (Table 3).

Hotspot analysis in *Scenario 0*, with the processing run on hydropower, indicated that global warming effects mainly originated from the raw material acquisition, followed by cleaning and waste disposal, and finally by processing. For *Scenario 1,* the highest environmental impacts were from the processing, followed by the raw material acquisition. *Scenario 2* showed similar results for raw material acquisition and processing, where cleaning and waste had the lowest impacts on the environment of the three categories. Packaging and backup power impacts on the environment were relatively negligible in all S*cenarios*.

The raw material acquisition had the same overall impacts (320 kg CO_2 eq in all *Scenarios*), or 69% of the total global warming effect in *Scenario 0*, 30% in *Scenario 1,* and 53% in *Scenario 2*. The shift in global warming impacts and other impact

categories reflects the different energy composition in the assessed *scenarios*. Looking at other relevant impact categories, ozone formation, both affecting the terrestrial ecosystems and human health, remained the highest environmental contributor in the raw material acquisition across all *Scenarios.*

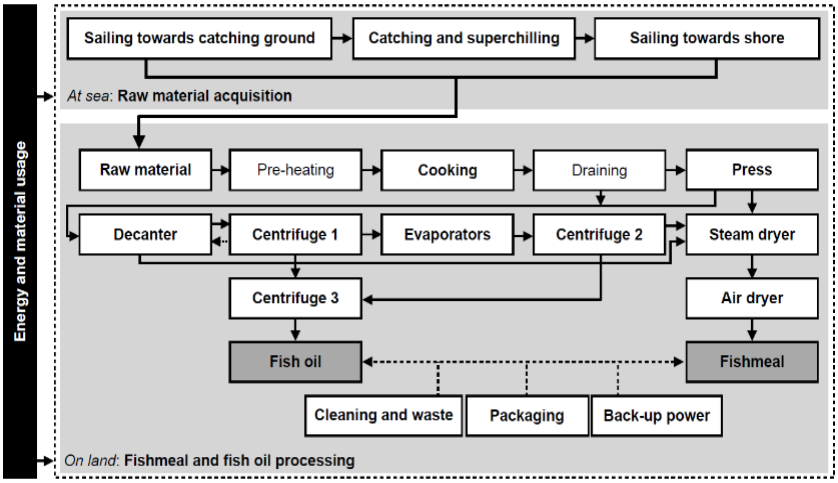


Figure 12: System boundaries and the fishmeal and fish oil production process flow. In bold are processes contributing to the LCA calculations. Pre-heating used excess heating from the evaporators and the steam-dryer and draining did not require any energy.

Observed averages from seven fishing trips showed that fuel usage also differed between fishing gear (lower fuel usage with purse seiner) during catching and whether the raw material was superchilled onboard (Table 4). However, sailing towards shore required slightly higher fuel usage with the purse seiner than trawl, but the results were not significantly different. On average, sailing towards shore required the highest fuel usage during the trip, or 44 ± 13% compared to sailing towards the catching ground, energy using during catching or superchilling as indicated by Table 4. This is due to vessels being heavier when sailing towards shore due to the refrigerated seawater tanks being filled with the catch, compared to sailing out to the catching grounds.

Table 3: Raw material acquisition for *all Scenarios*

Dates from capelin catching from one vessel in 2018	**Fuel usage**		
	Sailing towards catching ground	Catching and superchilling	Sailing towards shore
Average fuel and time with trawl	30 ± 3%	**31 ± 4%[a]**	39 ± 6%
Average fuel and time with purse seiner	31 ± 15%	**17 ± 7%[b]**	52 ± 18%
Overall average fuel and time	30 ± 9%	25 ± 9%	44 ± 13%

Table 4: Hotspot analysis for the three *Scenarios* studied on capelin fishmeal and fish oil production with 90°C cooking temperature. Compared *Scenarios* included *Scenario 0* (hydropower), *Scenario 1* (heavy fuel oil), and *Scenario 2* (75.4% hydropower and 24.6% heavy fuel oil). All results were generated at a 90°C cooking temperature. The color describes the percentage of the environmental impacts, whereas the darker color indicates higher environmental impacts. Backup power was 1% in terrestrial ecotoxicity in *Scenario 0*, but 0% in other categories in all of the *Scenarios*.

***Scenario 0*: Hydropower**	**Total results** (2.5^{th}-97.5^{th}%)	**Unit**	**Raw material**	**Processing**	**Packaging**	**Cleaning, waste and maintenance**
Global warming	4.6×10^{2} (4.4×10^{2}-4.9×10^{2})	kg CO_2 eq	69%	27%	0%	3%
Stratospheric ozone depletion	1.5×10^{-4} (1.1×10^{-4}-2.3×10^{-4})	kg CFC11 eq	64%	5%	0%	31%
Ionizing radiation	5.2 (1.7-1.6×10^{1})	kBq Co-60 eq	56%	20%	1%	22%
Ozone formation, Human health	7.1 (4.8-1.0×10^{1})	kg NO_x eq	99%	1%	0%	1%
Fine particulate matter formation	2.3 (2.0-2.7)	kg PM2.5 eq	98%	1%	0%	1%
Ozone formation, Terrestrial ecosystems	7.2 (4.8-1.0×10^{1})	kg NO_x eq	99%	1%	0%	1%
Terrestrial acidification	7.3 (6.4-8.4)	kg SO_2 eq	99%	1%	0%	1%
Freshwater eutrophication	1.3×10^{-2}(6.0×10^{-3}-2.8×10^{-2})	kg P eq	30%	28%	2%	41%
Marine eutrophication	1.7×10^{-3}(1.3×10^{-3}-2.2×10^{-3})	kg N eq	22%	17%	2%	59%
Terrestrial ecotoxicity	4.0×10^{2}(2.6×10^{2}-6.8×10^{2})	kg 1,4-DCB	69%	16%	0%	14%
Freshwater ecotoxicity	2.7 (1.8-4.1)	kg 1,4-DCB	26%	28%	1%	45%
Marine ecotoxicity	3.8 (2.7-5.7)	kg 1,4-DCB	32%	26%	1%	41%
Human carcinogenic toxicity	3.7 (2.0-7.8)	kg 1,4-DCB	37%	48%	1%	14%
Human non-carcinogenic toxicity	5.9×10^{1}(3.8×10^{1}-1.0×10^{2})	kg 1,4-DCB	33%	28%	1%	37%
Land use	1.2 (9.2×10^{-1}-1.6)	m^2a crop eq	44%	23%	2%	31%
Mineral resource scarcity	3.9×10^{-1} (2.4×10^{-1}-6.4×10^{-1})	kg Cu eq	26%	58%	0%	15%
Fossil resource scarcity	1.1×10^{2} (9.8×10^{1}-1.3×10^{2})	kg oil eq	90%	2%	0%	7%
Water consumption	8.5×10^{1}(-9.4×10^{3}-7.3×10^{3})	m^3	17%	82%	0%	0%
***Scenario 1*: Heavy fuel oil**						
Global warming	1.1×10^{3} (1.0×10^{3}-1.1×10^{3})	kg CO_2 eq	30%	68%	0%	1%
Stratospheric ozone depletion	3.0×10^{-4} (1.8×10^{-4}-5.6×10^{-4})	kg CFC11 eq	31%	53%	0%	15%
Ionizing radiation	9.9×10^{1} (3.8-2.4×10^{1})	kBq Co-60 eq	29%	58%	1%	12%
Ozone formation, Human health	8.6 (6.0-1.2×10^{1})	kg NO_x eq	82%	17%	0%	0%
Fine particulate matter formation	5.0 (2.8-1.5×10^{1})	kg PM2.5 eq	45%	54%	0%	1%
Ozone formation, Terrestrial ecosystems	8.6 (6.1-1.2×10^{1})	kg NO_x eq	82%	17%	0%	0%
Terrestrial acidification	1.5×10^{1} (8.1-5.0×10^{1})	kg SO_2 eq	47%	53%	0%	0%
Freshwater eutrophication	1.5×10^{-2} (6.7×10^{-3}-3.3×10^{-2})	kg P eq	26%	37%	2%	35%
Marine eutrophication	1.8×10^{-3} (1.4×10^{-3}-2.5×10^{-3})	kg N eq	20%	25%	2%	53%
Terrestrial ecotoxicity	3.6×10^{3} (2.2×10^{3}-6.0×10^{3})	kg 1,4-DCB	8%	91%	0%	2%
Freshwater ecotoxicity	2.8 (2.0-4.3)	kg 1,4-DCB	24%	32%	1%	42%
Marine ecotoxicity	6.3 (4.6-8.9)	kg 1,4-DCB	20%	55%	1%	25%
Human carcinogenic toxicity	5.2 (3.3-9.3)	kg 1,4-DCB	27%	62%	1%	10%
Human non-carcinogenic toxicity	1.3×10^{2} (7.9×10^{1}-2.1×10^{2})	kg 1,4-DCB	16%	66%	0%	18%
Land use	1.7 (1.1-2.7)	m^2a crop eq	32%	44%	2%	23%
Mineral resource scarcity	3.0×10^{-1} (1.9×10^{-1}-5.1×10^{-1})	kg Cu eq	35%	45%	1%	20%
Fossil resource scarcity	3.3×10^{2} (2.5×10^{2}-4.4×10^{2})	kg oil eq	31%	66%	0%	2%
Water consumption	1.5×10^{1}(-1.1×10^{1}-3.7×10^{1})	m^3	97%	0%	0%	2%
***Scenario 2*: Composition of hydropower (75.4%) and heavy fuel oil (24.6%)**						
Global warming	6.1×10^{2} (5.9×10^{2}-6.4×10^{2})	kg CO_2 eq	53%	44%	0%	3%
Stratospheric ozone depletion	1.9×10^{-4} (1.3×10^{-4}-3.1×10^{-4})	kg CFC11 eq	51%	24%	0%	25%
Ionizing radiation	6.4 (2.3-1.7×10^{1})	kBq Co-60 eq	46%	35%	1%	18%
Ozone formation, Human health	7.5 (5.2-1.0×10^{1})	kg NO_x eq	94%	5%	0%	1%
Fine particulate matter formation	3.0 (2.3-5.5)	kg PM2.5 eq	76%	23%	0%	1%
Ozone formation, Terrestrial ecosystems	7.5 (5.2-1.1×10^{1})	kg NO_x eq	94%	5%	0%	1%
Terrestrial acidification	9.2 (7.0-1.8×10^{1})	kg SO_2 eq	77%	22%	0%	1%
Freshwater eutrophication	1.4×10^{-2} (6.3×10^{-3}-3.1×10^{-2})	kg P eq	29%	30%	2%	39%
Marine eutrophication	1.7×10^{-3} (1.4×10^{-3}-2.2×10^{-3})	kg N eq	21%	19%	2%	58%
Terrestrial ecotoxicity	1.2×10^{3} (8.3×10^{2}-1.9×10^{3})	kg 1,4-DCB	23%	71%	0%	5%
Freshwater ecotoxicity	2.7 (1.9-4.2)	kg 1,4-DCB	26%	29%	1%	44%
Marine ecotoxicity	4.4 (3.3-6.4)	kg 1,4-DCB	28%	36%	1%	35%
Human carcinogenic toxicity	4.1 (2.4-7.9)	kg 1,4-DCB	34%	52%	1%	13%
Human non-carcinogenic toxicity	7.6×10^{1} (5.3×10^{1}-1.2×10^{2})	kg 1,4-DCB	26%	44%	1%	29%
Land use	1.3 (1.0-1.9)	m^2a crop eq	40%	29%	2%	28%
Mineral resource scarcity	3.7×10^{-1} (2.4×10^{-1}-6.0×10^{-1})	kg Cu eq	28%	55%	0%	16%
Fossil resource scarcity	1.7×10^{2} (1.4×10^{2}-2.0×10^{2})	kg oil eq	61%	33%	0%	5%
Water consumption	6.8×10^{1}(-7.1×10^{3}-5.7×10^{3})	m^3	22%	78%	0%	1%

Abbreviations: CO_2=carbon dioxide, Eq=equivalent, CFC11=trichlorofluoromethane or freon-11, Co-60=cobalt isotope ^{60}Co, NO_x=nitrogen oxide, PM2.5=fine particulate matter less than 2.5 micrometers, SO_2=sulfur dioxide, P=phosphorus, N=nitrogen, 1,4-DCB=1,4 dichnolorbenzene, Cu=copper

The fishmeal and fish oil production process showed different environmental impact results when compared between scenarios, as the processing includes energy sources of both green and oil origin. Most of the environmental impact categories shift from raw material acquisition being the largest hotspot when the processing is run on hydropower (*Scenario* 0), to be the processing, when operated on heavy fuel oil (*Scenario 1*). The impact categories which shift depending on the energy source exclude ozone formation which remain the highest in the raw material acquisition, and freshwater-, and marine eutrophication, and freshwater ecotoxicity of cleaning, and waste, which remain the highest contributors across all of the *Scenarios*. However, although running on hydropower in *Scenario 0* returns roughly half of the environmental impacts compared to heavy fuel oil, some categories increase when running on hydropower. Those categories include water consumption and mineral resource scarcity, which increase by 82%, and 24% when running on hydropower compared to heavy fuel oil, respectively.

Combined effects from the drying steps (steam dryer and air dryer), evaporation and cleaning, and waste accounted for >71% of the total environmental impact. Hence, alterations during drying, evaporation, cleaning, and waste could positively affect the environment. However, reducing the cooking temperature from 90°C to 85°C resulted in 0.1-0.6% overall lower environmental impacts in *Scenario 1*, when the fishmeal and fish oil production operated on heavy fuel oil. Furthermore, in theory, 205 kg CO_2 eq could have been saved during the capelin season in Iceland 2018 (*Scenario 2)* if all fishmeal and fish oil factories in Iceland had adjusted their cooking temperature to 85°C. This slight decrease in cooking temperature during the cooking process contributed to 13% lower global warming effects in *Scenario 0,* where the factory operated on hydropower. Hence, optimizing possibilities in energy-intensive processing steps could return even higher environmental gains when the factory runs on other fuel sources (*Scenarios 1* and *2*). Optimization possibilities for lowering the environmental impacts of producing 1 tonne fishmeal and fish oil are shown in Table 5.

Table 5: Optimization possibilities for lower environmental impacts of fishmeal and fish oil production.

Optimization possibilities	
Raw material acquisition	• Purse seiner resulted in lower energy usage compared to trawl • Changing energy source on the vessels is proposed
The fishmeal and fish oil processing	• A green energy source is proposed • Optimizing the drying and evaporation steps • Exchange cleaning agents for eco-friendly cleaning agents

Hydropower or other green energy sources are proposed as the primary energy source for minimal environmental impacts in Iceland, and elsewhere, where green energy sources are available. This would lower the environmental impacts of the fishmeal and fish oil production process. The heavy fuel oil was estimated to have more than five times higher impacts on global warming, while the impacts were four times higher on stratospheric ozone depletion, three times higher on ionizing radiation, at least seventeen times higher on ozone formation, fifty-two times higher on fine particulate matter formation, seventy-nine times higher on terrestrial acidification, twenty-six times higher on terrestrial ecotoxicity, and twenty-one times higher on fossil fuel scarcity compared to operating on hydropower. All impact categories resulted in higher values running on heavy fuel oil than hydropower, except mineral resource scarcity, which remained similar between scenarios, and water consumption, which decreased from 70 to 0.4 m^3 when using heavy fuel oil. Although the total environmental impacts mainly depend on the energy source, improved usage of cleaning agents would lower the environmental impacts, primarily for freshwater ecotoxicity, and freshwater-, and marine eutrophication.

Standard cleaning agents are proposed to be exchanged towards more environmentally friendly cleaning agents or improved monitoring of their use to some extent, as cleaning and waste (waste being relatively low compared to the cleaning agents) have relatively high environmental impacts. An overview of the average usage per 1 tonne fishmeal from 2010-2020 can be seen in Table 6, where chemical agents were grouped for a clearer overview. Furthermore, the average usage of cleaning agents was divided into two periods, from 2010-2016 and 2017-2020, as the company changed its energy source from heavy fuel oil to hydropower in 2017 (Table 6). As a result, the oil usage, sulfur dioxide-, and carbon dioxide release decreased significantly between these time periods (presented in bold in Table 6).

The cleaning agents differ between years where catch, weather, staff, delay prior to initiation of the fishmeal and fish oil production affects the energy and chemical use. Regulations or registrations currently limiting the usage of chemicals during fishmeal and fish oil production are currently none. Hence, it is currently in the company's interest to use excessive amounts of cleaning agents to fulfill rules for hygiene (FAO, 1986). Furthermore, a positive correlation is likely between shorter labor time for cleaning and higher usage of cleaning agents. Furthermore, chemicals may be bought in bulk and used for more than one year, possibly explaining the high standard deviations in the cleaning agent usage (Table 6). However, except for oil usage and release of carbon-, and sulfur dioxide, no significant changes were seen between the years, and the standard deviations in all categories were relatively high.

Table 6: The average usage of cleaning agents per 1 tonne of fishmeal and fish oil produced from 2010-2020 in the company studied, as affected by main energy source.

Time period	**2010-2016**	**2017-2020**
Main energy source	**Heavy fuel oil**	**Hydropower**
Power usage (kWh)	459.5 ± 75.1	501.5 ± 19.8
Oil usage (liters)	**17 ± 11.9**	**0.2 ± 0.3**
Cold water usage (m^3)	2.6 ± 0.9	1.7 ± 0.9
Water usage at sea (m^3)	16.9 ± 2.7	15.8 ± 0.0
Formaldehyde (liters)	0.02 ± 0.01	0.02 ± 0.01
Acetic acid with and without lignin (liters)	1.23 ± 0.47	0.96 ± 0.51
NaOH related solutions (liters)	0.44 ± 0.38	0.27 ± 0.02
NaOH in solid state (kg)	0.19 ± 0.39	0.04 ± 0.01
HNO_3 and HCl (liters)	0.15 ± 0.05	0.09 ± 0.01
Soaps and cleaning agents (liters)	0.03 ± 0.03	0.05 ± 0.03
Antioxidants for fish oil (liters)	0.05 ± 0.05	0.10 ± 0.03
Packaging (kg)	0.06 ± 0.04	0.05 ± 0.03
Diesel for cars (liters)	0.03 ± 0.02	0.03 ± 0.01
Trash (kg)	0.16 ± 0.16	0.06 ± 0.05
Recycled metal scraps (kg)	0.70 ± 0.36	0.06 ± 0.07
Sulfur dioxide (kg)	**0.36 ± 0.36**	**0 ± 0**
Carbon dioxide (kg)	**38.1 ± 32.4**	**0.6 ± 0.8**

5.5 NIR (Paper IV)

The utilization of pelagic species is increasing, although the raw material and the availability of pelagic species vary highly (Hilmarsdottir et al., 2021; Romotowska et al., 2016). For a high-value product, the fishmeal and fish oil production needs to produce a consistent product (Kristinsson & Rasco, 2000). Quick feedback from the production process is beneficial as repairs or attention can be brought to problematic operational steps to keep the final product consistent. Hence, applying near infrared spectroscopy (NIR) as a monitoring tool during production to predict the main chemical quality parameters of pelagic fishmeal and fish oil was assessed. Using NIR requires no sample preparation and gives quick response, and thus has the potential to save time, labor work, and chemical use, as well as resulting in faster process optimization.

NIR reflection spectra of the samples from Paper I-II were collected simultaneously as the physicochemical assessments of the samples were evaluated. The spectra were compared with results from water and lipid measurements, and fat-free dry matter (FFDM) was assessed as the remaining mass in the sample. The

spectra were, furthermore, compared to FFA, PL, and fatty acid composition results as presented in Papers I-II. Samples from each operational step during the production process were measured in triplicates, where two of the sampling replicates were used to build the Partial Least Square Regression (PLSR) models, and the third sample replicate was set as an independent test set for validation of the prediction model.

The water content varied highly during the fishmeal processing. Water content prediction from 0.3 to 93 g water/100 g sample was successful where both test and data set resulted in R^2 correlation coefficients >0.99 (Table 5.6). Due to the wide concentration range, the same prediction model could be used for processing, monitoring, and control purposes of water changes throughout the whole process. Lipid content prediction models had correlation coefficients in the range 0.93-0.98, except when applying the second derivative data pre-processing method, which showed a disappointing correlation of 0.53. However, by applying a baseline correction (Bl.C) or full multiplicative scatter correction (MSC) of the data prior to modeling, a calibration coefficient of 0.97 and independent validation coefficients of 0.96-0.98 could be achieved, depending on the chosen data pre-treatment. Water and lipid generally account for 85-90% of the raw material weight processed into fishmeal and fish oil (FAO, 1986), and it is hence of great value to monitor those compounds online during fishmeal processing. This holds especially true for processes such as fishmeal processing, where the extraction of the lipids and water are often problematic, in which inadequate process monitoring can easily result in high-lipid fishmeal, of less value than low-lipid fishmeal (Windsor, 2001).

The protein content is the majority of FFDM (around 15 g/100 g sample in the herring raw material), and ash content is normally relatively low (~4 g/100 g sample in the herring raw material) (Oterhals & Thoresen, 2021). However, as protein measurements give significantly different results depending on the extraction methods used (Mæhre et al., 2018), estimating the protein content from NIR spectra directly could provide a more accurate result. Further measurements and identification on vitamins, minerals, and other trace elements within the FFDM would be necessary to address this matter but are left to further studies, although being a promising subject. However, the FFDM concentrations in the current study ranged from 0-84.4%, where the prediction correlation was 0.92 during calibration, and 0.94 during validation when applying the MSC pre-treatment of the data. The high protein content is of interest for fishmeal and fish oil producers as higher prices tend to follow higher protein content, where 65% protein in fishmeal is used as a reference value for fishmeal buyers (Index Mundi, 2022). Hence, an online quality monitoring system could help prevent bottlenecks during production and keep a high protein content in the final product.

Table 7: NIR prediction model summary of attributes feasible to predict with NIR, from data in Paper I-II where Table 7 is adapted from Table S2 in Paper V. Green color indicates independent validation, and blue shows result from calibration of the model.

Components predicted with NIR	Range of sample	R^2_{CV} *n=60*	RMSEC	R^2_P *n=30*	RMSEP	Data pre-treatment
[a]Water content	0.3 – 93.0	0.9995	0.67	0.9938	2.41	None
[a]Lipid content	0 – 100	0.9669	3.94	0.9773	3.94	BILC.
		0.9681	3.86	0.9592	6.34	MSC
[a]Fat free DM	0 – 84.4	0.9183	6.23	0.9356	5.58	MSC
[a]Phospholipids	0 – 1.4	0.9617	0.06	0.8617	0.11	MSC
Fatty acid composition						
· [b]SFA	20 – 32	0.9953	0.20	0.9928	0.24	None
		0.9889	0.31	0.8363	1.13	MSC
· [b]MUFA	38 – 54	0.9062	1.15	0.7968	1.62	None
		0.9462	0.87	0.8291	1.49	MSC
· [b]PUFA	12 – 36	0.9371	1.53	0.8461	2.20	None
		0.9816	0.83	0.8588	2.11	MSC
· [b]DHA	4 – 17	0.9073	0.84	0.8146	1.09	None
		0.9623	0.54	0.8785	0.89	MSC
· [b]EPA	2 – 10	0.9536	0.40	0.8278	0.71	None
		0.9791	0.27	0.8689	0.62	MSC

Abbreviations: n=number of samples, R^2_{CV}=calibration correlation factor, R^2_P=prediction correlation factor, RMSEC=root-mean-square error of calibration, RMSEP=root-mean-square error of prediction, DM: Dry matter, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, MSC: multiplicative scatter correction, Bl.C.: baseline correction. *Superscript*: a=g/100 g sample, b=g/100 g lipids.

Omega-3 fatty acids, such as docosahexaenoic (DHA) and other polyunsaturated fatty acids (PUFA) are known to have multiple health benefits (Berquin et al., 2007; Gunnarsdottir et al., 2008; Larsen et al., 2011; Zheng et al., 2013) and are therefore of interest to minimize their loss during the production. As PUFAs can be destroyed during drying, as high temperatures drastically affect the long-chain PUFAs (Fournier et al., 2006), it is possible to monitor processing steps affecting the PUFAs with NIR spectroscopy. Excellent prediction models were achieved for SFA, DHA, and EPA ($R^{2>}$0.98) with low prediction error (<1% of lipids) in all lipid class parameters. The prediction error was slightly higher for MUFAs and PUFAs, although remaining within acceptable limits. This higher prediction error might be due to spectral similarities and a possible overlap of the absorption peaks between the unsaturated bonds in PUFA and MUFA, resulting in difficulties distinguishing the level of unsaturation. Although prediction models for DHA and EPA for optimization and monitoring purposes were successful, a wider range of samples would have been preferred. However, that is left for future studies to investigate.

5.6 Comparison of traditional fishmeal, commercial pet food, and human protein product

During the assessment of the traditional processes, questions arose on how the chemical characteristics of the traditionally produced fishmeal products were compared to commercial pet food and human protein sources. The following chapter describes investigations of where the traditional fishmeal products assessed in this study rank compared to commercial pet food and fish protein products for human consumption.

Water content and lipid quality results of the fishmeal products produced at 85°C, 90°C and 95°C from MHB cut-offs (Paper I) were compared to the water and lipid content of BW and C fishmeal (Paper II), as well as of a commercial protein product intended for human consumption and a commercial pet food product (Figure 13). The commercial protein product was a protein powder available in capsules and produced from Atlantic cod (*Gadus morhua*) cut-offs without bones and skin (Protis; Kópavogur, Iceland, www.protis.is). The commercial pet food product contained proteins made from cut-offs and side-streams which variated depending on the catch each time, where its processed by a company located at the north coast of France (Copalis; Le Portel, France www.copalis.fr), mainly consisting of pelagic fish species .

The water content of the commercial- protein product and the pet food was similar to the MHB fishmeal produced at 85°C (Figure 13). Although the fishmeal producers want to keep the water content around 6%, lowering the cooking temperature might be thus beneficial for the future development of higher value commercial products. The BW fishmeal was significantly lower in lipid content than the pet food, or 9.4±0.1% compared to 10.4±0.0% lipids. Hence, the BW fishmeal could be sold as pet food based on the lipid quality assessment. However, the protein product for human consumption only contained 0.3±0.1% lipids, emphasizing the importance of extracting the lipids out more effectively for a higher value product. Further developments of the fishmeal towards pet food production could, based on this, include relatively minor processing changes, as the BW fishmeal resulted in similar lipid qualities (lipid content, FFA and PL) as the commercial pet food product. However, to obtain a holistic view of the fishmeal quality compared to both pet food and human protein sources, the protein quality and amnio acid composition of the samples were also investigated.

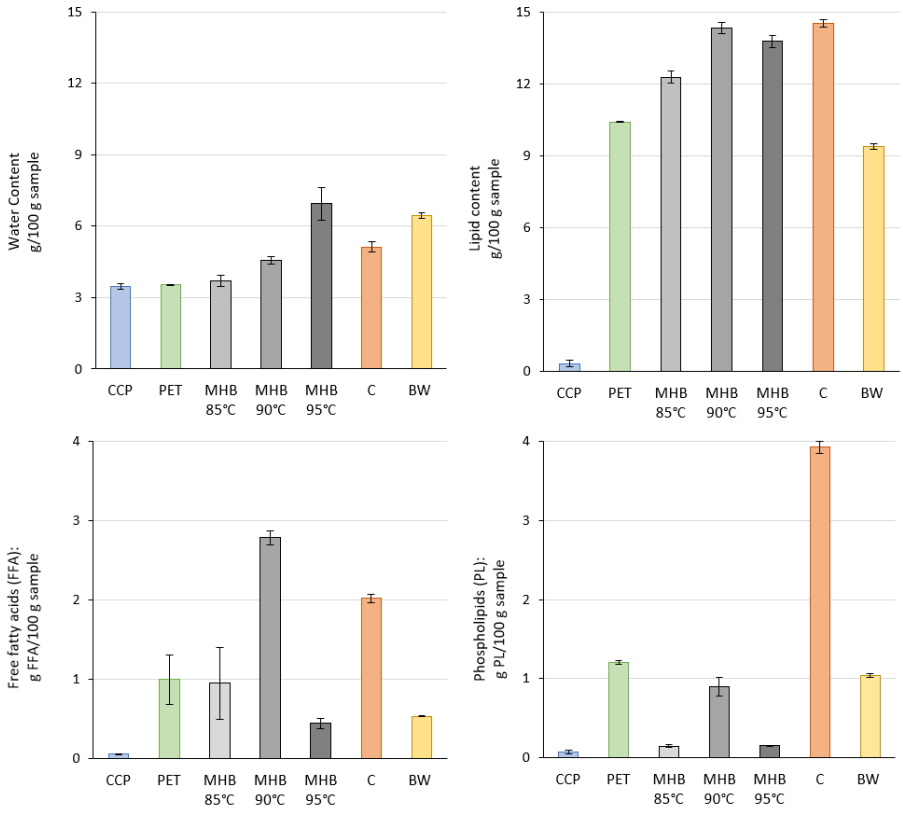


Figure 13: Water-, lipid-, FFA-, and PL content in the studied fishmeal products from traditional processing of a mackerel herring blend (MHB) with 85, 90 and 95°C cooking temperature, capelin (C) and blue whiting (BW) each processed at 90°C, compared to a commercial protein product for human consumption (CPP) and a commercial pet food product (PET).

Essential amino acids are amino acids that are crucial for successful growth and nitrogen balance, as they are not synthesized in humans or other vertebrates and must be obtained from the diet (Lopez & Mohiuddin, 2021). The essential amino acids are phenylalanine, valine, tryptophan, threonine, isoleucine, methionine, histidine, leucine, and lysine and usually derive from animal-based sources (Lopez & Mohiuddin, 2021), although plants are being engineered for improved levels of essential amino acids (Galili et al., 2005). However, as the amino acid composition is known to differ between fish species (Hall, 2010), the commercial protein, commercial pet food, and the MHB fishmeal produced at 90°C were further compared as amino acids per FFDM (Figure 14).

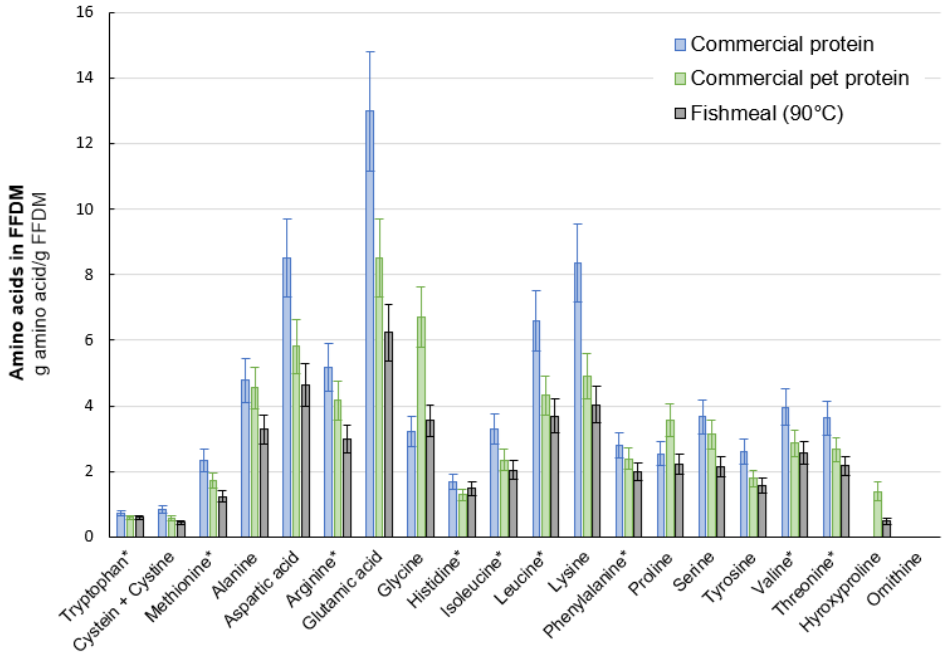


Figure 14: Amino acid profiles of protein commercially sold for human consumption (blue), for a commercial pet food product (green), and traditional fishmeal from a mackerel herring blend (MHB, gray) produced at 90°C. Essential amino acids are marked with *, where arginine is considered essential for children and young adults only.

The comparison generally showed higher amounts of all amino acids in the commercial protein for human consumption compared to the commercial pet food and the mackerel herring blend fishmeal (MHB) (Figure 14). However, the commercial protein for human consumption contained neither proline (Pro) nor hydroxyproline (Hyp). This may be explained by the fact that the cut-offs from Atlantic cod were used as the raw material for this product, and did not include fish skin, which generally is high in Pro and Hyp content (Akita et al., 2020). Furthermore, the glycine (Gly) was relatively low in the commercial protein compared to earlier reported values, but as Gly is commonly reported to be higher in white fishmeal compared to herring fishmeal (around 6 g and 10 g glycine/100g protein, respectively) (Hall, 2010). Furthermore, since the Gly-Pro-Hyp sequence is one of the main factors impacting collagen thermostability (Burjanadze, 2000; Karim & Bhat, 2009), lower amounts of Gly were not surprising in the commercial protein due to the lack of fish skin in the raw material.

The essential amino acid composition of the fishmeal was not significantly different from the essential amino acid composition of the pet food, apart from arginine (Figure 14). The reason for a slight decrease in most of the amino acids

between the commercial protein, commercial pet protein, and the fishmeal could be due to heat treatment, as higher heat increases the possibility of burning the material, increasing risk of protein denaturation affecting the protein solubility (Zayas, 1997b) and lipid oxidation (Jacobsen, 2015; Mozuraityte et al., 2016).

Hydration affects both polar and non-polar protein groups due to hydrogen bonds and Van der Waals interactions (Privalov & Makhatadze, 1993), it is also not unlikely that some amino acids were lost during the fishmeal production. Furthermore, during the steam-drying, the water content in the incoming material decreased significantly, possibly burning proteins while evaporating water from the mass. If the wet concentrate sticks to the metal plates in the steam-dryer, the risk of such burning increases even more.

The comparison between the fishmeal samples from the study and the commercial products show that the raw materials used for the fishmeal production contains various value-added potentials. Further process optimization and product development from the studied raw materials are promising for human consumption. That is though left for further studies.

7 Conclusions

The main goal of this Ph.D. project was to investigate the material quality changes at each processing step during traditional fishmeal and fish oil processing. The current thesis demonstrates the changes in water- and lipid quality at each step, along with protein quality changes in promising protein-rich processing streams. Furthermore, the environmental impact of the traditional processes was evaluated, both with regards to different energy sources and changes in cooking temperature. The study provides an overview of quality changes during fishmeal and fish oil production, and the results can thus be used as future reference, as detailed production analysis was missing from literature. Fishmeal and fish oil producers can thus test the efficiency and effects of a processing step of interest and compare it to values that were measured in the current study, as well as the environmental impacts of their processes.

Overall chemical composition results indicated that the processing streams should not be blended and recirculated but should rather be classified based on their lipid and protein quality. Hence, streams currently entering the dryers simultaneously (press cake, sludge, and concentrate) should be collected and dried separately, each with their optimal temperature and drying time. Optimizing the drying steps would benefit all streams, as lowering temperature exposure increases protein digestibility in the fishmeal (Opstvedt et al., 2003). This opens up the possibility of producing more diverse and higher quality products, which can be aimed at specific markets, increasing the overall value of the production. Thus, part of the production process could be targeted for different end-users, as the solid side-streams have different compositions and physical properties. For a product meant for human consumption, the focus should be on the lipids where drastic changes need to occur to obtain a product with lower lipid content than 0.5%. In addition, more efficient cooling protocols should be installed both after the landings and during the procurement of raw materials, in order to obtain products with lower FFAs, TVB-N and biogenic amines, and proteins that dissolve better in saline solutions. However, degradation should be minimized to produce higher value products.

Environmental consciousness has been getting more attention lately, and hence, incorporating sustainability into optimized processing is beneficial. The aim of the thesis included assessing the environmental impacts of current fishmeal and fish oil production, including the effects of reducing the cooking temperature. Hotspot analysis identified high impact areas of producing 1 tonne of fishmeal and fish oil with the main energy sources applied in Europe. Results showed different environmental impacts due to the energy source used. Furthermore, the obtained results could act as a guide towards cleaner production of fishmeal and fish oil, and affect priorities

when redesigning the fishmeal and fish oil processes for lower environmental impacts.

Furthermore, optimization or redesigning the evaporation and drying steps would return high environmental gain, as they combined account for 74-95% of the effect on each impact category. The processing and the raw material acquisition are the highest environmental contributors of producing 1 tonne capelin fishmeal and fish oil, although the impacts differentiated depending on the energy source, as presented in the different *Scenarios*. When the process was run on hydropower (*Scenario 0*), the raw material acquisition was the highest environmental contributor, but was shifted to the process when run on heavy fuel oil (*Scenario 1*) due to the energy source being fossil based. However, few impact categories were not affected by the different energy sources. Ozon formation remained highest across all *scenarios* during raw material acquisition, where freshwater- and marine eutrophication, and freshwater ecotoxicity, remained the highest environmental contributors across all *scenarios* in cleaning and waste. Other impact categories depended on the energy source during the process. However, reduction in the cooking temperature did not only result in a better lipid separation and higher lipid quality of the fishmeal, but also lowered environmental impacts of the production, independently of the energy source. Calculated with regards to the energy origin of fishmeal factories in Iceland (75.4% hydropower, and 24.6% heavy fuel oil described in *Scenario 2*), 205 kg CO_2 eq (further details in Table 7 in Paper IV) and could have been saved if all the factories in Iceland had decreased their cooking temperature by 5°C during the capelin season in Iceland 2018. Furthermore, chemical agents during cleaning showed higher impacts than expected, indicating that several opportunities lie ahead in exchanging the current cleaning agents or food-grade or more environmentally friendly cleaning agents in the future.

During optimization or redesign of a fishmeal and fish oil factory, using near infrared spectroscopy (NIR) as a measuring tool assessing quality characteristics of the final fishmeal is of great value, as traditional quality measurements can be financially and environmentally costly. The pelagic species vary highly in raw materials characteristics, and with increasing utilization of pelagic species, using NIR for online monitoring and processing redesigning purposes is of great value. Prediction models for water-, lipid-, and FFDM content were successful with high calibration correlation factors and prediction correlation factors within acceptable limits. Furthermore, prediction models for phospholipids, SFA, MUFA, PUFA, DHA, and EPA were also achieved. Thus, several quality attributes can be monitored simultaneously throughout the production, over each processing step, and significantly increase the value of the final product. Furthermore, using NIR for online

monitoring or redesigning purposes further seizes the opportunity for fast development and changes towards a cleaner production.

Today, fishmeal and fish oil producers in Iceland are occasionally forced to run on fossil fuels compared to renewable sources due to a low water reservoir status. More sustainable solutions are therefore ahead, where assessing the environmental impacts in each step of the production process can reduce the total environmental impacts. However, quality can be increased, while simultaneously returning both higher value products, a reduction in environmental impacts, and encouraging more sustainable fisheries.

8 Future perspectives

The future includes possibilities to change the fishmeal and fish oil production towards protein concentrate production intended for human consumption. The currently practiced trawling of pelagic species to feed aquaculture species is far from aligning within the Sustainable Developmental Goals set by the United Nations. To answer the responsibility of both producing a higher value product and achieving a cleaner production, the studied fishmeal and fish oil production company already has invested in a pilot-scale production process. The most energy-intensive processing steps will be exchanged for less heat invasive technologies, hopefully returning less environmental impacts.

Future perspectives include looking into several alternative processing steps in more detail, both from a quality perspective and with lower environmental impacts. A few operational steps which are currently being investigated at the processing facility as spin-off from this thesis' results are discussed below.

Homogenization of the raw material is vital to achieve, as discussed in Paper I. A mincer has been bought and added to the processes, to obtain a more homogenous raw material, which furthermore increases the effect of enzyme addition if applied and decreases the risk of inconsistent final product characteristics and quality.

Hydrolyzation has shown promise at an industrial scale to achieve protein degradation and ease of lipid and water separation from the solid streams. With enzyme addition, heat during the cooking step can be reduced, preserving the protein quality, which can increase the digestibility of the fishmeal (Manditsera et al., 2019). Furthermore, problems during pressing could be avoided, as large amounts of suspended particles in the stickwater would potentially decrease (FAO, 1986), and the yield of the liquid stream is likely to rise. However, the enzyme addition will need

to dissolve the PL from the FFDM, indicating that more than one type of enzymes need to be applied. However, although hydrolyzation shows promising results, a bitter taste remains one of the main bottlenecks during its application or protein production (Steinsholm et al., 2021). The combination of enzymes and their concentrations and applications will thus require optimization to each raw material.

Cooking alters proteins at different temperatures (Hastings, 1985). Therefore, when using milder heat processing procedures such as pasteurization, the proteins are expected to alter less, and might thus be recommended. Along with decreasing the risk of burning the raw material, higher separation could be achieved between the lipids and the FFDM due to less protein aggregation in the hydrolyzed liquid stream. Less heat would be applied to the hydrolyzation and pasteurization compared to the traditional processing steps. This lower potential energy could favor less protein folding (Zumdahl et al., 2016).

Membrane filtration is proposed to be added or exchanged for the evaporation step, as fractionation during industrial processing of fish protein concentrate with membrane filtration (both ultra and nano-filters) has shown promising results (Bourseau et al., 2009). Furthermore, with a membrane filtration system, peptides as small as 0.5-1 kDa can be extracted from the liquid stream, decreasing the product bitterness, which would be of interest for products development for human consumption (Steinsholm et al., 2021).

Drying turned out to be one of the most energy-intensive processing steps analyzed in Paper III, affecting lipid and protein quality (Paper I-IV). For higher quality and industrial application use, applying a spray drier is recommended rather than freeze-drying due to the long drying time and high production cost of freeze-drying (Barbosa et al., 2015). Furthermore, yield and quality losses vary due to drying techniques, and should thus be chosen wisely based on the aimed for product quality characteristics

As one of the main bottlenecks of the quality aspect of the studied fishmeal product, the lipid separation remained inadequate in all cases. Hence, application of enzyme hydrolyzation is currently being investigated with regards to the final product quality and its suitability in the process. Enzyme technology has shown promise to produce high-value products from the marine side-streams (Guerard, 2007). Higher portions of phospholipids and soluble proteins have been recovered during hydrolyzation compared to fishmeal production, measured in mackerel and herring cut-offs (Oterhals & Thoresen, 2021). Water streams includes polar lipids as they tend to dissolve in the water due to stable intermolecular bonds forming with water (Zumdahl et al., 2016), and would need an extra extraction process for recovery (Oterhals & Thoresen, 2021). However, heat treatment and mechanical separation

should remove most of the water and neutral lipids (TAGs) (Oterhals & Thoresen, 2021), which seems to be the current aim for fishmeal and fish oil producers, as high effort is put into using more effective machinery.

The focus at the processing company included in the present study is currently on redesigning a new production process, aiming for production of higher value products and hence, substantial changes and extensive development of the fishmeal and fish oil production are foreseen in the next years. However, the question remains: will these processing steps fit in a fishmeal factory with a continuous production with an input of 1200 tonnes per day and will the end production have a higher environmental impact than the current process. The production will perhaps be divided and optimized depending on the raw materials processed each time, as well as the intended market of the final product. However, as the pilot-scale processing will not include the main energy contributors of the fishmeal and fish oil processing processes, it will be interesting to re-evaluate the environmental impacts after the pilot-scale production has been fully established. Therefore, this study has shown that several opportunities lay ahead for producing higher quality protein and oil products, both from lean and fatty pelagic fish species, while simultaneously applying cleaner production practices.

This evaluation on the current outlook on fishmeal and fish oil production processes gives insight and a holistic assessment to the current processing methods and a baseline to further optimize or redesign the fishmeal and fish oil processes, with regards to higher quality and lower environmental impacts. However, with new designs and optimizations, ongoing environmental assessments can encourage both sustainable fisheries, and move further towards the Sustainable Developmental Goals set by the United Nations.

References

Ackman, R. G. (1967). The influence of lipids on fish quality. *International Journal of Food Science & Technology*, *2*, 169–181. https://doi.org/10.1111/j.1365-2621.1967.tb01339.x

Akita, M., Nishikawa, Y., Shigenobu, Y., Ambe, D., Morita, T., Morioka, K., & Adachi, K. (2020). Correlation of proline, hydroxyproline and serine content, denaturation temperature and circular dichroism analysis of type I collagen with the physiological temperature of marine teleosts. *Food Chemistry*, *329*, 126775. https://doi.org/10.1016/j.foodchem.2020.126775

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). *The lipid bilayer. Molecular biology of the cell* (4th ed.). https://www.ncbi.nlm.nih.gov/books/NBK26871/

AOCS. (1998). *Official method Ce 1b-89. Official method and recommended practices of the AOC* (7th ed.). American Oil Chemists' Society.

Arason. (1994). Production of fish silage. In A. M. Martin (Ed.), *Fisheries Processing* (pp. 244–272). Springer US. https://doi.org/10.1007/978-1-4615-5303-8_11

Arnold, S. H., & Brown, W. D. (1978). Histamine (?) Toxicity from Fish Products. In *Advances in Food Research* (Vol. 24, pp. 113–154). Elsevier. https://doi.org/10.1016/S0065-2628(08)60157-3

Bao, H. N. D., Arason, S., & Þórarinsdóttir, K. A. (2007). Effects of dry ice and superchilling on quality and shelf life of arctic charr (Salvelinus alpinus) fillets. *International Journal of Food Engineering*, *3*(3). https://doi.org/10.2202/1556-3758.1093

Barbosa, J., Borges, S., Amorim, M., Pereira, M. J., Oliveira, A., Pintado, M. E., & Teixeira, P. (2015). Comparison of spray drying, freeze drying and convective hot air drying for the production of a probiotic orange powder. *Journal of Functional Foods*, *17*, 340–351. https://doi.org/10.1016/j.jff.2015.06.001

Berquin, I. M., Min, Y., Wu, R., Wu, J., Perry, D., Cline, J. M., Thomas, M. J., Thornburg, T., Kulik, G., Smith, A., Edwards, I. J., D'Agostino, R., Zhang, H., Wu, H., Kang, J. X., & Chen, Y. Q. (2007). Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. *The Journal of Clinical Investigation*, *117*(7), 1866–1875. https://doi.org/10.1172/JCI31494

Bimbo, A. P., & Crowther, J. B. (1992). Fish meal and oil: Current uses. *Journal of the American Oil Chemists Society*, *69*(3), 221–227. https://doi.org/10.1007/BF02635890

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917. https://doi.org/10.1139/y59-099

Boissy, J., Aubin, J., Drissi, A., van der Werf, H. M. G., Bell, G. J., & Kaushik, S. J. (2011). Environmental impacts of plant-based salmonid diets at feed and farm scales. *Aquaculture*, *321*(1), 61–70. https://doi.org/10.1016/j.aquaculture.2011.08.033

Bourseau, P., Vandanjon, L., Jaouen, P., Chaplain-Derouiniot, M., Massé, A., Guérard, F., Chabeaud, A., Fouchereau-Péron, M., Le Gal, Y., Ravallec-Plé, R., Bergé, J.-P., Picot, L., Piot, J.-M., Batista, I., Thorkelsson, G., Delannoy, C., Jakobsen, G., & Johansson, I. (2009). Fractionation of fish protein hydrolysates by ultrafiltration and nanofiltration: Impact on peptidic populations. *Desalination*, *244*(1–3), 303–320. https://doi.org/10.1016/j.desal.2008.05.026

Bureau, D., Gibson, J., & El-Mowafi, A. (2002). Review: Use of Animal Fats in Aquaculture Feeds. *In: Cruz-Suárez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés M. G., Simoes N. (Eds.). Advances En Nutrición Acuícola VI. Memorias Del VI Simposium*.

Burjanadze, T. V. (2000). New analysis of the phylogenetic change of collagen thermostability. *Biopolymers*, *53*(6), 523–528. https://doi.org/10.1002/(SICI)1097-0282(200005)53:6<523::AID-BIP8>3.0.CO;2-7

Cozzolino, Chree, A., Murray, I., & Scaife, J. R. (2002). The assessment of the chemical composition of fishmeal by near infrared reflectance spectroscopy. *Aquaculture Nutrition*, *8*(2), 149–155. https://doi.org/10.1046/j.1365-2095.2002.00206.x

Cozzolino, Chree, A., Murray, I., & Scaife, J. R. (2009). Usefulness of near infrared spectroscopy to monitor the extent of heat treatment in fish meal. *International Journal of Food Science & Technology*, *44*(8), 1579–1584. https://doi.org/10.1111/j.1365-2621.2008.01845.x

Cozzolino, D., & Murray, I. (2012). A Review on the Application of Infrared Technologies to Determine and Monitor Composition and Other Quality Characteristics in Raw Fish, Fish Products, and Seafood. *Applied Spectroscopy Reviews*, *47*(3), 207–218. https://doi.org/10.1080/05704928.2011.639106

Cozzolino, Murray, I., Chree, A., & Scaife, J. R. (2005). Multivariate determination of free fatty acids and moisture in fish oils by partial least-squares regression and near-infrared spectroscopy. *LWT - Food Science and Technology*, *38*(8), 821–828. https://doi.org/10.1016/j.lwt.2004.10.007

Dang, H. T. T., Gudjónsdóttir, M., Karlsdóttir, M. G., Van Nguyen, M., Romotowska, P. E., Tómasson, T., & Arason, S. (2017). Influence of temperature stress on lipid stability of Atlantic herring (Clupea harengus) muscle during frozen storage. *Journal of the American Oil Chemists' Society*, *94*(12), 1439–1449. https://doi.org/10.1007/s11746-017-3053-4

Dang, H. T. T., Gudjónsdóttir, M., Ren, D., Karlsdóttir, M. G., Minh, V. N., Tómasson, T., & Arason, S. (2018). Effects of pre and postrigor freezing and temperature stress during frozen storage on physicochemical stability of Atlantic herring (Clupea harengus) muscle. *Journal of Food Processing and Preservation*, *42*(9), e13754. https://doi.org/10.1111/jfpp.13754

Davidson, J., Barrows, F. T., Kenney, P. B., Good, C., Schroyer, K., & Summerfelt, S. T. (2016). Effects of feeding a fishmeal-free versus a fishmeal-based diet on post-smolt Atlantic salmon Salmo salar performance, water quality, and waste production in recirculation aquaculture systems. *Aquacultural Engineering*, *74*, 38–51. https://doi.org/10.1016/j.aquaeng.2016.05.004

de Koning, A. J. (2002). Quantitative quality tests for fish meal. II. An investigation of the quality of South African fish meals and the validity of a number of chemical quality indices. *International Journal of Food Properties*, *5*(3), 495–507. https://doi.org/10.1081/JFP-120015487

de Koning, A. J., Milkovitch, S., Fick, M., & Wessels, J. P. H. (1986). The free fatty acid content of fish oil. An analysis of anchovy lipids at different stages in the manufacture of anchovy meal and oil. *Fette, Seifen, Anstrichmittel*, *88*(10), 404–406. https://doi.org/10.1002/lipi.19860881009

EFSA. (2010). Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. *EFSA Journal*, *8*(10), 1874. https://doi.org/10.2903/j.efsa.2010.1874

Einarsson, Bæk, A. M., Eliasen, O., Flesland, O., Jacobsen, C., Jokumsen, A., Pálsson, J., Pedersen, S. A., & Samuelsen, T. A. (2019). *Nordic center of excellence network in fishmeal and fish oil* (ISSN 1670-7192; p. 124). Matís ohf. https://doi.org/10.5281/zenodo.3243334

EUfishmeal. (2019). *EUfishmeal input to the BREF SA TWG* (p. 12). https://effop.org/wp-content/uploads/2019/03/EUfishmeal-BREF-SA-Report.pdf

Eysteinsson, S. T., Gudjónsdóttir, M., Jónasdóttir, S. H., & Arason, S. (2018). Review of the composition and current utilization of Calanus finmarchicus – Possibilities for human consumption. *Trends in Food Science & Technology*, *79*, 10–18. https://doi.org/10.1016/j.tifs.2018.06.019

Eysteinsson, S. Th., Arason, S., & Guðjónsdóttir, M. (2020). Chemical characterization and processing suitability of zooplankton-rich side-streams from Atlantic mackerel (Scomber scombrus) processing. *Journal of Food Composition and Analysis*, *89*, 103471. https://doi.org/10.1016/j.jfca.2020.103471

FAO. (1986). *The production of fish meal and oil* (FAO Fisheries Technical Paper - 142 ISBN 92-5-102464-2; Aquaculture Feed and Fertilizer Resources Information System). http://www.fao.org/3/X6899e/X6899E00.HTM

FAO. (1997). *FAOSTAT statistical database*. [Rome] : FAO, c1997-. https://search.library.wisc.edu/catalog/999890171702121

FAO. (2001a). *Feed and feed ingredient standards* [Aquaculture Feed and Fertilizer Resources Information System]. Food and Agriculture Organization of the United Nations. http://www.fao.org/fishery/affris/feed-and-feed-ingredient-standards/en/

FAO. (2001b). Handling and processing blue whiting. In *Torry advisory note No. 81*. The Ministry of Agriculture, fisheries and food. http://www.fao.org/3/x5952e/x5952e00.htm#Contents

FAO. (2018). *The state of World fisheries and aquaculture. Meeting the sustainable development goals* (ISBN 978-92-5-130562-1; The State of the World, p. 210). Food and Agriculture Organization of the United Nations. http://www.fao.org/3/I9540EN/i9540en.pdf

FAO. (2020). *The state of world fisheries and aquaculture 2020: Sustainability in action* (ISBN: 978-92-5-132692-3; The State of the World, p. 224). Food and Agriculture Organization of the United Nations. https://doi.org/10.4060/ca9229en

Fellows, P. (1988). *Food processing technology: Principles and practice* (3rd ed.). Published jointly by Ellis Horwood and VHC.

Fernandez-Segovia, I., Camacho, M. M., Martinez-Navarrete, N., Escriche, I., & Chiralt, A. (2003). Structure and color changes due to thermal treatments in desalted cod. *Journal of Food Processing and Preservation*, *27*(6), 465–474. https://doi.org/10.1111/j.1745-4549.2003.tb00530.x

FIF. (2019). *Energy usage in the factories from 2003*. The Association of Icelandic Fishmeal and Fish Oil Producers. fif.is

Fish Anatomy. (1913). In *Webster's Revised Unabridged Dictionary*. C. & G. Merriam Co. http://www.artscape.us/aquaculture/fish/fish_anatomy.jpg

Fontaine, J., Hörr, J., & Schirmer, B. (2001). Near-Infrared Reflectance Spectroscopy Enables the Fast and Accurate Prediction of the Essential Amino Acid Contents in Soy, Rapeseed Meal, Sunflower Meal, Peas, Fishmeal, Meat Meal Products, and Poultry Meal. *Journal of Agricultural and Food Chemistry*, *49*(1), 57–66. https://doi.org/10.1021/jf000946s

Food and Agriculture Organization of the United Nations. (1997). *FAOSTAT statistical database*. [Rome] : FAO, c1997-. https://search.library.wisc.edu/catalog/999890171702121

Fournier, V., Destaillats, F., Juanéda, P., Dionisi, F., Lambelet, P., Sébédio, J.-L., & Berdeaux, O. (2006). Thermal degradation of long-chain polyunsaturated fatty acids during deodorization of fish oil. *European Journal of Lipid Science and Technology*, *108*(1), 33–42. https://doi.org/10.1002/ejlt.200500290

Frankel, E. N. (2012). Oxidation in multiphase systems. In *Lipid Oxidation* (pp. 259–297). Elsevier. https://doi.org/10.1533/9780857097927.259

Fréon, P., Durand, H., Avadí, A., Huaranca, S., & Orozco Moreyra, R. (2017). Life cycle assessment of three Peruvian fishmeal plants: Toward a cleaner production. *Journal of Cleaner Production*, *145*, 50–63. https://doi.org/10.1016/j.jclepro.2017.01.036

Galili, G., Amir, R., Hoefgen, R., & Hesse, H. (2005). Improving the levels of essential amino acids and sulfur metabolites in plants. *Biological Chemistry*, *386*(9), 817–831. https://doi.org/10.1515/BC.2005.097

Galkanda-Arachchige, H. S. C., Wilson, A. E., & Davis, D. A. (2020). Success of fishmeal replacement through poultry by-product meal in aquaculture feed formulations: A meta-analysis. *Reviews in Aquaculture*, *12*(3), 1624–1636. https://doi.org/10.1111/raq.12401

Gregory, J., Fredholm, S., & Kirchain, R. (2009). *Is economic value an effective proxy for embodied energy and environmental impact in material systems?* (p. 5). https://doi.org/10.1109/ISSST.2009.5156777

Guðjónsdóttir, M. (2011). *Quality changes during seafood processing as studied with NMR and NIR spectroscopy* [Ph.D thesis, Norwegian University of Science and Technology]. https://orbit.dtu.dk/en/publications/quality-changes-during-seafood-processing-as-studied-with-nmr-and

Guerard, F. (2007). Enzymatic methods for marine by-products recovery. In F. Shahidi (Ed.), *Maximising the Value of Marine By-Products* (pp. 107–136). Woodhead Publishing.

Gunnarsdottir, I., Tomasson, H., Kiely, M., Martinéz, J. A., Bandarra, N. M., Morais, M. G., & Thorsdottir, I. (2008). Inclusion of fish or fish oil in weight-loss diets for young adults: Effects on blood lipids. *International Journal of Obesity*, *32*(7), 1105–1112. https://doi.org/10.1038/ijo.2008.64

Hall, G. M. (2010). Fishmeal production and sustainability. In G. M. Hall (Ed.), *Fish Processing* (pp. 207–235). Wiley-Blackwell. https://doi.org/10.1002/9781444328585.ch9

Hastings, R. J., Rodger, G. W., Park, R., Matthews, A. D., & Anderson, E. M. (1985). Differential scanning calorimetry of fish muscle: The effect of processing and species variation. *Journal of Food Science*, *50*(2), 503–506. https://doi.org/10.1111/j.1365-2621.1985.tb13437.x

Hauschild, M. Z., Rosenbaum, R. K., & Olsen, S. I. (Eds.). (2018). *Life Cycle Assessment*. Springer International Publishing. https://doi.org/10.1007/978-3-319-56475-3

Henderson, R. J., & Almatar, S. M. (1989). Seasonal changes in the lipid composition of herring (Clupea harengus) in relation to gonad maturation. *Journal of the Marine Biological Association of the United Kingdom*, *69*(2), 323–334. https://doi.org/10.1017/S0025315400029441

Hilmarsdottir, G. S., Ogmundarson, Ó., Arason, S., & Gudjónsdóttir, M. (2021). Efficiency of fishmeal and fish oil processing of different pelagic fish species: Identification of processing steps for potential optimization toward protein production for human consumption. *Journal of Food Processing and Preservation*, 15. https://doi.org/10.1111/jfpp.15294

Hilmarsdottir, Ogmundarson, Arason, & Gudjónsdóttir, M. (2020). The Effects of Varying Heat Treatments on Lipid Composition during Pelagic Fishmeal Production. *Processes*, *8*(9), 1142. https://doi.org/10.3390/pr8091142

Hilmarsson, Ó. Þ., Valsdóttir, Þ., Reykdal, Ó., & Einarsdóttir, G. (2015). *Fiskbókin*. Matís. https://fiskbokin.is/uppsjavarfiskar/lodna/

Huss, H. H. (1995). *Quality and quality changes in fresh fish* (Technical Paper FAO Fisheries Technical Paper-348; Food and Agriculture Organization of the United Nations). Ministry of Agriculture and Fisheries, Denmark. http://www.fao.org/3/V7180E/V7180E00.htm

Huy, D., Nam, V. Q., Hanh, H. T., Minh, P., & Huong, L. T. T. (2021). A review and further analysis on seafood processing and the development of the fish Pangasius from the food industry perspective. *Food Science and Technology*. https://doi.org/10.1590/fst.76421

ICES. (2011). *Report of the working group on widely distributed stocks (WGWIDE)* (ICES CM 2011/ACOM:15; ICES WGWIDE Report, p. 642). International Council for the Exploration of the Sea.

ICES. (2018). *Report of the Working Group on Widely Distributed Stocks (WGWIDE)* (ICES CM 2018/ACOM: 23; ICES WGWIDE Report, p. 576). International Council for the Exploration of the Sea.

Ienistea. (1971). Bacterial production and destruction of histamine in foods, and food poisoning caused by histamine. *Die Nahrung*, *15*(1), 109–113.

Index Mundi. (2022). *Fishmeal—Monthly price (euro per metric ton)—Commodity prices—Price charts, data, and news*. Indexmundi.Com. https://www.indexmundi.com/commodities/?commodity=fish-meal&months=120

ISO. (1999). *Animal feeding stuffs—Determination of moisture and other volatile matter content*. International Organization for Standardization. https://www.iso.org/obp/ui/#iso:std:iso:6496:ed-2:v1:en

ISO. (2009). *ISO 5983-2 Animal feeding stuff—Determination of nitrogen content and calculation of crude protein content*. International Organization for Standardization. https://www.iso.org/obp/ui/#iso:std:iso:5983:-2:ed-2:v1:en

Jacobsen, C. (2010). Enrichment of foods with omega-3 fatty acids: A multidisciplinary challenge. *Annals of the New York Academy of Sciences*, *1190*(1), 141–150. https://doi.org/10.1111/j.1749-6632.2009.05263.x

Jacobsen, C. (2015). Some strategies for the stabilization of long chain n-3 PUFA-enriched foods: A review. *European Journal of Lipid Science and Technology*, *117*(11), 1853–1866. https://doi.org/10.1002/ejlt.201500137

Jayathilakan, K., Sultana, K., Radhakrishna, K., & Bawa, A. S. (2012). Utilization of Byproducts and Waste Materials from Meat, Poultry and Fish Processing Industries: A Review. *Journal of Food Science and Technology*, *49*(3), 278–293. https://doi.org/10.1007/s13197-011-0290-7

Kamps, J. J. A. G., Hopkinson, R. J., Schofield, C. J., & Claridge, T. D. W. (2019). How formaldehyde reacts with amino acids. *Communications Chemistry*, *2*(1), 126. https://doi.org/10.1038/s42004-019-0224-2

Karim, A. A., & Bhat, R. (2009). Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids*, *23*(3), 563–576. https://doi.org/10.1016/j.foodhyd.2008.07.002

Kelleher, S. D., & Hultin, H. O. (1991). Lithium Chloride as a Preferred Extractant of Fish Muscle Proteins. *Journal of Food Science*, *56*(2), 315–317. https://doi.org/10.1111/j.1365-2621.1991.tb05269.x

Keller, S. (1990). *Making profits out of seafood wastes: International Conference on Fish By-products*. *no. 90-07*. http://nsgl.gso.uri.edu/aku/akuw90001/akuw90001index.html

Kose, S. (2010). On-board fish processing. In G. M. Hall (Ed.), *Fish Processing* (pp. 167–206). Wiley-Blackwell. https://doi.org/10.1002/9781444328585.ch8

Köse, S., Quantick, P., & Hall, G. (2003). Changes in the levels of histamine during processing and storage of fish meal. *Animal Feed Science and Technology*, *107*(1–4), 161–172. https://doi.org/10.1016/S0377-8401(03)00127-5

Kristinsson, H. G., & Rasco, B. A. (2000). Fish Protein Hydrolysates: Production, Biochemical, and Functional Properties. *Critical Reviews in Food Science and Nutrition*, *40*(1), 43–81. https://doi.org/10.1080/10408690091189266

Larsen, R., Eilertsen, K.-E., & Elvevoll, E. O. (2011). Health benefits of marine foods and ingredients. *Biotechnology Advances*, *29*(5), 508–518. https://doi.org/10.1016/j.biotechadv.2011.05.017

Li, D.-Y., Zhou, D.-Y., Yin, F.-W., Dong, X.-P., Xie, H.-K., Liu, Z.-Y., Li, A., Li, J.-X., Rakariyatham, K., & Shahidi, F. (2020). Impact of Different Drying Processes on the Lipid Deterioration and Color Characteristics of Penaeus Vannamei. *Journal of the Science of Food and Agriculture*, *n/a*(n/a). https://doi.org/10.1002/jsfa.10280

Lopez, M. J., & Mohiuddin, S. S. (2021). Biochemistry, Essential Amino Acids. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK557845/

Lowry, R. R., & Tinsley, I. J. (1976). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists Society*, *53*(7), 470–472. https://doi.org/10.1007/BF02636814

Luten, J. B. (2009). *Marine Functional Food*. Wageningen Academic Pub. https://books.google.is/books?hl=en&lr=&id=CWLFXvslYx0C&oi=fnd&pg=PA115&dq=thorkelsson+and+kristinnsson+2009&ots=3n66pielus&sig=Yp19D6OqjgEAJ2bhr4RZxyBXua4&redir_esc=y#v=onepage&q=thorkelsson%20and%20kristinnsson%202009&f=false

Lv, X., Li, H., Wang, J., Dong, Y., Li, J., & Qin, Y. (2013). Quantification of Fishmeal in Compound Feed Using NIR Spectroscopy. *Food Analytical Methods*, *6*(3), 906–914. https://doi.org/10.1007/s12161-012-9501-8

Mæhre, H. K., Dalheim, L., Edvinsen, G. K., Elvevoll, E. O., & Jensen, I.-J. (2018). Protein Determination—Method Matters. *Foods*, *7*(1), 5. https://doi.org/10.3390/foods7010005

Malle, P., & Poumeyrol, M. (1989). A New Chemical Criterion for the Quality Control of Fish: Trimethylamine/Total Volatile Basic Nitrogen (%). *Journal of Food Protection*, *52*(6), 419–423. https://doi.org/10.4315/0362-028X-52.6.419

Manditsera, F. A., Luning, P. A., Fogliano, V., & Lakemond, C. M. M. (2019). Effect of domestic cooking methods on protein digestibility and mineral bioaccessibility of wild harvested adult edible insects. *Food Research International*, *121*, 404–411. https://doi.org/10.1016/j.foodres.2019.03.052

Margeirsson, B., Lauzon, H. L., Thorvaldsson, L., Arnason, S. V., Arason, S., Valtýsdóttir, K. L., & Martinsdóttir, E. (2010). *Optimized chilling protocols for fresh fish* (Report Matis 15-10). Matis Food Research, Innovation and Safety. https://rafhladan.is/bitstream/handle/10802/1508/54-10-Optimised-Chilling-Protocols.pdf?sequence=1

Masoum, S., Alishahi, A., Shekarchi, M., & Farahmand, H. (2011). Evaluation of the Mineral Contents in Fish Meal by FT-NIR using PLS and Kernel PLS. *Iranian Journal of Mathematical Chemistry*, *2*(1), 31–38.

Matís. (2020). *Loðna / Capelin* [Educational website from Matís ehf]. Fræðsluvefur Matís | Loðna | Fiskitegundir | Fiskur | Fræðsluvefur Matís | Allt Um Mat. http://www.alltummat.is/fiskur/fiskitegundir/lodna/

Mozuraityte, R., Kristinova, V., Standal, I. B., Carvajal, A. K., & Aursand, M. (2016). Oxidative stability and shelf life of fish oil. In *Oxidative Stability and Shelf Life of Foods Containing Oils and Fats* (pp. 209–231). Elsevier. https://doi.org/10.1016/B978-1-63067-056-6.00005-7

Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliot, M., Farrell, A. P., Forster, I., Gatlin, D. M., Goldburg, R. J., Hua, K., & Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 15103–15110. https://doi.org/10.1073/pnas.0905235106

Nygaard, H. (2010). *Standard Norwegian fishmeal- and fishoil process* (Heat Treatment Requirements No. 33/2010; p. 25). Nofima Marine AS.

Ocaño-Higuera, V. M., Maeda-Martínez, A. N., Marquez-Ríos, E., Canizales-Rodríguez, D. F., Castillo-Yáñez, F. J., Ruíz-Bustos, E., Graciano-Verdugo, A. Z., & Plascencia-Jatomea, M. (2011). Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. *Food Chemistry*, *125*(1), 49–54. https://doi.org/10.1016/j.foodchem.2010.08.034

Oehlenschläger, J. (2014). Seafood Quality Assessment. In *Seafood Processing* (pp. 359–386). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118346174.ch14

Olajos, I. (2015). *Implementation and verification of an analytical method for the quantification of biogenic amines in seafood products* [M.Sc. Thesis, University of Iceland]. http://hdl.handle.net/1946/20573

Opstvedt, J., Nygård, E., Samuelsen, T. A., Venturini, G., Luzzana, U., & Mundheim, H. (2003). Effect on protein digestibility of different processing conditions in the production of fish meal and fish feed: Processing effects on protein quality of extruded fish feed. *Journal of the Science of Food and Agriculture*, *83*(8), 775–782. https://doi.org/10.1002/jsfa.1396

Oterhals, Å., & Thoresen, L. (2021). *Phospholipid levels in mackerel and herring residuals and intermediate products from the fish meal and fish protein hydrolysate process* (Nofima Rapportserie 11/2021, p. 15). Nofima AS. https://hdl.handle.net/11250/2771853

Oterhals, Å., & Vogt, G. (2013). Impact of extraction, refining and concentration stages on the stability of fish oil. In C. Jacobsen, N. S. Nielsen, A. F. Horn, & A.-D. M. Sørensen (Eds.), *Food Enrichment with Omega-3 Fatty Acids* (pp. 111–129). Woodhead Publishing. https://doi.org/10.1533/9780857098863.2.111

Pérez-Villarreal, B., Amárita, F., Bald, C., Pardo, M. A., & Sagardia, I. (2008). Validation of traceability in the seafood production chain. In *Improving Seafood Products for the Consumer* (pp. 539–566). Elsevier. https://doi.org/10.1533/9781845694586.5.539

Pike, I. H., & Hardy, R. W. (1997). Standards for assessing quality of feed ingredients. In L. R. D]Abramo, D. E. Conklin, & D. M. Akiyama (Eds.), *Crustacean Nutrition: Vol. Advances in World Aquaculture No. 6* (pp. 473–491). World Aquaculture Society.

Privalov, P. L., & Makhatadze, G. I. (1993). Contribution of Hydration to Protein Folding Thermodynamics: II. The Entropy and Gibbs Energy of Hydration. *Journal of Molecular Biology*, *232*(2), 660–679. https://doi.org/10.1006/jmbi.1993.1417

Prokopchuk, I., & Sentyabov, E. (2006). Diets of herring, mackerel, and blue whiting in the Norwegian sea in relation to Calanus finmarchicus distribution and

temperature conditions. *ICES Journal of Marine Science*, *63*(1), 117–127. https://doi.org/10.1016/j.icesjms.2005.08.005

Refsgaard, H. H. F., Brockhoff, P. M. B., & Jensen, B. (2000). Free Polyunsaturated Fatty Acids Cause Taste Deterioration of Salmon during Frozen Storage. *Journal of Agricultural and Food Chemistry*, *48*(8), 3280–3285. https://doi.org/10.1021/jf000021c

Romotowska, P. E., Gudjónsdóttir, M., Karlsdóttir, M. G., Kristinsson, H. G., & Arason, S. (2017). Stability of frozen Atlantic mackerel (Scomber scombrus) as affected by temperature abuse during transportation. *LWT - Food Science and Technology*, *83*, 275–282. https://doi.org/10.1016/j.lwt.2017.05.024

Romotowska, P. E., Karlsdóttir, M. G., Gudjónsdóttir, M., Kristinsson, H. G., & Arason, S. (2016). Seasonal and Geographical Variation in Chemical Composition and Lipid Stability of Atlantic Mackerel (Scomber scombrus) Caught in Icelandic Waters. *Journal of Food Composition and Analysis*, *49*, 9–18. https://doi.org/10.1016/j.jfca.2016.03.005

Samuel-Fitwi, B., Meyer, S., Reckmann, K., Schroeder, J. P., & Schulz, C. (2013). Aspiring for environmentally conscious aquafeed: Comparative LCA of aquafeed manufacturing using different protein sources. *Journal of Cleaner Production*, *52*, 225–233. https://doi.org/10.1016/j.jclepro.2013.02.031

Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews*, *39*(11), 4067–4079. https://doi.org/10.1039/B922183M

Shen, G., Han, L., Fan, X., Liu, X., Cao, Y., & Yang, Z. (2017). Classification of fish meal produced in China and Peru by online near infrared spectroscopy with characteristic wavelength variables. *Journal of Near Infrared Spectroscopy*, *25*(1), 63–71. https://doi.org/10.1177/0967033516686041

Síldarvinnslan. (2018). *Green Accounting for Síldarvinnslan hf.* (p. 4). The Environmental Agency of Iceland.

Smárason, B. O., Ogmundarson, Ó., Árnason, J., & Bjornsdóttir, R Davídsdóttir, B. (2017). Life cycle assessment of Icelandic arctic char fed three different feed types. *Turkish Journal of Fisheries and Aquatic Sciences*, *17*, 1387–1395. https://doi.org/10.4194/1303-2712-v17

Smarason, B. O., Ogmundarson, O., Arnason, J., Bjornsdottir, R., & Daviosdottir, B. (2017). Life cycle assessment of Icelandic arctic char fed three different feed types. *Turkish Journal of Fisheries and Aquatic Sciences*, *17*(1). https://doi.org/10.4194/1303-2712-v17_1_10

Standal, I. B., Mozuraityte, R., Rustad, T., Alinasabhematabadi, L., Carlsson, N.-G., & Undeland, I. (2018). Quality of filleted Atlantic mackerel (Scomber scombrus) during chilled and frozen storage: Changes in lipids, vitamin D, proteins, and small metabolites, including biogenic amines. *Journal of Aquatic Food*

Product Technology, *27*(3), 338–357. https://doi.org/10.1080/10498850.2018.1436107

Statistics Iceland. (2019). *Profitability in fishing and fish processing 2018* (Statistical Series 104 (14); Statistical Series). Statistics Iceland; ISSN: 1670-4770. https://statice.is/publications/publication-detail/

Statistics Iceland. (2020a). *Catch by type of processing, fishing grounds and fish species 1992-2019* [Statistics database]. Hagstofa Íslands. https://px.hagstofa.is/pxis/pxweb/is/Atvinnuvegir/Atvinnuvegir__sjavarutvegur__aflatolur__radsofun_afla_vinnsla/SJA09110.px/table/tableViewLayout1/?rxid=a27798d0-60e7-46dd-a8b2-d4563ebbc167

Statistics Iceland. (2020b). Hagstofa Íslands. https://px.hagstofa.is/pxis/pxweb/is/Atvinnuvegir/Atvinnuvegir__sjavarutvegur__aflatolur__londunartegundir/SJA09032.px/chart/chartViewLine/?rxid=fdd9c170-2fa8-4e84-9160-9add4325660d

Steinsholm, S., Oterhals, Å., Thoresen, L., Underhaug, J., Kousoulaki, K., & Aspevik, T. (2021). Reduction in flavor-intense components in fish protein hydrolysates by membrane filtration. *Journal of Food Science*, *86*(9), 3855–3867. https://doi.org/10.1111/1750-3841.15855

Stewart, J. C. M. (1980). Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Analytical Biochemistry*, *104*(1), 10–14. https://doi.org/10.1016/0003-2697(80)90269-9

Stroud, G. D. (2001). The Herring. In *Torry advisory note No. 57*. Ministry of Agriculture, fisheries and food. http://www.fao.org/3/x5933e/x5933e00.htm#Contents

Sveinsdóttir, H. I., Karlsdóttir, M. G., Arason, S., Stefánsson, G., Sone, I., Skåra, T., Rustad, T., Larsson, K., Undeland, I., & Gudjónsdóttir, M. (2020). Effect of antioxidants on the sensory quality and physicochemical stability of Atlantic mackerel (Scomber scombrus) fillets during frozen storage. *Food Chemistry*, *321*, 126744. https://doi.org/10.1016/j.foodchem.2020.126744

Tarhouni, A., Ben Zid, M., Talbi, O., Elbour, M., Sadok, S., & Mihoubi Boudhrioua, N. (2019). New Integrated Process for Production of Edible and Fishmeal Powders from Sardines: Drying Kinetics and Quality Attributes. *Process Safety and Environmental Protection*, *122*, 352–365. https://doi.org/10.1016/j.psep.2018.12.019

Tena, N., Lobo-Prieto, A., Aparicio, R., & Gonzalez, D. (2018). *Storage and preservation of fats and oils*. https://doi.org/10.1016/B978-0-08-100596-5.22268-3

Tengku-Rozaina, T. M., & Birch, E. J. (2013). Physicochemical characterisation and oxidative stability of refined hoki oil, unrefined hoki oil and unrefined tuna oil. *International Journal of Food Science & Technology*, *48*(11), 2331–2339. https://doi.org/10.1111/ijfs.12222

Thorkelsson, G., Slizyte, R., Gildberg, A., & Kristinsson, H. G. (2009). Fish proteins and peptides. Processing methods, quality and functionality. In Luten (Ed.), *Marine functional foods*. Wageningen Academic Publishers.

Toldrá, F., & Reig, M. (2011). The stability and shelf life of seafood. In *Food and Beverage Stability and Shelf Life* (pp. 779–792). Elsevier. https://doi.org/10.1533/9780857092540.3.779

Torrecillas, S., Mompel, D., Caballero, M. J., Montero, D., Merrifield, D., Rodiles, A., Robaina, L., Zamorano, M. J., Karalazos, V., Kaushik, S., & Izquierdo, M. (2017). Effect of fishmeal and fish oil replacement by vegetable meals and oils on gut health of European sea bass (Dicentrarchus labrax). *Aquaculture*, *468*, 386–398. https://doi.org/10.1016/j.aquaculture.2016.11.005

Vilhjálmsson. (2002). Capelin (Mallotus villosus) in the Iceland–East Greenland–Jan Mayen Ecosystem. *ICES Journal of Marine Science*, *59*(5), 870–883. https://doi.org/10.1006/jmsc.2002.1233

Visciano, P., Schirone, M., & Paparella, A. (2020). An Overview of Histamine and Other Biogenic Amines in Fish and Fish Products. *Foods*, *9*(12), 1795. https://doi.org/10.3390/foods9121795

Windsor, M. L. (2001). *Fish Meal*. FAO in partnership with Support unit for International Fisheries and Aquatic Research, SIFAR, 2001. https://www.fao.org/3/x5926e/x5926e00.htm

Xiong, Y. L. (1997). Protein denaturation and functionality losses. In M. C. Erickson & Y.-C. Hung (Eds.), *Quality in Frozen Food* (pp. 111–140). Springer Science & Business Media. https://doi.org/10.1007/978-1-4615-5975-7

Zayas, J. F. (1997a). Emulsifying Properties of Proteins. In J. F. Zayas (Ed.), *Functionality of Proteins in Food* (pp. 134–227). Springer. https://doi.org/10.1007/978-3-642-59116-7_4

Zayas, J. F. (1997b). Solubility of Proteins. In J. F. Zayas (Ed.), *Functionality of Proteins in Food* (pp. 6–75). Springer. https://doi.org/10.1007/978-3-642-59116-7_2

Zhang, F., Jiang, S., Feng, X., Wang, R., Zeng, M., & Zhao, Y. (2020). Effect of heat treatment on physicochemical state and in vitro digestion of salt-soluble protein from Pacific oyster (Crassostrea gigas). *LWT*, *134*, 110126. https://doi.org/10.1016/j.lwt.2020.110126

Zheng, J.-S., Hu, X.-J., Zhao, Y.-M., Yang, J., & Li, D. (2013). Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: Meta-analysis of data from 21 independent prospective cohort studies. *BMJ*, *346*(jun27 5), f3706–f3706. https://doi.org/10.1136/bmj.f3706

Zumdahl, S. S., Zumdahl, S., A., & DeCoste, Donald J.,. (2016). *Chemistry* (10th ed.). Cengage Learning.

Original publications

Paper I

Received: 17 August 2020 | Revised: 23 November 2020 | Accepted: 13 January 2021

DOI: 10.1111/jfpp.15294

ORIGINAL ARTICLE

Journal of Food Processing and Preservation | WILEY

Efficiency of fishmeal and fish oil processing of different pelagic fish species: Identification of processing steps for potential optimization toward protein production for human consumption

Gudrun Svana Hilmarsdottir[1] | Ólafur Ogmundarson[1] | Sigurjon Arason[1,2] | María Gudjónsdóttir[1,2]

[1]Faculty of Food Science and Nutrition, University of Iceland, Reykjavík, Iceland

[2]Matis ohf., Icelandic Food and Biotech R&D, Reykjavík, Iceland

Correspondence
Gudrun Svana Hilmarsdottir, Faculty of Food Science and Nutrition, University of Iceland, Aragata 14, 102 Reykjavík, Iceland.
Email: gsh9@hi.is

Funding information
Rannís Technology Development Fund, Grant/Award Number: 198883-0611; The University of Iceland research fund; The Added Value of Seafood fund of the Ministry of Fisheries and Agriculture in Iceland, Grant/Award Number: R18 031-18

Abstract

Most fish meal and fish oil production facilities run with outdated processes, producing low-value products, typically not intended for human consumption. The aim of the study was to perform a detailed analysis of the current fishmeal and oil production processes of capelin (*Mallotus villosus*), and compare the key locations of both fattier and leaner pelagic raw material to identify which steps need to be improved for the production of higher-value products. Results indicated inefficient draining and concentration during the production process and ineffective break-down of the raw material, as lipid separation was ineffective in all studied species. Although the raw material initially differed in lipid content (2%–20%, depending on the species), all fishmeal produced resulted in high-lipid fishmeal (9%–14%). Chemical composition variations in the solid steams entering the dryers suggested that drying these streams individually could lead to more process and product flexibility, which can result in higher-value products, such as fish meal and protein powders for human consumption.

Practical applications

Traditional production lines of fishmeal and fish oil factories have remained the same for decades, resulting in low-quality products with a low market price. Therefore, optimization and redesign of the production processes are needed to increase the product quality. This study analyzed the lipid quality and water content in all processing steps. The current evaluations will help to shift the traditional fishmeal and fish oil production process from low-quality to high-quality products intended for human consumption by presenting a detailed analysis of the production process not available in the literature to date. Our results can act as reference values for other fishmeal and fish oil producers to assess their production quality. That includes identification of bottlenecks affecting the efficiency and effectiveness of their production processes.

1 | INTRODUCTION

The total world fish production in 2018 was 179 million tons, where fishmeal and fish oil accounted for 10% (18 million tons) (FAO, 2020). The overall production of fishmeal has been declining since 1994, when it reached 30 million tons (FAO, 2018). The fishmeal price was 0.133 EUR/100 g (Index Mundi, 2020) in August 2014, when at the same time, protein for strength/muscle build (intended for human consumption) was, on average, 4.11 EUR/100 g (European Commission et al., 2016). The fishmeal prices have remained similar, as in September 2020 and the fishmeal price was 0.125 EUR/100 g (Index Mundi, 2020). The low fishmeal prices force the industry to change and adapt, where optimizing the production line toward fish protein powder is of high potential.

The primary raw materials processed into fishmeal and fish oil are by-catch, cut-offs, and small pelagic species (FAO, 2020; Thorkelsson et al., 2009), where the main species around Iceland are capelin (*Mallotus villosus*), blue whiting (*Micromesistius poutassou*), Atlantic herring (*Clupea harengus*), and Atlantic mackerel (*Scomber scombrus*) (Statistics Iceland, 2019). However, the catch within the Icelandic jurisdiction varies both in quantity and quality between seasons and catching grounds (Statistics Iceland, 2019).

Traditional fishmeal and fish oil processing lines have remained unchanged for decades (Bimbo & Crowther, 1992; Einarsson et al., 2019; FAO, 1986; Hall, 2010; Oterhals & Vogt, 2013) even though the catch is of higher value when reaching the harbor (Bao et al., 2007) due to improved handling and chilling protocols onboard (Margeirsson et al., 2010). Until recently, the industrial focus has been on obtaining a high throughput in the plants and less on the quality of the products. Since the fishmeal price depends primarily on its protein and lipid contents, minimizing the lipid content of the meal is of high interest. Generally fishmeal with a higher lipid content are considered of lower quality than fishmeal with a lower lipid content (Einarsson et al., 2019; Windsor, 2001). The lipid content and composition of the fishmeal are also important factors influencing the stability of the fishmeal during storage (Bragadóttir et al., 2004). However, information on the effects of processing on lipid quality, such as the fatty acid composition, formation of free fatty acids (FFAs), and phospholipids (PLs) of fishmeal, is scarce but necessary for the development of higher-value protein products for human consumption.

To control the quality and stability of the final product and to open up for the possibility of producing more valuable products for human consumption, the fishmeal process needs to be understood in detail. An understanding of the effects of each processing step on the lipid quality and quantity is essential as the separation between the lipids and the dry matter is often problematic.

The objective of this study was hence to investigate the effectiveness of the current fishmeal and fish oil processing practices and to make a detailed mapping of the effects of each processing step on the lipid quality as affected by species variations (capelin, blue whiting, and mixture of herring and mackerel). Moreover, the study aimed at identifying problematic processing steps for both fatty and lean pelagic species in order to optimize an established commercial fishmeal processing line from producing fishmeal to producing fish protein powders intended for human consumption.

2 | MATERIALS AND METHODS

2.1 | The fishmeal and fish oil plant

During the fishmeal and fish oil production, the water-, lipid-, free fatty acid-, and phospholipid content of the raw material was monitored throughout the production. Figure 1 shows a flow chart of the fishmeal and oil plant studied. The raw material was *pre-heated* for approximately 20 min at 55°C, followed by *cooking* for approximately 20 min at 90°C. The cooking temperature was continuously adjusted to keep the two cookers at 90°C on average. After cooking, the raw material was *drained* and *pressed* mainly for water and oil removal. The liquid from the draining and the press are combined and enter a decanter. The liquid from the decanter, *separated press liquid*, was led through a centrifuge for oil recovery, followed by concentration via two-step evaporation. Oil from the decanter (*separated oil*) and concentration (*concentrate oil*) form the *final oil* after washing with hot water and another centrifugation. The solid streams from the press (*press cake*), decanter (*sludge*), and the evaporation steps (*the latter concentrate*) were mixed and dried down to approximately 40% water at a rotary disk *steam-dryer* (30 ± 5 min at steam temperature 160°C, drying temperature 95°C). The next drying step included a Hetland *air-dryer* (16 ± 2 min, input air temperature < 450°C, center dryer temperature 150°C, and approximate wet bulk temperature 65°C), which lowered the water content down to 5%–10% water. The final fishmeal consisted of both fine meal and fishmeal. The fine meal was collected from a cyclone installed for the removal of dust entrained in the exit gas stream, while the fishmeal refers to the commercial fishmeal end-product of the process.

2.2 | Raw materials

Raw materials and processing samples were collected during three runs of the fishmeal factory (Table 1). The raw materials processed during these runs were (i) *capelin* (*Mallotus villosus*) (ii) *a blend of Atlantic mackerel* (*Scomber scombrus*) *and Atlantic herring* (*Clupea harengus*), and (iii) *blue whiting* (*Micromesistius poutassou*). Initiation of the fishmeal and fish oil processing line was 1–3 days post catch to secure enough raw materials. Approximately 1,200 tons of raw materials enter the fishmeal processing facility per day, where the production capacity of the factory is around 10 tons/hour of fishmeal. The fishmeal production yield usually is around 20%, while the fish oil yield can vary from 0% to 20% dependent on the fat content of the species.

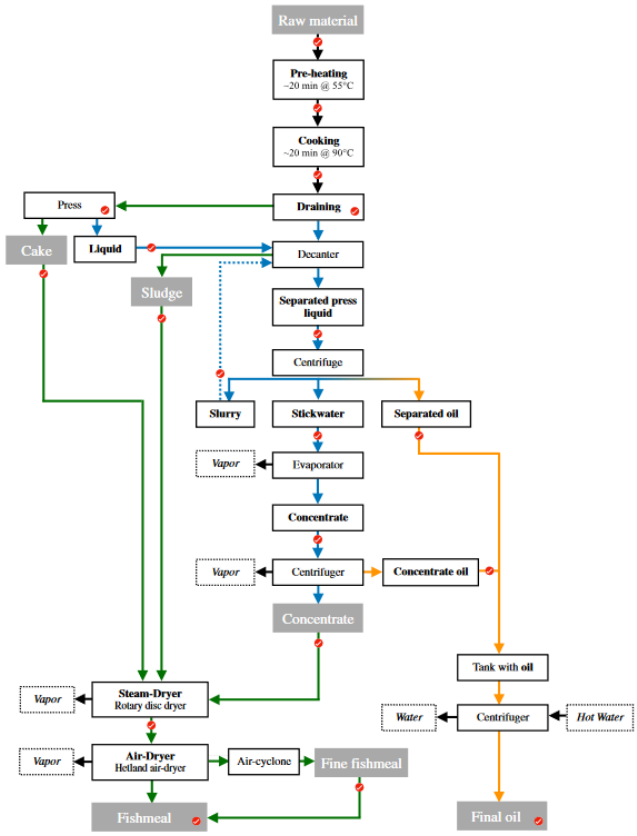


FIGURE 1 Traditional fishmeal processing plant. Solid streams are presented with green lines, liquid streams with blue lines, and oil streams with yellow lines. Red dots indicate sampling points in the capelin production, and gray boxes indicate sampling points in the mackerel/herring blend and blue whiting production

2.3 | Sampling

After establishing a steady state for commercial fishmeal production, samples were collected throughout the fishmeal factory, as shown in Figure 1. Samples were taken more frequently throughout the capelin production to give a detailed analysis of the processing. Samples were collected at key locations in the mackerel/herring blend and blue whiting production (raw material, press cake, sludge, concentrate, and final fishmeal and fish oil) to compare the different raw materials while keeping the experimental costs within an acceptable range.

TABLE 1 Detailed description of the species processed into fishmeal and oil regarding catching time and date, processing time, catching ground, fishing equipment, total catch, and temperature and salt content during landing of the catch at the harbor. Range is given in case of data from more than one trawler

Name in text	Blue whiting *(whole)*	Capelin *(whole)*	Mackerel/herring blend *(cut-offs from mackerel and cut-offs herring)*
Species and by-catch	100% *Blue whiting* (*Micromesistius poutassou*)	100% *Capelin* (*Mallotus villosus*)	58% *Atlantic mackerel* (*Scomber scombrus*) 37% *Atlantic herring* (*Clupea harengus*) 4.5% *Blue whiting* (*Micromesistius poutassou*) <0.5% By-catch
Catching date	30.04.19	28.02.18	03.09.17–07.09.17
Dates fishmeal processed	02.05.19–03.05.19 (waited for ~ 24 hr)	01.03.18–02.03.18	07.09.17–08.09.17
Catching grounds (of Iceland)	South of Faroe Island (60°North 7°West)	320 (south), 616 (northeast)*	400, 511, 512, 553–555, 600 (southeast)*
Fishing equipment	Midwater trawling	Purse seine	Midwater trawling
Total catch	2,170 tons	2,450 tons	884 tons
Temperature at landing	2.1°C (one trawler)	4 ± 1.5°C	3 ± 1.5°C
Salt content at landing	0.5 g/100 g sample	0.6 ± 0.1 g/100 g sample	0.9 ± 0.4 g/100 g sample
TVN at landing	16.5 (one trawler)	23.4–76.8	24.6–38.8
TVN in the tanks	32.4–37.9	25.5–40.2	20.0–48.0
TVN in the meal	85.8–108.6	100.6–108.4	136.5 (one meal party)
Cadav in the meal (g/kg)	0.64–1.67	0.64–0.83	0.76 (one meal party)

Abbreviation: TVN, total volatile nitrogen.

Three individual samples were taken from each sample location to investigate if the production was homogenous. The samples were transported to the research facility and kept at −25°C until analysis. Samples were thawed at 0–4°C for up to 36 hr prior to chemical composition analysis, where the thawing time depended on the water content of the samples. Each sample triplicate was measured twice to confirm the measurements.

2.4 | Chemical composition analysis

The water content was measured according to the ISO 6496 (ISO, 1999), where samples (~3 g) were kept at 104°C ± 1°C for a minimum of 4 hr. Water-in-oil was measured using calorimetric titration performed by 851 Titrando (Metrohm, Herisau, Switzerland).

Total lipids were extracted and determined gravimetrically, according to Bligh and Dyer (1959) with modifications described by Dang et al. (2017) and Romotowska et al. (2016). The total lipid extract was further used to measure lipid content, phospholipids (PLs), free fatty acids (FFAs), and fatty acid composition (FAC).

Phospholipids (PLs, phosphatidylcholine) were measured by a colorimetric method described by Stewart (1980), with modifications described by Dang et al. (2017) and Romotowska et al. (2016). The method describes a complex formed between PLs and ammonium ferrothiocyanate, which has an absorbance at 488 nm. The samples were measured in a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The standard curve was prepared with phosphatidylcholine.

Free fatty acids (FFAs) were measured in the total lipid extract according to the method by Lowry and Tinsley (1976), modified by Bernárdez et al. (2005) and described by Dang et al. (2017) and Romotowska et al. (2016), at 710 nm, using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The standard curve was prepared with oleic acid.

The fatty acid composition (FAC) of the samples was analyzed with an adjusted methylation method based on the AOCS Official Method Ce1b-89 (AOCS, 1998), described by Dang et al. (2017) and Romotowska et al. (2016). The samples were determined by a gas chromatography (Varian 3,900 GC, Varian, Inc., Walnut Creek, CA), where the oven was set at 100°C for the first 4 min, and with a rate of 3°C/min, ended at 240°C. Helium was used as the gas carrier at 8 ml/min column flow. Software used was Galaxie Chromatography Data System, (Version 1.9.3.2 software, Varian Inc.).

2.5 | Statistical analysis

Data summaries, handling, figures, and tables were performed in Microsoft Office Excel (Microsoft Inc., Redmond, WA, USA), while analysis of variance (ANOVA) and Tukey's HSD test were performed in RStudio (RStudio Inc., Boston, MA, USA). The significance level was set $p < .05$. Results were shown as mean values ± standard deviation (*SD*) from the three triplicates for each sample.

3 | RESULTS AND DISCUSSION

3.1 | Chemical composition changes during capelin processing

3.1.1 | Water content changes during capelin processing

The water content in the capelin raw material (82.0 ± 1.2 g/100 g sample) was stable until the streams were divided into solid (represented by green colored dashed lines) and liquid streams (blue colored dashed lines) (Figure 2a-2b). These results indicate that the raw material entering the processing plant was homogenous and in line with the fact that no water was added or removed during the heating steps (pre-heating and cooking) at the beginning of the process. Earlier reported measurements show a water content in high-lipid capelin of 72.7 ± 0.7 g/100 g sample and 76.9 ± 0.74 g/100 g sample in low-lipid capelin (Cyprian et al., 2015). The water content observed in the current study is, therefore, slightly higher than in the raw material studied by Cyprian et al. (2015), but the water and lipid contents of capelin are highly dependent on the catching season and location of the species (Arason et al., 2014; Bragadóttir et al., 2002; Vilhjálmsson, 1994).

No significant differences were detected between the raw material and the draining step during the capelin processing, although the draining was intended to remove most of the water and hence lower the water content of the main processing stream. Lower amounts of solid particles in the liquid stream might result in a lower water content of the concentration, as viscosity greatly affects the degree of evaporation (Einarsson et al., 2019). However, the water content of the press cake decreased significantly (54.5 ± 0.4 g/100 g sample) from the draining step (82.0 ± 1.2 g/100 g sample), indicating that the press was successful in water removal from the solid stream, as the press cake of fatty fish has been reported to be 53 g/100 g water content. After the concentration of the stickwater, a water content of 84.3 ± 0.1 g/100 g sample was observed in the latter concentrate, which was higher than expected, as stickwater has been reported to be 65 g/100 g water content (Hall, 2010) and 30%–50% dry matter (Einarsson et al., 2019). However, no significant differences were observed in the water content between the two concentration steps, indicating that the second concentration step was ineffective in water removal and required adjustments. After the concentration steps, the latter concentrate was rejoint into the solid stream along with the press cake and the sludge (63.7 ± 6.3 g/100 g sample), and the streams were mixed immediately in the steam-dryer. The steam-dryer reduced the water content of the solid streams down to

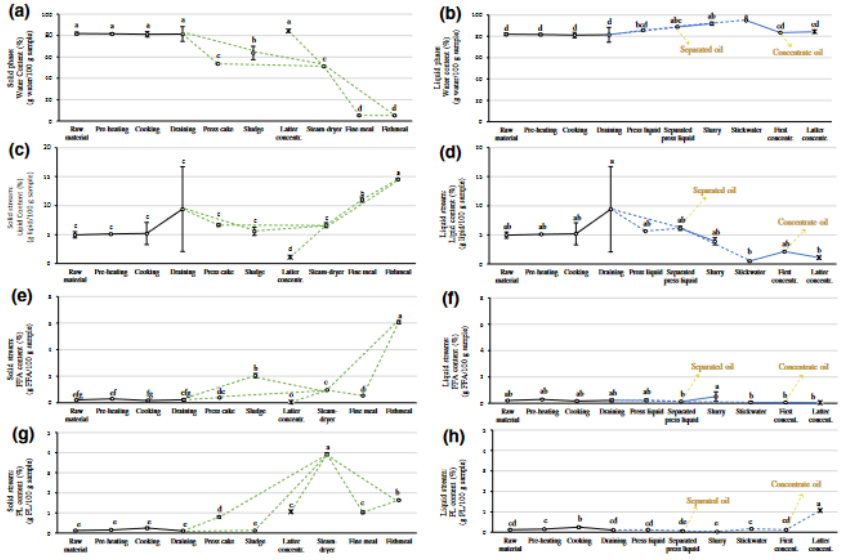


FIGURE 2 Solid streams (presented in green, a) and liquid streams (presented in blue, b) from the fishmeal and oil processing lines from Figure 1. Oil streams are presented with yellow color. Water content on Figure 2a–2b, lipid content on Figure 2c–2d, free fatty acids (FFA) on Figure 2e–2f, and phospholipids (PL) on Figure 2g–2h. The dashed lines indicate where more than one stream was connected between the processing steps, but unbroken lines indicate only one connection

51.2 ± 0.5 g/100 g sample. The air-dryer lowered the water content further down to 5.1 ± 0.2 g/100 g sample of the capelin fishmeal. Water content was expected to be higher in the fishmeal, as it has been reported to be around 9–10 g/100 g water content (Hall, 2010; de Koning, 2002). The fine meal and final capelin fishmeal did not vary significantly in water content.

The water content did not significantly change between the press cake and the steam-dryer due to the addition of the latter concentrate and the sludge to the solid stream. This raises questions of the effectiveness of processing these streams together with regards to water removal. It is highly likely that the water removal could be improved considerably by drying these streams (the latter concentrate, press cake, and sludge) individually.

Oil streams were collected from two places during processing, from the centrifuges installed after the pressing and evaporation, respectively (Figure 1). When combined again these oil streams form the final commercial oil. The water content was highest in the concentrate oil (2.2 ± 0.5 g/100 g sample), while the separated oil (0.6 ± 0.2 g/100 g sample) and final oil (0.6 ± 0.2 g/100 g sample) were lower in water content.

The European Food Safety Authority (EFSA) has defined the quality guidelines for crude oils (Bimbo, 1998), refined fish oil (Hamm, 2009), and omega-3 concentrates (GRAS by FDA in the USA) where the allowed water content in crude oil is 0.5%–1%, unspecified in refined oil (Hamm, 2009) and 0.1% in omega-3 concentrates (EFSA, 2010). Therefore, the final oil from the capelin processing falls into the crude oil category.

Low water content in oil is beneficial, as the exposure of water in the oil increases the microorganisms and enzyme activity (Rodriguez, 2013; Roos, 2003). Moreover, the droplet size of the water is critical, where small water droplets in water-in-oil (w/o) emulsions are reported to be more stable in oil phases than large droplets, as the sedimentation speed is lower (Ushikubo & Cunha, 2014) and the amount of growth compounds are limited on the radius of the w/o droplets (Verrips & Zaalberg, 1980). Unfortunately, the w/o droplet size was not investigated in the current study. However, the small water droplets were separated from the oil, by pressurizing hot water through the oil prior to centrifugation, leading to improved water-oil separation. During further process optimization, actions toward limiting the amount of hot water pressurized through the oil and the water droplet size should be taken.

3.1.2 | Lipid content changes during capelin processing

The capelin raw material had a lipid content of 5.0 ± 0.5 g/100 g sample. Previously, capelin has been reported to vary from 4% to 20% in lipid content (Vilhjalmsson, 1994), where adult capelin are 2- to 4-fold higher in lipid content in the autumn, due to its feeding in the summer (Vilhjálmsson, 2002). During measurements, it was noted that eggs were seen in the samples, indicating that the catch consisted of both female and male capelin (Vilhjálmsson, 2002). Reported values in lipid content in capelin have been 10.2 ± 0.2 g/100 g sample (high-lipid capelin) and 5.8 ± 0.5 g/100 g sample (low-lipid capelin) (Cyprian et al., 2015), which is slightly higher than in the current study.

Several changes were observed in the lipid content during processing. The lipid content of the press cake (6.6 ± 0.2 g/100 g sample) and sludge (5.6 ± 0.7 g/100 g sample) was significantly higher than in the capelin raw material due to the liquid removal during pressing. However, the press cake has been reported to be lower, or 4 g/100 g sample in fatty species (Hall, 2010). Analysis of the liquid streams indicated that some lipids were pressed out with the water during pressing, leaving the latter concentrate with a lipid content of 1.1 ± 0.0 g/100 g sample, and has been reported to be 2 g/100 g sample previously (Hall, 2010). These streams were then fed to the dryers, increasing the relative lipid content of the material, again in relation to the water removal obtained during the two drying steps. After steam-drying, the measured lipid content was 6.6 ± 0.4 g/100 g sample, which rose further during the air-drying to 11.0 ± 0.5 g/100 g sample in the fine meal, and to 14.5 ± 0.2 g/100 g sample in the final capelin fishmeal. The fishmeal resulted in high-lipid content, but previously reported values were 6 g/100 g sample for fatty species (Hall, 2010) up to 12.9 ± 1.4 g/100 g sample fishmeal produced with steam-drying (de Koning, 2002).

The obtained results indicate that lipid removal from the solid stream was inefficient using the current processing techniques and must be improved. One of the primary purposes of using heating during early processing is to break down the protein–lipid bonds to ease the separation of lipids from the solid stream. As different temperatures affect different parts of the muscle (Hastings et al., 1985), the heating step, therefore, requires adjustment to serve its purpose. Furthermore, a higher focus on the first steps during the processing line is suggested, as these steps are the only steps breaking down the protein–lipid bonds for material entering the press. Several methods could be used to optimize the production line, including adding a mincer, which has been shown to be effective for blending raw materials in fish silage production (Arason, 1994). Enzyme addition has also successfully been used in the industry to catalyze the hydrolysis during the first steps of fish protein hydrolysates production, or to apply pH manipulation (Hultin et al., 2005; Kristinsson, 2007).

Results also showed that each solid stream (press cake, slurry, and concentrate) entering the dryers had different lipid content. Drying these streams individually could lead to a higher drying efficiency and, to the production of higher-quality protein products with a low-lipid content.

3.1.3 | Free fatty acids (FFA) changes during capelin processing

The formation of FFA was monitored in the samples to assess the effects of lipid hydrolysis, both due to delays between catching and processing, and during the processing itself. All the capelin was caught on the same day, and the majority at the same catching ground. Hence, the waiting time between catch and processing was

approximately 1.5 days. Measured FFA values in the raw material were 0.2 ± 0.0 g/100 g sample, or 4.4 ± 0.6 g/100 g lipid. Capelin caught in the spring have been reported to have the highest FFA values of the seasons, varying from 3.3 ± 0.0 g FFA/100 g lipid at the start of landing, up to 4.1 ± 0.2 g FFA/100 g lipid at the end of the landing, where the landing took 10–15h and started within 24h from harvesting (Bragadóttir et al., 2002). The FFA value of the raw material in the current study was hence similar to the FFA content in fish at the end of landing in the Bragadóttir et al. (2002) study.

Moreover, when looking at the formation of FFA during the process on a lipid basis, the FFA values increased and varied highly in the liquid stream. Low FFA values were observed in the separated press liquid (2.1 ± 0.2 g FFA/100 g lipid), while high values were observed in the slurry (14.2 ± 10.4 g FFA/100 g lipid) and stickwater (11.5 ± 11.5 g FFA/100 g lipid). Decreased FFA values were then observed in the separated oil (1.9 ± 0.1 g FFA/100 g lipid). The reason for a high FFA in slurry and stickwater could be the recirculation of the slurry, as more heat and oxygen is then introduced to the stream, increasing the lipid oxidation (Jacobsen, 2015). Moreover, as FFAs bind to proteins both through hydrophobic and hydrophilic interactions (Xiong, 1997), it was not surprising to see high FFA values in samples with relatively high-lipid content as well as water. Relative FFA levels rose during steam- and air-drying to 6.1 ± 0.1 g FFA/100 g sample in the final capelin fishmeal. In addition to being an effect of the water removal, this increase could be the result of the formation of lipoprotein complexes due to reduced polarity during dehydration (protein–FFA bindings) (Xiong, 1997). Those lipoproteins deteriorate when subjected to high temperatures, which also drastically affect the long-chain PUFA (Fournier et al., 2006). As the input temperature of the air-dryer is 450°C, the lipids are likely partially expressed as FFAs. Furthermore, lipoproteins might lead to an unwanted increase in insoluble proteins (Xiong, 1997), which have lower digestibility toward fish larvae than soluble proteins (Tonheim et al., 2007). Moreover, as a higher fraction of crude proteins are in the insoluble fraction (Tonheim et al., 2007), adjusting the dehydration step is valuable to ensure maximum yield of the soluble protein fraction in the final commercial fishmeal.

Interestingly, a significant difference was observed in FFA between the final fishmeal (6.1 ± 0.1 g FFA/100 g sample) and the fine meal (0.5 ± 0.0 g FFA/100 g sample), although the same processing procedure was applied to both samples. Lower values have been reported for fishmeal than in the current study, where the final fishmeal, on a lipid basis, resulted in 13.9 ± 0.3 g FFA/100 g lipid, and previously reported values from fishmeal produced with steam-drying was 8.5–9.5 g FFA/100 g lipid (de Koning, 2002). It is unknown which solid stream(s), entering the dryers, form the fine meal. A suggestion could be the latter concentrate, as it is more hydrolyzed than the other solid streams due to more water, heat, and oxygen during the liquid stream processing, and hence could be lower in kDa and swirl up the air duct. However, these suggestions are not confirmed and are a research topic by itself.

The development of FFA has been recognized as occurring concurrently with protein denaturation, where protein–FFA binding is the driving force in lipid hydrolysis (Ackman, 1967). Not only FFA is a product from lipid hydrolyzation, but also involved in the formation of secondary oxidation products and has no beneficial effects on health (Tena et al., 2018). Higher water content was, therefore, assumed to accumulate a higher degree of lipid hydroxylation, as observed in the current study. Therefore, mixing the press cake, sludge, and the latter concentrate is not recommended to keep the formation of FFA low. Moreover, during the processing, an increase in FFA levels was observed in the sludge compared to other samples, indicating that the majority of the FFA could be separated from the main solid stream through the press and decanter. This gives yet another argument for not mixing the streams.

Results from the oil streams showed that the FFA content was significantly lower in the separated oil (1.9 ± 0.0 g/100 g sample) compared to the concentrate oil (2.1 ± 0.1 g/100 g sample) and the final oil (2.2 ± 0.1 g/100 g sample). Allowed FFA values (oleic acid) in crude oil are 2%–5% FFA (Bimbo, 1998), while 0.1% oleic acid is allowed for refined oil (Hamm, 2009), but not specified for omega-3 concentrates (EFSA, 2010). FFA values obtained in the current study indicate that the produced oils can be defined as crude oils. Lowering FFA values of the produced oils by processing of fresher raw materials and by process optimization would, therefore, be beneficial if the production is intended for higher-value products.

3.1.4 | Phospholipids (PL) changes during capelin processing

Phospholipids (phosphatidylcholine) were measured throughout the processing, and the raw material had a PL value of 2.6 ± 0.5 g/100 g lipid. Cyprian et al., (2015) reported PL values of 5.8 ± 0.5 g/100 g lipid in high-lipid capelin, and 10.2 ± 0.2 g/100 g lipid in low-lipid capelin, at the same season and at the same storage conditions. Interestingly, the measured PL content was significantly lower than previously reported, suggesting higher degradation of PL, which could relate to the higher FFA values in the current study, as FFAs are among the products of hydrolyzed PLs (Ackman, 1967) and triacylglycerols (TAGs) (Arason, 1994).

When in contact with water, PLs can be hydrolyzed, resulting in the removal of the tail fatty acids from the polar head group. This may explain the observed lowered PL concentrations and simultaneous increase in FFA concentrations. Results show that most of the PLs measured follow the solid streams peaking after steam-drying (3.9 ± 0.1 g/100 g sample). The increase in PLs in the steam-dried samples was primarily explained by the water removal experienced in the drying process. The air-dryer lowered the water content even further, the PL decreased from 3.9 ± 0.1 g/100 g sample to 1.6 ± 0.0 g/100 g sample. Due to evaporation, a higher concentration of the PL would be expected in the fishmeal. However, high temperatures can cause accelerated deterioration of the lipids to FFAs (Fellows, 1988), which could explain the loss of PLs after air-drying in the fishmeal. Moreover, high heat affects the long-chain PUFA, which are easily degraded at temperatures between 180°C

and 220°C (Fournier et al., 2006) as well as in the presence of oxygen (Jacobsen, 2010). As the lipids exceeded 250°C in the driers and were exposed to added oxygen during recirculation earlier in the process, a simultaneous decrease in PL concentration and an increase in FFAs during the last steps of drying can be explained. Previous values of fishmeal include average values from 24 to 28 g Pl/100 g lipid (de Koning, 2002), where results in the current study were similar, or 27.0 ± 0.3 g Pl/100 g lipid.

In the sampled oil streams, no PLs were detected. Moreover, there are no quality guidelines for minimum PL requirements in omega-3 concentrates (EFSA, 2010).

3.1.5 | Fatty acid composition (FAC) changes during capelin processing

The FAC of the capelin was dominated by monounsaturated fatty acids (MUFA: 36.3–69.4 g/100 g lipid), followed by polyunsaturated fatty acids (PUFA: 9.4–44.0 g/100 g lipid) and saturated fatty acids (SFA: 17.7–23.9 g/100 g lipid) (Table 2). Kas'yanov et al., (2002) reported a similar FAC of 44.2–51.0 g MUFA/100 g lipid, 19.8%–25.5% g PUFA/100 g lipid, and 22.9%–29.9% g SFA/100 g lipid in capelin caught between July and October. Bragadóttir (2004) also reported seasonal changes in the FAC of capelin, indicating that both the lipid content and composition are highly dependent on season and catching ground.

During processing, higher concentrations of MUFA were detected in the separated press liquid (67.6 ± 0.3 g MUFA/100 g lipid) than in the press cake (41.0 ± 1.0 g MUFA/100 g lipid), suggesting that MUFAs mainly follow the liquid stream. MUFAs concentrations decreased during processing between sampling of the stickwater and the latter concentration, while concentrations of the other fatty acid classes (SFA and PUFA) increased in concentration between the same processing steps. Moreover, SFAs were higher in the press cake than in the separated press liquid, suggesting that the majority of the SFA followed the solid streams. Furthermore, the press cake contained higher values of PUFAs, including *n-3* PUFAs and eicosapentaenoic acid (EPA), compared to the separated press liquid. During the process, a systematic trend was detected in PUFA, *n-3* PUFA, and EPA levels, which were highest in the sludge, followed by

TABLE 2 Fatty acid composition of capelin samples during fishmeal and fish oil processing (see Figure 1). The capelin was caught the February 28th and processed on March 2nd 2018. Samples were collected from south and northeast of Iceland. All data are explained as g X/100 g lipid and mean ± *SD* (*n* = 3)

Capelin samples at 90°C	Lipid content	SFA	MUFA	PUFA	EPA	EPA/DHA	*n–3* PUFA	*n–3/n–6*
Raw materials	5.0 ± 0.5^{def}	19.2 ± 1.2^{bc}	57.1 ± 2.1^{cd}	20.4 ± 1.6^{cd}	6.9 ± 0.3^{bc}	0.8 ± 0.1^{a}	16.8 ± 1.5^{cd}	5.3 ± 0.2^{cde}
Pre-heating	5.1 ± 0.1^{def}	19.5 ± 0.2^{bc}	59.0 ± 1.0^{cd}	19.0 ± 1.1^{cde}	6.5 ± 0.3^{bcd}	0.9 ± 0.0^{a}	15.5 ± 1.0^{cde}	5.1 ± 0.1^{def}
Cooking	5.2 ± 1.9^{cdef}	19.4 ± 0.4^{bc}	58.2 ± 5.1^{cd}	19.6 ± 4.7^{cd}	6.7 ± 1.3^{bcd}	0.9 ± 0.2^{a}	16.2 ± 4.4^{cde}	5.4 ± 0.9^{cde}
Draining	9.4 ± 7.3^{bcd}	18.9 ± 0.9^{bc}	63.9 ± 3.5^{abc}	15.0 ± 3.0^{def}	5.5 ± 0.9^{cdef}	1.2 ± 0.3^{a}	11.8 ± 3.0^{defz}	4.2 ± 0.9^{defz}
Press liquid	5.6 ± 0.0^{cdef}	18.5 ± 0.4^{c}	64.0 ± 0.3^{abc}	15.0 ± 0.3^{def}	5.4 ± 0.1^{cdef}	1.1 ± 0.0^{a}	11.5 ± 0.2^{defz}	4.1 ± 0.0^{efz}
Press cake	6.6 ± 0.2^{cde}	23.6 ± 0.3^{a}	41.0 ± 1.0^{f}	33.4 ± 0.7^{a}	10.1 ± 0.1^{a}	0.6 ± 0.0^{a}	29.4 ± 0.9^{a}	8.7 ± 0.3^{a}
Separated press liquid	6.1 ± 0.4^{cdef}	17.9 ± 0.2^{c}	67.6 ± 0.3^{ab}	11.5 ± 0.2^{ef}	4.3 ± 0.1^{ef}	1.5 ± 0.0^{a}	8.3 ± 0.1^{fz}	3.3 ± 0.2^{fz}
Slurry	3.9 ± 0.6^{def}	19.3 ± 0.1^{bc}	58.2 ± 0.6^{cd}	19.7 ± 0.7^{cd}	6.6 ± 0.2^{bcd}	0.8 ± 0.0^{a}	16.4 ± 0.7^{cde}	5.9 ± 0.2^{bcd}
Stickwater	0.5 ± 0.1^{f}	19.7 ± 0.4^{bc}	60.2 ± 1.5^{abc}	17.8 ± 1.1^{cdef}	6.1 ± 0.4^{bcde}	0.9 ± 0.0^{a}	14.4 ± 1.1^{def}	5.1 ± 0.3^{def}
Separated oil	99.4 ± 0.2^{a}	18.2 ± 0.3^{c}	68.4 ± 1.0^{a}	10.6 ± 1.2^{f}	3.7 ± 1.1^{f}	1.5 ± 0.5^{a}	7.2 ± 1.1^{z}	2.7 ± 0.3^{z}
Sludge	5.6 ± 0.7^{cdef}	23.7 ± 0.9^{a}	38.1 ± 1.8^{f}	35.1 ± 1.0^{a}	11.3 ± 0.3^{a}	0.7 ± 0.0^{a}	30.8 ± 1.0^{a}	7.6 ± 0.2^{ab}
First concentrate	2.2 ± 0.0^{ef}	19.5 ± 0.1^{bc}	59.9 ± 0.7^{bc}	18.7 ± 0.5^{cde}	6.4 ± 0.1^{bcd}	0.8 ± 0.0^{a}	15.3 ± 0.5^{cde}	5.4 ± 0.1^{cde}
Latter concentrate	1.1 ± 0.0^{ef}	20.6 ± 1.7^{b}	51.0 ± 6.6^{de}	25.1 ± 6.3^{bc}	7.7 ± 0.9^{b}	0.7 ± 0.1^{a}	21.7 ± 6.3^{bc}	7.3 ± 1.4^{ab}
Concentrate oil	97.8 ± 0.5^{a}	18.0 ± 0.1^{c}	67.9 ± 0.1^{ab}	11.7 ± 0.0^{ef}	4.4 ± 0.0^{ef}	1.8 ± 0.0^{a}	7.9 ± 0.0^{fz}	2.9 ± 0.0^{z}
Steam-dryer	6.6 ± 0.4^{cde}	23.4 ± 0.2^{a}	43.0 ± 0.7^{ef}	31.6 ± 0.5^{ab}	9.9 ± 0.1^{a}	0.6 ± 0.0^{a}	27.6 ± 0.5^{ab}	7.8 ± 0.0^{a}
Fine meal	11.0 ± 0.5^{bc}	22.6 ± 0.0^{cd}	43.2 ± 0.3^{ef}	32.6 ± 0.9^{abc}	10.1 ± 0.3^{abc}	0.6 ± 0.0^{a}	28.7 ± 1.0^{ab}	8.7 ± 0.4^{a}
Fishmeal	14.5 ± 0.2^{b}	23.1 ± 0.7^{a}	40.8 ± 4.2^{f}	39.1 ± 4.9^{a}	10.8 ± 1.0^{a}	1.6 ± 1.5^{a}	30.9 ± 1.0^{a}	7.0 ± 0.9^{abc}
Final oil	99.1 ± 0.4^{a}	18.5 ± 0.5^{c}	65.5 ± 4.1^{abc}	13.4 ± 3.0^{def}	4.9 ± 0.9^{def}	1.5 ± 0.5^{a}	9.8 ± 3.2^{efz}	3.5 ± 1.0^{fz}

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; *n-3* PUFA, omega–3 PUFA; *n-3/n-6*, ratio between omega-3 and omega-6 fatty acids); PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

[a-z]Letter indicates a significant difference between vertical results, where $p < .05$.

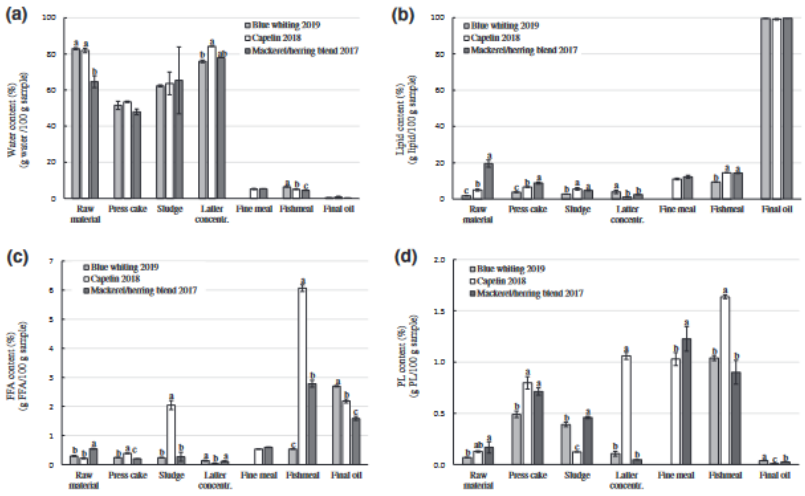


FIGURE 3 Water content (3a), lipid content (3b), free fatty acids content (3c), and phospholipid content (3d) at key locations in fishmeal processing in blue whiting (gray column), capelin (white column), and mackerel/herring blend (dark gray column). Key locations include press cake, slurry, and latter concentrate, which were all solid streams entering the drying steps

the stickwater, and lowest in the separated oil. This indicated further that the PUFA, *n-3* PUFA, and EPA mainly followed the solid stream. If these lipids were to be successfully separated from the solid stream, the final oil would increase in PUFA, *n-3* PUFA, and EPA concentrations. As a PUFA-rich diet suggests multiple health benefits (Jacobsen, 2010; Larsen et al., 2011; Ruxton et al., 2004; Tocher, 2015), optimization of the solid and oil streams would be of focus when developing higher-value products from the processing plant. However, *n-3* PUFA are also highly susceptible to lipid oxidation, which may lead to the formation of undesirable fishy and rancid off-flavors (Jacobsen, 2010). Both the potential health benefits and the oxidative stability, therefore, depend on the FAC of each individual product matrix (Jacobsen, 2010), which needs to be considered during further process optimization.

3.2 | Process efficiency comparison between species

Fishmeal from three different runs were compared to evaluate the efficiency of the processing facilities toward a variation in raw materials. The types of fish that entered the processing were (i) *capelin*, (ii) a *mackerel/herring blend*, and (iii) *blue whiting* (Table 1). All types of fishmeal were produced under their standard conditions, as described earlier.

Samples from chosen key sampling locations during processing (raw materials, press cakes, sludges, latter concentrate, final fishmeal, and final oil) were compared during processing of the different fish species (capelin, mackerel/herring blend, blue whiting). As the fine meals differentiated significantly in composition from the final fishmeal of both the capelin and mackerel/herring blend, the fine meal was investigated in these species as well.

3.2.1 | Process comparison of water content from different raw materials

The water content was significantly higher in the blue whiting (82.9 ± 0.6 g/100 g sample) and capelin (82.0 ± 1.2 g/100 g sample) raw materials compared to the mackerel/herring blend (64.6 ± 3.3 g/100 g sample) (Figure 3a). The sludge and the latter concentrate introduced higher amounts of water into the driers than expected, as the evaporation system should lower the water content down to 50%–70% water content and depends on the viscosity/dry matter relationship (Einarsson et al., 2019; FAO, 1986; Hall, 2010). No significant difference was observed in the water content of the sludge from the three species. Simultaneously, the large standard deviation obtained in the water content of the mackerel/herring blend during processing indicated high heterogeneity of these samples, was possibly due to the different chemical composition of the two mixed species (mackerel and herring). Centrifuging the latter concentrate was expected to remove the oil and water from the liquid stream, and thus feed the dryers with relatively similar material in water content. However, this was not the case, and the water content of the capelin latter concentrate

was nearly 10% higher compared to the latter concentrate of the other species. This increases the drying load during the capelin processing. The fine meals and fish oils did not differ significantly in water content between the species. A trend was observed in the water content of the raw material and the fishmeal, where higher water content in the raw material resulted in a higher water content of the fishmeal. Hence, mackerel/herring blend fishmeal resulted in the lowest water content of the species.

According to the Feed and Feed Ingredient Standards from FAO, the water content should not exceed 10 g/100 g sample for fishmeal, while minimum requirements are not specified (FAO, 2001). The water content of all fishmeal samples was hence within the required range for feed production. No standards regarding water content were found for fish protein concentrate for human consumption, other than a protein content of 65%–80%, which limits the water content (Einarsson et al., 2019; FAO, 2001). The factory currently aims to produce fishmeal with 7–8 g water/100 g sample, but as the value of fishmeal depends on protein content, the water content is adjusted depending on the amounts of cut-offs and different species available each time. Hence, the mackerel/herring blend fishmeal was considered the most promising fishmeal regarding water content.

3.2.2 | Process comparison of lipid content from different raw materials

The lipid content of the raw material differed significantly between the blue whiting (1.8 ± 0.1 g/100 g sample), capelin (5.0 ± 0.5 g/100 g sample), and mackerel/herring blend (19.5 ± 2.0 g/100 g sample), as expected (Figure 3b). The lipid content of the press cakes varied significantly between the species, where the blue whiting had the lowest lipid content (3.7 ± 0.2 g/100 g sample), followed by capelin (6.6 ± 0.2 g/100 g sample), while the mackerel/herring blend press cake still had the highest lipid content (8.8 ± 0.6 g/100 g sample). Interestingly, although the blue whiting fishmeal was significantly lower in lipid content (9.4 ± 0.1 g/100 g sample), no significant differences were observed in the lipid content between the capelin (14.3 ± 0.2 g/100 g sample) and mackerel/herring blend (14.5 ± 0.2 g/100 g sample) fishmeal samples. Furthermore, no significant differences were observed in the lipid content of the fish oils between the species.

Most of the lipids dispersed into the drying steps were from the press cake of the fattiest raw material (mackerel/herring blend) but from the latter concentrate in the leanest raw material (blue whiting). The highest lipid content dispersed into the dryers of the solid streams from capelin came from the sludge. The lipids of lean fish mostly consist of PLs (Ackman, 1980; Huss, 1995) as an integral part of the structure of the cells (Huss, 1995). Separating the PLs from the lipid–protein bond is thus likely to have a higher threshold compared to TAGs during processing. Moreover, as fattier fish species store their lipids in fat cells throughout their body (Huss, 1995), the press cakes of fattier fish were expected to contain higher lipid content, which was indeed observed.

Fishmeal is divided into fish protein concentrate (FPC) types A–C depending on their lipid content (Einarsson et al., 2019; Windsor, 2001). As the streams are all fed into the dryers and blended, the fishmeal ends up as the low-grade Type C, since the lipid content exceeded 3 g/100 g sample in the fishmeal of all three species. To reach lower lipid content in the products, redesigning of the fishmeal factory is suggested. The obtained lipid contents of the fishmeal samples were similar to fishmeal from South Africa (de Koning, 1999), which all are too high in lipid content for human consumption.

The separated oil from the capelin production contained 99.4 ± 0.2 g lipids/100 g sample and differed significantly from the separated oil from the mackerel/herring blend (98.8 ± 0.1 g lipid/100 g sample). The lipid content in the final oil between the species was relatively similar and fell within the range of crude oil where the allowed water content ranges between 0.5% and 1% (EFSA, 2010; Hamm, 2009).

3.2.3 | Process comparison of FFA content from different raw materials

In the raw materials, FFAs were significantly highest in the mackerel/herring blend, possibly both due to a high-lipid content, and more heterogeneity of the raw material (Figure 3c). When comparing the solid streams entering the dryers, the sludge contributed to the highest FFA values between the species, which was even more evident on a lipid base. It could be assumed that the recirculation of the slurry introduced additional heat, oxygen, and water to the process, inducing lipid oxidation (Jacobsen, 2015), increasing the formation of FFAs. Moreover, as the latter concentrate contributed to the lowest amounts of FFA in all species, it could be assumed that most of the FFAs were extracted out in the sludge. This was supported by the observation of a peak in FFA in the capelin sludge (2.0 ± 0.2 g/100 g sample or 37.0 ± 1.8 g/100 g lipid), which was also reflected in the capelin fishmeal (6.1 ± 0.1 g/100 g sample or 41.8 ± 0.4 g/100 g lipid) compared to the other species. Even though a peak was only observed in the capelin sludge, separating the streams (press cake, sludge, and latter concentrate), and processing them further individually could have prevented this FFA peak in the capelin fishmeal. The highest concentrations of FFA in the final fishmeal samples were measured in the capelin (6.1 ± 0.1 g FFA/100 g sample) and mackerel/herring blend productions (2.8 ± 0.1 g FFA/100 g sample). The highest concentration of FFA in the blue whiting process was observed in the fish oil. The amount of FFA ranged between 1.5 and 3 g FFA/100 g in the final oil from all species, which is considered to be in the crude oil range (Bimbo, 1998). However, FAO has not specified any maximum FFA values for fishmeal intended for feed (FAO, 2001), although fishmeal buyers might have a limit of FFA values.

3.2.4 | Process comparison on PL content from different raw materials

No significant differences were observed in the PL content of the three raw materials on a 100 g sample basis (Figure 3d). However,

TABLE 3 Fatty acid composition of capelin (caught in 2018), mackerel/herring blend (caught in 2017), and blue whiting (caught in 2019) samples produced under standard condition at 90°C. All data are explained as g X/100 g lipid and mean ± *SD*

	Sample	Lipid content	SFA	MUFA	PUFA	EPA	EPA/DHA	*n*-3 PUFA	*n*-3/*n*-6
Capelin (g/100 g lipid)	Raw materials	5.0 ± 0.5^{gh}	19.2 ± 1.2^{hi}	57.1 ± 2.1^{b}	20.4 ± 1.6^{de}	6.9 ± 0.3^{e}	0.8 ± 0.1^{a}	16.8 ± 1.5^{c}	5.3 ± 0.2^{d}
	Press cake	6.6 ± 0.2^{g}	23.6 ± 0.3^{cd}	41.0 ± 1.0^{gh}	33.4 ± 0.7^{ab}	10.1 ± 0.1^{a}	0.6 ± 0.0^{a}	29.4 ± 0.9^{ab}	8.7 ± 0.3^{b}
	Sludge	5.6 ± 0.7^{gh}	23.7 ± 0.9^{c}	38.1 ± 1.8^{h}	35.1 ± 1.0^{ab}	11.3 ± 0.3^{a}	0.7 ± 0.0^{a}	30.8 ± 1.0^{a}	7.6 ± 0.2^{bc}
	Latter conc.	1.1 ± 0.0^{i}	20.6 ± 1.7^{ghi}	51.0 ± 6.6^{bcde}	25.1 ± 6.3^{cd}	7.7 ± 0.9^{cde}	0.7 ± 0.0^{a}	21.7 ± 6.3^{ef}	7.3 ± 1.4^{bc}
	Fine meal	11.0 ± 0.5^{de}	22.6 ± 0.0^{cdefg}	43.2 ± 0.3^{fgh}	32.6 ± 0.9^{ab}	10.1 ± 0.3^{ab}	0.5 ± 0.0^{a}	28.7 ± 1.0^{abc}	8.7 ± 0.4^{b}
	Fishmeal	14.5 ± 0.2^{c}	23.1 ± 0.7^{cdef}	40.8 ± 4.2^{gh}	39.1 ± 4.9^{a}	10.8 ± 1.0^{a}	0.7 ± 0.2^{a}	30.9 ± 1.0^{a}	7.0 ± 0.9^{c}
	Final oil	99.1 ± 0.4^{a}	18.5 ± 0.5^{i}	65.5 ± 4.1^{a}	13.4 ± 3.0^{e}	4.9 ± 0.9^{f}	1.5 ± 0.5^{a}	9.8 ± 3.2^{h}	3.5 ± 1.0^{efg}
Mackerel/herring blend (g/100 g lipid)	Raw materials	19.5 ± 2.0^{b}	23.0 ± 0.5^{cdef}	42.2 ± 2.6^{fgh}	31.4 ± 2.5^{bc}	8.7 ± 0.4^{bc}	0.7 ± 0.0^{a}	23.6 ± 1.7^{bcde}	3.3 ± 0.2^{fg}
	Press cake	8.8 ± 0.6^{f}	28.2 ± 1.8^{b}	49.0 ± 1.1^{cdef}	19.5 ± 3.1^{de}	4.6 ± 0.8^{f}	0.6 ± 0.0^{a}	14.9 ± 2.7^{gh}	3.8 ± 0.2^{efg}
	Sludge	4.7 ± 0.2^{h}	30.9 ± 0.3^{a}	53.7 ± 0.4^{bc}	13.3 ± 0.7^{e}	2.8 ± 0.2^{g}	0.6 ± 0.0^{a}	9.6 ± 0.6^{h}	3.3 ± 0.3^{fg}
	Latter conc.	2.4 ± 0.2^{i}	21.4 ± 0.1^{defgh}	45.6 ± 0.1^{defg}	28.9 ± 0.2^{bc}	7.7 ± 0.0^{cde}	0.7 ± 0.0^{a}	21.6 ± 0.2^{ef}	3.4 ± 0.0^{efg}
	Fine meal	12.3 ± 0.8^{d}	23.4 ± 0.2^{cde}	38.8 ± 0.7^{gh}	34.1 ± 0.6^{ab}	8.4 ± 0.1^{cd}	0.6 ± 0.0^{a}	27.6 ± 0.6^{abcde}	4.8 ± 0.1^{def}
	Fishmeal	14.3 ± 0.2^{c}	23.3 ± 0.3^{cde}	38.3 ± 0.6^{h}	34.6 ± 0.7^{ab}	8.6 ± 0.1^{c}	0.5 ± 0.0^{a}	28.0 ± 0.7^{abcd}	4.9 ± 0.1^{de}
	Final oil	99.7 ± 0.1^{a}	22.0 ± 0.1^{cdefg}	43.7 ± 0.2^{fgh}	31.3 ± 0.4^{bc}	8.6 ± 0.1^{c}	0.8 ± 0.0^{a}	23.2 ± 0.3^{cde}	3.2 ± 0.0^{g}
Blue whiting (g/100 g lipid)	Raw materials	1.8 ± 0.1^{i}	21.9 ± 0.2^{cdefg}	44.3 ± 0.7^{defgh}	30.6 ± 0.3^{bc}	8.4 ± 0.1^{cd}	0.5 ± 0.0^{a}	27.5 ± 0.3^{abcde}	11.7 ± 0.0^{a}
	Fishmeal	9.4 ± 0.1^{a}	21.3 ± 0.0^{efgh}	44.0 ± 0.4^{efgh}	31.4 ± 0.5^{bc}	8.6 ± 0.2^{c}	0.5 ± 0.0^{a}	28.4 ± 0.4^{abc}	12.2 ± 0.1^{a}
	Final oil	99.6 ± 0.0^{ef}	20.9 ± 0.1^{fgh}	51.5 ± 0.3^{bcd}	24.9 ± 0.1^{cd}	7.5 ± 0.1^{cde}	0.7 ± 0.0^{a}	22.1 ± 0.1^{def}	11.7 ± 0.0^{a}

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; *n*-3 PUFA, omega-3 PUFA; *n*-3/*n*-6, ratio between omega-3 and omega-6 fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

[a–i]Letter indicates a significant difference between vertical results, where $p < .05$.

when calculating the PLs in the raw materials on a lipid basis, the mackerel/herring blend had the lowest amount of PLs (0.9 ± 0.2 g Pl/100 g lipid), followed by capelin (4.4 ± 0.6 g Pl/100 g lipids), while the highest amount of PLs was measured in the blue whiting (16.4 ± 0.6 g Pl/100 g lipid). PLs in capelin were low in the sludge due to the low-lipid content, but when compared on a lipid basis, capelin and blue whiting had the highest value, 14.4 ± 0.7 g/100 g lipid and 14.5 ± 0.8 g Pl/100 g lipid, respectively, while the mackerel/herring blend contained only 9.9 ± 0.4 g Pl/100 g lipid. However, high amounts of PL in the latter concentrate of the capelin (1.1 ± 0.0 g Pl/100 g sample or 11.3 ± 0.5 g Pl/100 g lipid), was suggested to be mirrored in the fishmeal, as the capelin was significantly higher in PLs (1.6 ± 0.0 g Pl/100 g sample or 27.0 ± 0.3 g Pl/100 g lipid) compared to the latter concentrate of both mackerel/herring blend and blue whiting. Moreover, the PLs were significantly lower in the capelin sludge compared to the other species, which can be explained by the high FFA of this sample. The fine meal of the capelin was lower in PL than the final fishmeal, while the opposite was observed in the mackerel/herring fine and final meal samples. The highest contributors to a PL-rich fishmeal from blue whiting or mackerel/herring blend were the press cake and sludge. On a lipid base, the press cake, sludge, and the latter concentrate all contributed to the high PL values in capelin fishmeal.

Reported PL values from seven pelagic factories were higher than measured in the current study, where the water content from those factories had double the amount measured in the current study (de Koning, 2002). Furthermore, de Koning (2002) concluded that PL values should be included in establishing the quality criteria of fishmeal to know the history of the meal. The results from the current study support that PL values should be taken into consideration when defining the quality of fish meal products.

3.2.5 | Process comparison of FAC from different raw materials

The FAC of all species was dominated by MUFAs, followed by PUFAs, while concentrations of SFAs were the lowest (Table 2,3). Measured values of the raw material from mackerel and herring blend are in line with previously reported values from mackerel (Romotowska et al., 2016) and herring (Dang et al., 2017). The blue whiting raw material has been reported with relatively similar SFAs (19–21 g/100 g lipids) (Jónsdóttir et al., 2007; Kolade, 2015), where MUFAs and PUFAs differentiate highly from measured values in blue whiting (Jónsdóttir et al., 2007; Kolade, 2015). MUFAs in blue whiting have been reported from 23.4 g/100 g lipids (Jónsdóttir et al., 2007) to 38.2 g/100g lipids (Kolade, 2015) and MUFAs in blue whiting in the current study was 44.3 ± 0.7 g/100g lipid. PUFAs in blue whiting has been reported from 21.6 g/100 g lipids (Kolade, 2015) to 46.4 g/100 g lipid (Jónsdóttir et al., 2007), and was measured 30.6 ± 0.3 g/100 g lipid in the current study.

When comparing the capelin raw material to the mackerel/herring blend and blue whiting, capelin had higher concentrations of MUFA (57.1 ± 1.6 g/100 g lipid) than the mackerel/herring blend and blue whiting, but had lower concentrations of SFA, PUFA, EPA, and *n-3* PUFA. Simultaneously the raw materials differentiated significantly in *n-3/n-6 ratios*, where blue whiting had the highest ratio, or 11.7 ± 0.0 g/100 g lipid, and the mackerel/herring blend significantly the lowest ratio (3.3 ± 0.2 g/100 g lipid). Although different concentrations of the raw material were observed, the *n-3/n-6* ratio increased during the processing of the solid streams, but *n-3/n-6* PUFA content strongly influences the membrane function and numerous cellular processes (Schmitz & Ecker, 2008). Omega (*n-3*, such as EPA, or docosahexaenoic acid (DHA)) and *n-6* (arachidonic acid, AA) fatty acids are competitive substrates involved in autacoid biosynthesis. A higher *n-3/n-6* ratio in is beneficial since *n-6* fatty acids are mostly pro-active, while the *n-3* fatty acids show inhibitory characteristics of inflammatory signaling (Schmitz & Ecker, 2008).

Higher concentrations of SFA and MUFA were observed in the press cake and sludge of the mackerel/herring blend when compared to the same sample places in capelin. However, higher concentrations of PUFA, EPA, *n-3* PUFA, and *n-3/n-6* ratio were observed in the capelin press cake compared to the mackerel/herring blend press cake. The *n-3/n-6* ratio was overall highest in blue whiting, followed by capelin, where the mackerel/herring blend had the lowest ratio. Interestingly, the fine capelin meal was higher in *n-3* PUFA than the capelin fishmeal, which was not observed in the mackerel/herring blend. The blue whiting fishmeal had the highest *n-3/n-6* ratio (12.2 ± 0.1 g/100 g lipid), followed by capelin (7.0 ± 0.9 g/100 g lipid), while the lowest ratio was measured in the mackerel/herring blend (4.9 ± 0.1 g/100 g lipid).

Moreover, the final oil of the blue whiting had a significantly higher ratio of *n-3/n-6* compared to the oils from the capelin and mackerel/herring blend. Fish oil from capelin had the highest concentration of MUFAs (65.5 ± 4.1 g/100 g lipid), followed by blue whiting (51.5 ± 0.3 g/100 g lipid), where the mackerel/herring blend had the lowest concentration of MUFA (43.7 ± 0.2 g/100 g lipid). Moreover, the mackerel/herring blend final oil had the highest amounts of PUFAs (31.3 ± 0.4 g/100 g lipid), followed by blue whiting (24.9 ± 0.1 g/100 g lipid), where lowest concentration was measured in capelin final oil (13.4 ± 3.0 g/100 g lipid).

Processing of individual streams may be optimized depending upon the species processed each time. During the capelin processing, optimization of further processing of the sludge and the press cake would be the most promising streams regarding the production of PUFAs. When producing the mackerel/herring blend, the latter concentrate would be the most promising stream for collection of PUFAs, the sludge for collection of MUFA, and the press cake for SFA. Moreover, as these values are all on lipid base, the mackerel/herring blend would be the most promising since the raw material was four times higher in lipid content than the capelin. However, all raw materials need to be broken down more effectively at the start of the production process, leaving the press cakes with a lower lipid content and a higher yield of PUFAs in the oil streams. Moreover, as *n-3* PUFAs are highly susceptible to lipid oxidation (Jacobsen, 2010), extracting them from

the fishmeal solid stream into the oil streams would not only increase the value of the fishmeal but is likely to prolong the shelf life of the fishmeal as well (Mozuraityte et al., 2016; Shahidi & Zhong, 2010). Furthermore, in order to maintain the PUFA quality during the final steps of the oil streams, temperatures, oxygen, and water usage during the fishmeal process and oil extraction might require further optimization.

4 | CONCLUSIONS

Traditional fishmeal factories worldwide have been run with the same technology for decades without a detailed analysis of the quality changes occurring during processing. These processing lines are mainly oriented toward water removal, while lipid removal and lipid quality are not of focus, resulting in low-value products. Hence, the objective of the current study was to investigate how the physicochemical characteristics of the raw material are changing during processing and identify which processing steps need to be changed in order to produce higher-value products in the future.

Detailed analysis of the capelin fishmeal production indicated that optimization of draining and cooking was needed, as the water content remained unchanged before and after cooking. Furthermore, with effective draining, a lower proportion of dry matter would enter the liquid stream. The press was working adequately as the press cake was low in water content and resulted in a relatively good lipid quality. Despite that, the water content of the concentrate samples remained too high, or 75%–85% in all species studied, where 65% would be expected (Hall, 2010). This indicates that the concentration process requires further optimization as well.

A high FFA increase was observed in the slurry and stickwater, possibly due to the recirculation of the slurry, which introduced more water, heat, and oxygen to the processing stream. With a more effective way of breaking down the protein–lipid bonds during the initial processing steps, recirculation of processing streams can be minimized. Hence, minimizing oxygen, heat, and water could lower the FFA values.

Lipid analysis indicated that the process did not result in low-lipid fishmeal in any of the fish species investigated. However, lower lipid content was observed in both capelin, and mackerel/herring blend fine meal, and is hence recommended to be collected separately. Moreover, solid streams entering the drying steps (press cake, sludge, and the latter concentrate) are recommended to be processed and dried separately. As the solid streams differed in water-, lipid-, FFA, and PL concentrations, and obtained different physicochemical characteristics, any optimization of the process calls for optimization of the drying times of each individual stream. Streams high in FFA values could thus also be easily bypassed during the production.

Optimization of early processing steps, such as homogenizing the raw material with a mincer or heat treatment optimization, is suggested to aid with lipid separation from the solid streams in all species. Furthermore, separate optimized drying of each solid stream may then lead to the production of high-quality products.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support from the AVS (The Added Value of Seafood) fund of the Ministry of Fisheries and Agriculture in Iceland (grant number: R18 031-18), the Rannís Technology Development Fund (no. 198883-0611), and the University of Iceland research fund as well as Síldarvinnslan hf. for access to their facilities, assistance, and raw materials.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Gudrun Svana Hilmarsdottir https://orcid.org/0000-0003-3869-1487
Ólafur Ogmundarson https://orcid.org/0000-0003-3171-2388
Sigurjon Arason https://orcid.org/0000-0002-7358-8103
María Gudjónsdóttir https://orcid.org/0000-0001-7577-1190

REFERENCES

Ackman, R. G. (1967). The influence of lipids on fish quality. *International Journal of Food Science & Technology*, *2*, 169–181. https://doi.org/10.1111/j.1365-2621.1967.tb01339.x

Ackman, R. G. (1980). Advances in fish science and technology. In J. J. Connell (Ed.), *Fish lipids* (pp. 86–103). Fishing News (Books) Ltd.

AOCS. (1998). *Official method Ce 1b–89. Official method and recommended practices of the AOC* (7th ed.). American Oil Chemists' Society.

Arason, S. (1994). Production of fish silage. In A. M. Martin (Ed.), *Fisheries processing* (pp. 244–272). Springer US. https://doi.org/10.1007/978-1-4615-5303-8_11

Arason, S., Nguyen, M. V., Thorarinsdottir, K. A., & Thorkelsson, G. (2014). Preservation of fish by curing. In I. S. Boziaris (Ed.), *Seafood processing: Technology, quality and safety* (1st ed., pp. 129–160). John Wiley and Sons Ltd. https://doi.org/10.1002/9781118346174.ch6

Bao, H. N. D., Arason, S., & Þórarinsdóttir, K. A. (2007). Effects of dry ice and superchilling on quality and shelf life of arctic charr (*Salvelinus alpinus*) fillets. *International Journal of Food Engineering*, *3*. https://doi.org/10.2202/1556-3758.1093

Bernárdez, M., Pastoriza, L., Sampedro, G., Herrera, J. J. R., & Cabo, M. L. (2005). Modified method for the analysis of free fatty acids in fish. *Journal of Agricultural and Food Chemistry*, *53*, 1903–1906. https://doi.org/10.1021/jf040282c

Bimbo, A. P. (1998). Guidelines for characterizing food grade fish oil. *International Fishmeal & Oil—manufacturers Association*, *9*, 473–483.

Bimbo, A. P., & Crowther, J. B. (1992). Fish meal and oil: Current uses. *Journal of the American Oil Chemists Society*, *69*, 221–227. https://doi.org/10.1007/BF02635890

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*, 911–917. https://doi.org/10.1139/y59-099

Bragadóttir, M., Pálmadóttir, H., & Kristbergsson, K. (2002). Seasonal changes in chemical composition and quality parameters in capelin (*Mallotus villosus*). *Journal of Aquatic Food Product Technology*, *11*, 87–103. https://doi.org/10.1300/J030v11n03_08

Bragadóttir, M., Pálmadóttir, H., & Kristbergsson, K. (2004). Composition and chemical changes during storage of fish meal from Capelin (*Mallotus villosus*). *Journal of Agricultural and Food Chemistry*, *52*, 1572–1580. https://doi.org/10.1021/jf034677s

Bragadóttir, M, Pálmadóttir, H, & Kristbergsson, K (2004). Composition and Chemical Changes during Storage of Fish Meal from Capelin (Mallotus villosus). *Journal of Agricultural and Food Chemistry, 52*, 1572–1580. https://doi.org/10.1021/jf034677s.

Cyprian, O. O., Nguyen, M. V., Sveinsdottir, K., Jonsson, A., Tomasson, T., Thorkelsson, G., & Arason, S. (2015). Influence of smoking and packaging methods on lipid stability and microbial quality of Capelin (*Mallotus villosus*) and sardine (*Sardinella gibbosa*). *Food Science & Nutrition, 3*, 404–414. https://doi.org/10.1002/fsn3.233

Dang, H. T. T., Gudjónsdóttir, M., Karlsdóttir, M. G., Van Nguyen, M., Romotowska, P. E., Tómasson, T., & Arason, S. (2017). Influence of temperature stress on lipid stability of Atlantic herring (*Clupea harengus*) muscle during frozen storage. *Journal of the American Oil Chemists' Society, 94*, 1439–1449. https://doi.org/10.1007/s11746-017-3053-4

de Koning, A. J. (1999). Properties of South African fish oils: A review. *International Journal of Food Properties, 2*, 205–216. https://doi.org/10.1080/10942919909524605

de Koning, A. J. (2002). Quantitative quality tests for fish meal. II. An investigation of the quality of South African fish meals and the validity of a number of chemical quality indices. *International Journal of Food Properties, 5*, 495–507. https://doi.org/10.1081/JFP-120015487

EFSA. (2010). Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. *EFSA Journal, 8*, 1874. https://doi.org/10.2903/j.efsa.2010.1874

Einarsson, M. I., Bæk, A. M., Eliasen, O., Flesland, O., Jacobsen, C., Jokumsen, A., Pálsson, J., Pedersen, S. A., & Samuelsen, T. A. (2019). *Nordic center of excellence network in fishmeal and fish oil* (ISSN 1670–7192; p. 124). Matís ohf. https://doi.org/10.5281/zenodo.3243334

European Commission, Directorate-General for Health and Food Safety, & Agra CEAS Consulting. (2016). *Study on food intended for sports-people final report*. Publications Office. http://bookshop.europa.eu/uri?target=EUB:NOTICE:EW0116224:EN:HTML

FAO. (1986). *The production of fish meal and oil* (FAO Fisheries Technical Paper—142 ISBN 92-5-102464-2; Aquaculture Feed and Fertilizer Resources Information System). http://www.fao.org/3/X6899e/X6899E00.HTM

FAO. (2001). *Feed and feed ingredient standards* [Aquaculture Feed and Fertilizer Resources Information System]. Food and Agriculture Organization of the United Nations. http://www.fao.org/fishery/affris/feed-and-feed-ingredient-standards/en/

FAO. (2018). *The State of World Fisheries and Aquaculture. Meeting the sustainable development goals* (ISBN 978-92-5-130562-1; The State of the World, p. 210). Food and Agriculture Organization of the United Nations. http://www.FAO.org/3/I9540EN/i9540en.pdf

FAO. (2020). *The state of world fisheries and aquaculture 2020: Sustainability in action* (ISBN: 978-92-5-132692-3; The State of the World, p. 224). Food and Agriculture Organization of the United Nations. https://doi.org/10.4060/ca9229en

Fellows, P. (1988). *Food processing technology: Principles and practice* (3rd ed.). Published jointly by Ellis Horwood and VHC.

Fournier, V., Destaillats, F., Juanéda, P., Dionisi, F., Lambelet, P., Sébédio, J.-L., & Berdeaux, O. (2006). Thermal degradation of long-chain polyunsaturated fatty acids during deodorization of fish oil. *European Journal of Lipid Science and Technology, 108*, 33–42. https://doi.org/10.1002/ejlt.200500290

Hall, G. M. (2010). Fishmeal production and sustainability. In G. M. Hall (Ed.), Fish processing (pp. 207–235). Wiley-Blackwell. https://doi.org/10.1002/9781444328585.ch9

Hamm, W. (2009). Processing of fish oil. In B. Rossell (Ed.), *Fish oils* (pp. 81–98). Wiley-Blackwell.

Hastings, R. J., Rodger, G. W., Park, R., Matthews, A. D., & Anderson, E. M. (1985). Differential scanning calorimetry of fish muscle: The effect of processing and species variation. *Journal of Food Science, 50*, 503–506. https://doi.org/10.1111/j.1365-2621.1985.tb13437.x

Hultin, H., Kristinsson, H., Lanier, T., & Park, J. (2005). *Process for recovery of functional proteins by pH shifts* (pp. 107–139). https://doi.org/10.1201/9781420028041.ch3

Huss, H. H. (1995). *Quality and quality changes in fresh fish* (Technical Paper FAO Fisheries Technical Paper-348; Food and Agriculture Organization of the United Nations). Ministry of Agriculture and Fisheries. http://www.fao.org/3/V7180E/V7180E00.htm

ISO. (1999). *Animal feeding stuffs* (ISO 6496:1999; Determination of Moisture and Other Volatile Matter Content, p. 7). International Organization for Standardization. https://www.iso.org/obp/ui/#iso:std:iso:6496:ed-2:v1:en

Jacobsen, C. (2010). Enrichment of foods with omega-3 fatty acids: A multidisciplinary challenge. *Annals of the New York Academy of Sciences, 1190*, 141–150. https://doi.org/10.1111/j.1749-6632.2009.05263.x

Jacobsen, C. (2015). Some strategies for the stabilization of long chain n-3 PUFA-enriched foods: A review. *European Journal of Lipid Science and Technology, 117*, 1853–1866. https://doi.org/10.1002/ejlt.201500137

Jónsdóttir, R., Tryggvadóttir, S. V., Bragadóttir, M., Einarsson, H., Björnsson, H., & Jónsson, S. (2007). *Bait from fishery byproducts*. Processing and product development, biotechnolgoy and food safety (Vol. *43–07*, p. 12). Matís Food Research, Innovation and Safety. https://www.matis.is/media/frettir/AVS-lokaskyrsla_Feitt_er_agnid__3_.pdf

Kas'yanov, S. P., Sayapina, T. A., Gor'kavaya, G. M., Naumenko, E. A., & Akulin, V. N. (2002). Correlation between the lipid composition of the Anadyr capelin Mallotus villosus and its physiological state. *Journal of Evolutionary Biochemistry and Physiology, 38*, 7. https://doi.org/10.1023/A:1015569305499

Kolade, O. Y. (2015). Fatty acid profile investigation of blue whiting fish (*Micromesistius poutassou*) flesh from Agbalata Market Badagry, Lagos West, Nigeria. *Emergent Life Sciences Research, 1*, 20–25.

Kristinsson, H. G. (2007). Aquatic food protein hydrolysates. In *Maximising the value of marine by-products* (pp. 229–248). Elsevier. https://doi.org/10.1533/9781845692087.2.229

Larsen, R., Eilertsen, K.-E., & Elvevoll, E. O. (2011). Health benefits of marine foods and ingredients. *Biotechnology Advances, 29*, 508–518. https://doi.org/10.1016/j.biotechadv.2011.05.017

Lowry, R. R., & Tinsley, I. J. (1976). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists Society, 53*, 470–472. https://doi.org/10.1007/BF02636814

Margeirsson, B., Lauzon, H. L., Thorvaldsson, L., Arnason, S. V., Arason, S., Valtýsdóttir, K. L., & Martinsdóttir, E. (2010). *Optimized chilling protocols for fresh fish (Report Matis 15-10)*. Matis Food Research, Innovation and Safety. https://rafhladan.is/bitstream/handle/10802/1508/54-10-Optimised-Chilling-Protocols.pdf?sequence=1

Mozuraityte, R., Kristinova, V., Standal, I. B., Carvajal, A. K., & Aursand, M. (2016). Oxidative stability and shelf life of fish oil. In *Oxidative stability and shelf life of foods containing oils and fats* (pp. 209–231). Elsevier. https://doi.org/10.1016/B978-1-63067-056-6.00005-7

Mundi, I. (2020). Fishmeal—Monthly price (euro per metric ton)—Commodity prices—Price charts, data, and news. *Indexmundi.Com*. https://www.indexmundi.com/commodities/?commodity=fish-meal&months=120&currency=eur

Oterhals, Å., & Vogt, G. (2013). Impact of extraction, refining and concentration stages on the stability of fish oil. In C. Jacobsen, N. S. Nielsen, A. F. Horn, & A.-D.-M. Sørensen (Eds.), *Food enrichment with omega-3 fatty acids* (pp. 111–129). Woodhead Publishing. https://doi.org/10.1533/9780857098863.2.111

Rodriguez, A. C. (2013). Effects of food processing on disease agents. In J. G. Morris, & M. E. Potter (Eds.), *Foodborne infections and intoxications*

(4th ed., pp. 455–478). Academic Press. https://doi.org/10.1016/B978-0-12-416041-5.00033-0

Romotowska, P. E., Karlsdóttir, M. G., Gudjónsdóttir, M., Kristinsson, H. G., & Arason, S. (2016). Seasonal and geographical variation in chemical composition and lipid stability of atlantic mackerel (*Scomber scombrus*) caught in icelandic waters. *Journal of Food Composition and Analysis*, *49*, 9–18. https://doi.org/10.1016/j.jfca.2016.03.005

Roos, Y. H. (2003). Water activity. Effect on food stability. In B. Caballero (Ed.), *Encyclopedia of food sciences and nutrition* (2nd ed., pp. 6094–6101). Academic Press. https://doi.org/10.1016/B0-12-227055-X/01275-X

Ruxton, C. H. S., Reed, S. C., Simpson, M. J. A., & Millington, K. J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: A review of the evidence. *Journal of Human Nutrition and Dietetics*, *17*, 449–459. https://doi.org/10.1111/j.1365-277X.2004.00552.x

Schmitz, G., & Ecker, J. (2008). The opposing effects of n−3 and n−6 fatty acids. *Progress in Lipid Research*, *47*, 147–155. https://doi.org/10.1016/j.plipres.2007.12.004

Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews*, *39*, 4067–4079. https://doi.org/10.1039/B922183M

Statistics Iceland. (2019). *Profitability in fishing and fish processing 2018* (Statistical Series 104 (14); Statistical Series). Statistics Iceland; ISSN: 1670–4770. https://statice.is/publications/publication-detail/

Stewart, J. C. M. (1980). Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Analytical Biochemistry*, *104*, 10–14. https://doi.org/10.1016/0003-2697(80)90269-9

Tena, N., Lobo-Prieto, A., Aparicio, R., & Gonzalez, D. (2018). *Storage and preservation of fats and oils*. https://doi.org/10.1016/B978-0-08-100596-5.22268-3

Thorkelsson, G., Slizyte, R., Gildberg, A., & Kristinsson, H. G. (2009). Fish proteins and peptides. Processing methods, quality and functionality. In Luten (Ed.), *Marine functional foods*. Wageningen Academic Publishers.

Tocher, D. R. (2015). Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. *Aquaculture*, *449*, 94–107. https://doi.org/10.1016/j.aquaculture.2015.01.010

Tonheim, S. K., Nordgreen, A., Høgøy, I., Hamre, K., & Rønnestad, I. (2007). In vitro digestibility of water-soluble and sater-insoluble protein fractions of some common fish larval feeds and feed ingredients. *Aquaculture*, *262*, 426–435. https://doi.org/10.1016/j.aquaculture.2006.10.030

Ushikubo, F. Y., & Cunha, R. L. (2014). Stability mechanisms of liquid water-in-oil emulsions. *Food Hydrocolloids*, *34*, 145–153. https://doi.org/10.1016/j.foodhyd.2012.11.016

Verrips, C. T., & Zaalberg, J. (1980). The intrinsic microbial stability of water-in-oil emulsions. *European Journal of Applied Microbiology and Biotechnology*, *10*, 187–196. https://doi.org/10.1007/BF00508606

Vilhjálmsson, H. (1994). The Icelandic capelin stock. Capelin, *Mallotus villosus* (Müller), in the Iceland–Greenland–Jan Mayen area. *Marine Research Institute*, *13*, 281.

Vilhjálmsson, H. (2002). Capelin (*Mallotus villosus*) in the Iceland-East Greenland–Jan Mayen ecosystem. *ICES Journal of Marine Science*, *59*, 870–883. https://doi.org/10.1006/jmsc.2002.1233

Windsor, M. L. (2001). Fish protein concentrate [Torrey advisory note nr. 39]. FAO Fisheries and Aquaculture Department. http://www.fao.org/3/x5917e00.htm#Contents

Xiong, Y. L. (1997). Protein denaturation and functionality losses. In M. C. Erickson, & Y.-C. Hung (Eds.), *Quality in frozen food* (pp. 111–140). Springer Science & Business Media. https://doi.org/10.1007/978-1-4615-5975-7

How to cite this article: Hilmarsdottir GS, Ogmundarson Ó, Arason S, Gudjónsdóttir M. Efficiency of fishmeal and fish oil processing of different pelagic fish species: Identification of processing steps for potential optimization toward protein production for human consumption. *J Food Process Preserv*. 2021;45:e15294. https://doi.org/10.1111/jfpp.15294

Paper II

processes

MDPI

Article

The Effects of Varying Heat Treatments on Lipid Composition during Pelagic Fishmeal Production

Gudrun Svana Hilmarsdottir [1,*], Ólafur Ogmundarson [1], Sigurjón Arason [1,2] and María Gudjónsdóttir [1,2]

[1] Faculty of Food Science and Nutrition, University of Iceland; Aragata 14, 102 Reykjavík, Iceland; olafuro@hi.is (Ó.O.); sigurjar@hi.is (S.A.); mariagu@hi.is (M.G.)

[2] Matís ohf, Icelandic Food and Biotech R&D, Vínlandsleið 12, 113 Reykjavík, Iceland

* Correspondence: gsh9@hi.is

Received: 20 August 2020; Accepted: 10 September 2020; Published: 12 September 2020

Abstract: The study aimed to provide insight into the lipid quality of pelagic fishmeal and fish oil processing of mackerel and herring cut-offs, and the effect of temperature changes in the cooker (85–95 °C) during production. Samples were collected after each processing step at a traditional processing line where water and lipid content, free fatty acids (FFA), phospholipids (PL) and fatty acid composition (FAC) were measured. Results showed that the standard procedures at 90 °C included ineffective draining and concentration steps. Moreover, the solid streams entering the driers variated in chemical composition, suggesting that processing each stream separately could be beneficial for maintaining the lipid quality. The cooking temperature affected the lipid removal from the fishmeal processing, where lowering the temperature to 85 °C resulted in a lower lipid content of the final fishmeal, along with lower FFA and PL values. Hence, the fishmeal and fish oil factories could save energy by lowering the cooking temperature, as well as obtaining more stable and higher value products. Further recommendations include more focus on the initial steps for a better homogenization and breakdown of the raw material, as well as investigation of different drying techniques applied on each processing stream entering the drying steps.

Keywords: fishmeal; fish oil; process optimization; heat treatment

1. Introduction

Marine rest raw materials (including remains from main production lines, cut-offs, heads, guts, by-catch, etc.) are a great source of lipids, proteins and minerals and have been used in fishmeal and fish oil production, along with small pelagic species [1]. Fishmeal and fish oil are considered the most nutritious and digestible ingredients for farmed fish, and with no major increases in raw material, any increase in fishmeal production needs to come from byproducts [2]. The current estimate of cut-offs from the main production that enter the fishmeal and fish oil factories is 25–35% of the total volume [2]. Although it is a positive development, the traditional fishmeal and fish oil processes were developed in the 1940s to 1960s [3], and little improvements have been made to the land-based processes since then [1,4–7]. However, applied handling improvements from catch to landing have resulted in higher fishmeal quality [8]. Hence, most of the produced fishmeal remains with a high lipid content, which the Food and Agriculture Organization (FAO) of the United Nations defines as a Type C fish protein concentrate (FPC), or fishmeal processed under sufficient hygienic conditions. Type C FPCs contain rancid lipids that can lower the nutritive value of the proteins, affect the product flavor and odor, and increase the risk of cumulative toxic effects if consumed regularly over a long period [1,9]. However, if the lipid content of the FPC is lowered below 0.75 g/100 g sample, the highest FPC class (Type A) would be reached, allowing improved FPC for human consumption [1,9]. Further

regulations for fishmeal and fish oil come from the Marine Ingredient Organization (IFFO), which is guided by the FAO regulations and accounts for more than 75% of the fishmeal and fish oil trade worldwide [10].

Heating is one of the most critical processing steps in fishmeal and fish oil production, both during cooking and drying. Cooking is the main step intended to separate the lipids and the proteins, making the lipid extractions more efficient at later stages of the processing line. To keep the lipid content low in the final fishmeal, it is important to remove the majority of the lipids early in the process, as the lipids cannot be extracted during drying. Therefore, by cooking the raw material at a temperature where the separation of the lipids and the proteins is the most effective, the lipid content in the fishmeal could be lowered. Moreover, above 90 °C, intermolecular disulfide bonds start to form and protein coagulation thereafter [11], leaving unfavorable interactions with solvent water [12]. Furthermore, cod and herring muscle proteins deform at different temperatures, starting from approximately 30 °C, while most of the proteins were fully unfolded at approximately 90 °C [13]. These results indicate that muscle protein degradation and denaturation is highly dependent on the chosen heat treatment [14].

Iceland's most caught pelagic species are the Atlantic herring (*Clupea harengus*) and capelin (*Mallotus villosus*). However, in recent years, catchings of oceanic redfish (*Sebastes mentella*), blue whiting (*Micromesistius poutassou*), and Atlantic mackerel (*Scomber* scombrus) have increased [15,16]. When the mackerel is caught in Icelandic waters, it has generally been feeding on the zooplankton species *Calanus finmarchicus* [17,18]. *C. finmarchicus* is very rich in enzymes, which can have fast degradative effects on the landed mackerel raw material if not treated properly. Hence, the processing companies tend to behead and gut the mackerel to prolong mackerel shelf life. The heads and guts are collected for fishmeal production along with bycatch and other potentially remaining raw materials. Since it takes a long time to collect the appropriate amount of these side streams prior to process initiation, the raw material must wait several days in the tanks until they are full, and enough material has been collected to initiate the process. This delay prior to operation increases the risk of degradation of the material due to microbial, enzymatic and oxidative processes [19,20]. Moreover, the raw material is highly heterogeneous, as it includes bycatch, heads, viscera, stomach content and damaged whole fish, which is all blended and collected over time. Moreover, as the mackerel catching season overlaps with the herring season, fishmeal is often produced from a mixed catch, i.e., including multiple species, increasing the heterogeneity of the raw material even further as the production pace of fishmeal is too high for separating the catch.

In this context, the aim of this study was to make a detailed investigation of the fishmeal and fish oil production processes of highly diverse and fat raw material from Atlantic herring and Atlantic mackerel and to assess the effects of three different temperatures in the cooker (85 °C, 90 °C and 95 °C) on the final fishmeal and fish oil quality.

2. Materials and Methods

2.1. Raw Materials and Sampling

Fish were caught east and southeast of the Icelandic coast by purse seiners from September 3 to September 6, 2017. The raw materials entering the fishmeal production line weighed in total 885 tons, and consisted of 513 tons (58%) of Atlantic mackerel cut-offs (*Scomber scombrus*), 330 tons (37%) of Atlantic herring cut-offs (*Clupea harengus*) and 40 tons (4.5%) of blue whiting (*Micromesistius poutassou*). Initiation of the fishmeal processing line was three days post catch as the mackerel and herring blend mainly consisted of cut-offs and damaged fish. The production capacity of the factory is around 10 tons per hour of fishmeal, with 1200 tons of raw material entering the production line per day. During September 2017, the average fishmeal production yield was 22.5%, and the fish oil production yield was 17.0%.

An overview of the fishmeal and oil process can be seen in Figure 1. Upon initiation of the fishmeal and fish oil process, the raw material entered a ***pre-heating*** step, where the temperature was kept at

approximately 55 °C for 20 min, followed by a ***cooking step at 85–95 °C*** for 20 min. The pre-heating step is powered by excess steam or condensate from the evaporators and other equipment for better energy efficiency, lowering the energy cost in the fishmeal plant [1]. Next, the raw material was drained before the press to remove excess water. The press liquid was combined with the drained liquid, which both entered a decanter. These liquid streams combined are called the ***separated press liquid***, which was treated both with centrifuges and evaporators to separate the fish oil from the solid streams. Next, a large part of the water was evaporated in a vaporizer before the material entered the drying steps. The solid streams from the press (***press cake***) and the decanter (***sludge***) were combined with the latter ***concentrate*** in a two-step drying process. The first drying step consisted of a rotary disc steam dryer (steam temperature 160 °C, drying temperature 95 °C and duration time 30 ± 5 min). The second drying step was a Hetland air dryer (maximum input air temperature 450 °C, dryer temperature 150 °C at the middle of the dryer, wet bulb temperature of approximately 65 °C and the drying time was 16 ± 2 min). The steam drying decreased the moisture content of the solid streams to approximately 40–50%, while the air dryer reduced the moisture further to approximately 5–10%. Some fine particle meal (***fine meal***) swirled up in the air duct during the air drying. This meal was lighter than the fishmeal and was collected and blended with the rest of the dried meal to make the final ***fishmeal*** product.

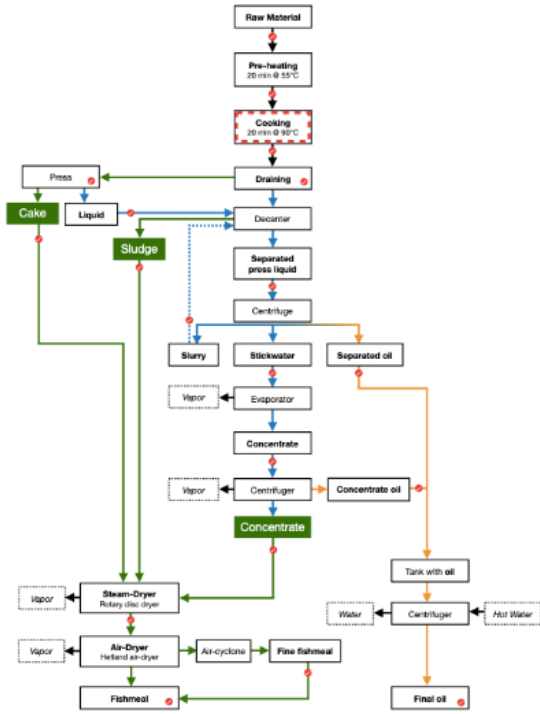


Figure 1. A traditional fishmeal and fish oil processing line. The green color represents the solid streams throughout the processing line, the blue streams identify the liquid streams and the yellow streams represent the oil streams. Red dots indicate sampling points in the production. Green-filled boxes highlight the solid streams entering the drying steps, and a red dashed line highlights the cooking step, which was investigated at three different temperatures.

After a steady state process had been established to produce commercial fishmeal, samples were collected throughout the process, with fishmeal and fish oil as end products. Standard cooking conditions include cooking the raw material at 90 °C for 20 min. However, upon changing the cooking temperatures between 85 °C, 90 °C and 95 °C, samples were collected to investigate the effect of the cooking temperature throughout the production line. All samples were cooled to 0 °C ± 2 °C overnight and transported the following morning to the laboratory, where the samples were kept at −25 °C until analysis, which took up to 6 months. Prior to analysis, samples were thawed at 0–4 °C for 12 h or up to 36 h, depending on the water content and the sample size. Three individual samples (triplicates) were collected at each point to investigate if the production was homogenous. Each triplicate was measured twice to confirm the consistency and reproducibility of the measurements.

2.2. Chemical Analysis

The water content of the samples, except the oil samples, was measured according to ISO 6496 [21]. Water content in the oil samples was measured using calorimetric titration, performed by an 851 Titrando (Metrohm, Herisau, Switzerland). Total lipids (TL)* were extracted and measured [22], and the TL extracts used both to measure enzymatic lipid hydrolysis in the form of free fatty acids (FFA) [23] with modifications [24] and phospholipid (PL) content [25]. In the current study, PL measurements refer to measurements of phosphatidylcholine, as it is the most abundant phospholipid class in the membrane [26]. The fatty acid composition (FAC) of the samples was determined by gas chromatography (Varian 3900 GC, Varian, Inc., Walnut Creek, CA, USA) of fatty acid methyl esters, based on the AOCS Official Method Ce 1b-89 [27], with minor adjustments. Results for water and lipid content, FFA and PL are shown as g/100 g sample. FAC results are presented as g/100 g lipid.

2.3. Statistical Analysis

Data summaries, tables and statistical analyses were performed in Microsoft Office 365 with Excel (Microsoft, Redmond, WA, USA) while one-way ANOVA, Tukey's HSD test and Pearson's correlation were done in RStudio (RStudio Inc., Boston, MA, USA). The significance level was set to $p < 0.05$ for all statistical analyses, and results were shown as mean ± SD from the three triplicates for each sample.

3. Results and Discussion

3.1. Chemical Composition of Mackerel and Herring Blend at Standard Conditions (90 °C)

3.1.1. Water Content Changes during Standard Processing (90 °C)

The water content increased significantly when the raw material (64.6 ± 2.0 g/100 g sample) was pre-heated (74.6 ± 1.8 g/100 g sample) and cooked (72.9 ± 1.0 g/100 g sample) (Figure 2). This increase in water content can be explained by heterogeneous raw material, as the mackerel and herring blend included different-sized mackerel and herring, in addition to mackerel heads and guts. Due to these large variations in the raw material during the initial processing steps, it can be asserted that during processing of multiple side streams and species, the processing line cannot produce a homogenous blend of raw materials during pre-heating and cooking. No additional solvents or liquids of any kind were added during the fishmeal process. After cooking, the material was drained to separate the heated raw material into solid streams (Figure 1, green-colored dashed line) and liquid streams (Figure 1, blue-colored dashed line).

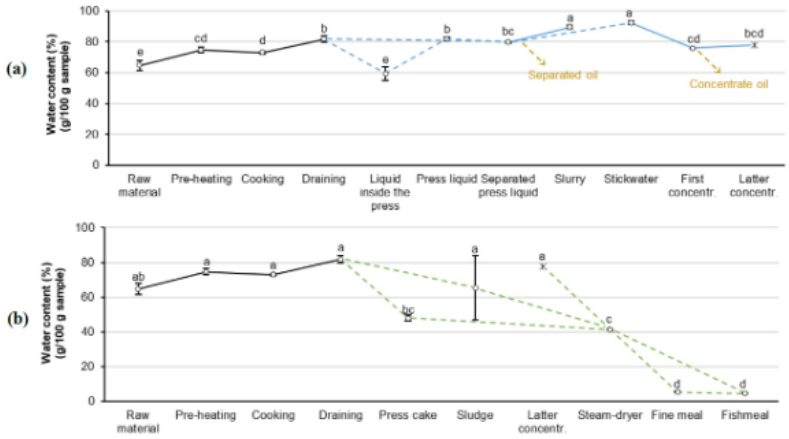


Figure 2. Water content in liquid streams (**a**) and solid streams (**b**) from the traditional fishmeal and fish oil processing facilities shown in Figure 1. Liquid streams are identified by a blue color, solid streams by a green color (**b**) and oil streams by yellow color. A dashed line indicates where the process breaks up into multiple streams or where they join each other again. Solid lines indicate only one possible gateway. Letters indicate significant differences where $p < 0.05$.

The draining did not result in a reduction of water content compared with the following step (separated press liquid) of the liquid stream (Figure 2a), indicating the inefficiency of the draining step. However, after pressing, a significant decrease in water content from 81.8 ± 2.0 g/100 g sample to 47.9 ± 1.6 g/100 g sample was observed, showing effective water removal in the press (Figure 2b). Any remaining solids in the liquid stream were removed in the decanter (sludge), or by centrifugation (slurry). The slurry was recirculated to the decanter because of its high lipid content and large particles. Meanwhile, the stickwater continued throughout the evaporation steps to form the first and second concentrates (Figure 2a). The second concentrate, the sludge (from decanter) and the press cake were then joined in the solid stream and entered the steam dryer. After the steam dryer, the water content had been lowered to 41.5 ± 0.1 g/100 g sample, followed by further drying in an air dryer, resulting in a water content of 4.6 ± 0.2 g/100 g sample in the final fishmeal (Figure 2b).

Interestingly, the fine meal (that swirls up to the drying cylinder) had a significantly higher water content (5.4 ± 0.1 g/100 g sample) than the final fishmeal. The process overview of the water changes indicated that the press and the air dryer were highly effective in removing water from the fishmeal. However, the draining, concentration and evaporation and steam drying steps were ineffective and required optimization.

During the oil extraction process, water was extracted from the oil by centrifugation, resulting in a final oil with a purity of 99.7 g lipid/100 g sample. As microorganisms and enzymes are primarily active in the water phases of biological samples [28,29], a higher water content would increase the risk of oxidation or other degradation of the oil during storage.

3.1.2. Lipid Content during Standard Processing (90 °C)

The raw material had a lipid content of 19.5 ± 2.0 g/100 g sample, which is an intermediate lipid content for mackerel (Figure 3). Mackerel are known to vary in lipid content between catching times, seasons and locations, with an average range between 15–25% [30,31]. In comparison, herring has a lipid content of 5–8% in the white muscle and a 15–20% lipid content in the dark muscle [32]. Herring heads, frames and viscera commonly have a 9–12% lipid content [33]. A negative correlation ($r = -0.66$) was observed between the water and lipid contents of samples over the whole processing line ($p < 0.05$),

which is in agreement with earlier findings both in mackerel [30] and herring [32]. Thus, the significant water content increase observed during these first processing steps was mirrored by a decrease in lipid content. Approximately 59% of the analyzed lipids in the raw material were removed during the cooking and draining steps, indicating that these processing steps serve an important role in overall lipid separation and removal from the solid stream into the liquid stream. However, the separation might become more effective with the use of other cooking temperatures or other appropriate settings, which will be analyzed in Section 3.2.

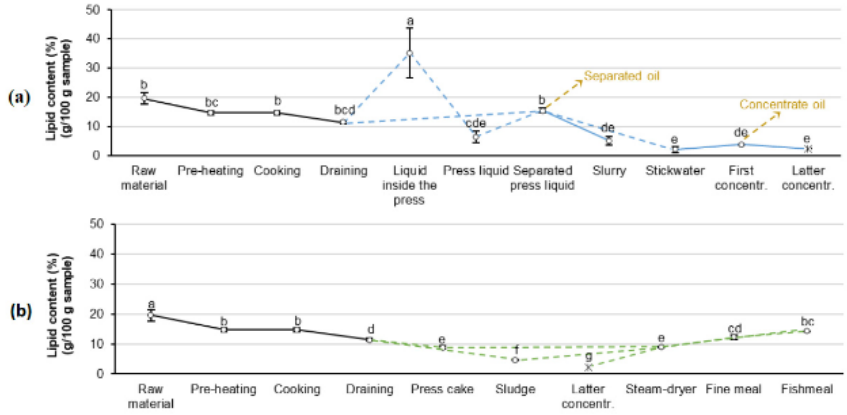


Figure 3. Lipid content in liquid streams (**a**) and solid streams (**b**) from the traditional fishmeal and fish oil processing line in Figure 1. Liquid streams are identified by a blue color, solid streams by a green color (**b**) and oil streams by a yellow color. A dashed line indicates where the process breaks up into multiple streams or where they join each other again. Solid lines indicate only one possible gateway. Letters indicate significant differences where $p < 0.05$.

Minor changes in lipid content in the liquid stream (Figure 3a) between the draining and separated press liquid suggest that the small amount of lipid added from within the press (lipid content of 35.2 ± 8.6 g/100 g sample) only had a minimal effect on the overall lipid content of the separated press liquid (15.5 ± 0.9 g/100 g sample). The separated press liquid was centrifuged and the oil extracted (the separated press oil), hence decreasing the lipid content in both the slurry and the stickwater. The first concentrate (3.9 ± 0.1 g lipid/100 g sample) underwent a similar oil extraction in addition to evaporation, resulting in a lower lipid content in the second concentrate (2.4 ± 0.2 g/100 g sample).

An overall decrease was observed in the lipid content of the solid stream (Figure 3b) throughout the processing line, until reaching the drying steps (8.9 ± 0.2 g lipid/100 g sample). However, after drying, the relative lipid content in the fishmeal increased to 14.3 ± 0.3 g/100 g sample, mainly due to water removal. A significant difference between the fishmeal and the fine meal (12.3 ± 0.8 g/100 g sample) was observed in both the water and lipid contents. The studied fishmeal is considered to be Type C, as it contained a lipid content above 3 g/100 g sample [5,9]. However, high-lipid fishmeal is not uncommon and has been reported before [34], but is hence unsuitable for human consumption, according to the FAO [1,9].

3.1.3. Free Fatty Acids (FFA) during Standard Processing (90 °C)

Although up to three days had passed from the catching of the fish until the fishmeal and oil production was initiated, a relatively low free fatty acid (FFA) content (0.4–0.6 g FFA/100 g sample) was observed in the raw material compared with earlier studies [20,32], indicating that enzymatic degradation of the raw material was not severe upon processing. Measured FFA values (Figure 4) were

close to the reported values of a dark herring muscle [32] and, taking into account easier exposure to oxygen for blended cut-offs compared with a part of a muscle, the FFA values are considered relatively low. Keeping FFA values low when catching mackerel can be difficult as its stomach is often full of the enzyme-rich zooplankton *Calanus finmarchicus* [6,17]. Along with gastric enzymes, these enzymes can initiate severe raw material degradation if not treated properly [6,17].

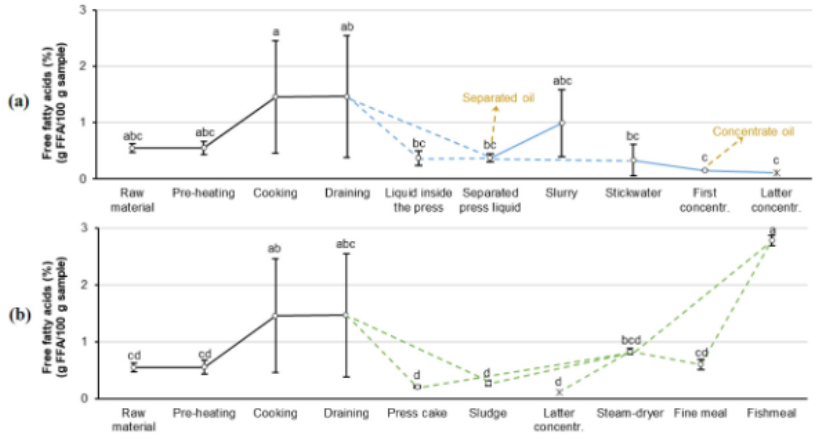


Figure 4. Free fatty acid (FFA) content in liquid streams (**a**) and solid streams (**b**) from the traditional fishmeal and fish oil processing line in Figure 1. Liquid streams are identified by a blue color, solid streams by a green color (**b**) and oil streams by a yellow color. A dashed line indicates where the process breaks up into multiple streams or where they join each other again. Solid lines indicate only one possible gateway. Letters indicate significant differences where $p < 0.05$.

The raw materials studied had higher FFA values than fresh mackerel muscle [31], including the effects of seasonal changes. The different parts of mackerel have been reported to have <0.7, <0.5 and <0.4 g FFA/100 g lipid in dark muscle, light muscle and whole mackerel, respectively, if stored at 4 °C for 4 days [20]. The dark muscle of herring has been shown to contain ~2.0 g FFA/100 g lipid and the light muscle ~1.1 g FFA/100 g lipid [32]. The FFA content in the raw material was 2.8 ± 0.7 g FFA/100 g lipid, or 0.5 ± 0.1 g FFA/100 g sample. This implies that the delay before processing enables the activation of enzymes and microbiological spoilage, which induces the formation of FFA through degradation of the raw material and should be kept as short as possible.

Free fatty acids (FFA) were also measured in the liquid, solid and oil streams during processing (Figure 4). Most of the FFA were, however, observed in the solid stream samples, peaking in the final fishmeal (19.3 ± 0.4 g FFA/100 g lipid).

The large standard deviations in FFA during cooking and draining can be explained by the heterogeneous nature of the raw material and the (yet) unoptimized production line. However, the FFA content remained relatively low in the press cake, sludge and latter concentrate, which all contained less than 0.35 g FFA/100 g sample. FFA levels were expected to rise during the concentration of aqueous materials, as observed during drying of the solid streams, but not between the concentration steps [35]. During the air drying, the water content decreased from 41.5 ± 0.1 g/100 g sample down to 4.6 ± 0.2 g/100 g sample, while the lipid content became a proportionally larger part of the sample. Hence, it is not surprising to observe an increase in FFA formation during drying, due to the high temperatures [36] and the raw materials' exposure to oxygen [37]. The FFA content in the fine meal and the fishmeal were 4.3 ± 0.2 g/100 g lipid and 19.3 ± 0.4 g/100 g lipid, respectively. Hence, the lipids of the fine meal were less hydrolyzed or denatured compared with the fishmeal, although the fine meal was lower in water content.

Published results from anchovy meal, processed directly after landing, showed an FFA content of 6.8 g FFA/100 g lipid [38], which is lower than the commercial fishmeal obtained in this study (19.3 ± 0.4 g FFA/100 g lipid). Hence, it is suggested that the processing of fishmeal and fish oil should not wait three days as in the current study. Lower FFA values could be reached by storing the raw material at 2–3 °C or even lower during the wait, or by removing the dark muscle and viscera from the raw material [39].

Moreover, the hydrolysis of PL seems to be a contributor to the increase in FFA if the raw material is not heated and the lipid hydrolysis inactivated [39]. It is reported that the primary cause for FFA escalation in fish oils is contamination by bacteria (genus *Alcaligenes*) which thrives at the oil–water interface, located at the bottom of oil storage tanks, and converts the phospholipids into oil-soluble FFA and water-soluble phosphate esters [35]. As the oil-soluble FFA have a lower density than the oil, they disperse upwards and hence contaminate the oil [35]. In the present study, the final oil contained 0.06 g FFA/100 g lipid, which is within acceptable margins of FFA values of fish oil intended for human consumption [1]. However, the oil would still need to undergo an additional refining process composed of deacidification, transesterification, concentration, deodorization and earth treatment, antioxidant addition and fill off to be considered for human consumption [1,40], in addition to FFA levels below 0.1 g FFA/100 g lipid [1].

3.1.4. Phospholipids (PL) during Standard Processing (90 °C)

Phospholipids (mostly phosphatidylcholine [26]) were measured throughout the processing line (Figure 5). Phospholipid values in the raw material were 0.9 ± 0.2 g PL/100g lipid, which is in good agreement with PL values in other pelagic species, as earlier studies have reported PL values ranging between 1.5–3.0 g PL/100 g lipid in herring and 0.7–4.0 g PL/100 g lipid in mackerel [31,32]. Upon cooking and draining, PL were almost non-existent in the liquid streams (Figure 5a).

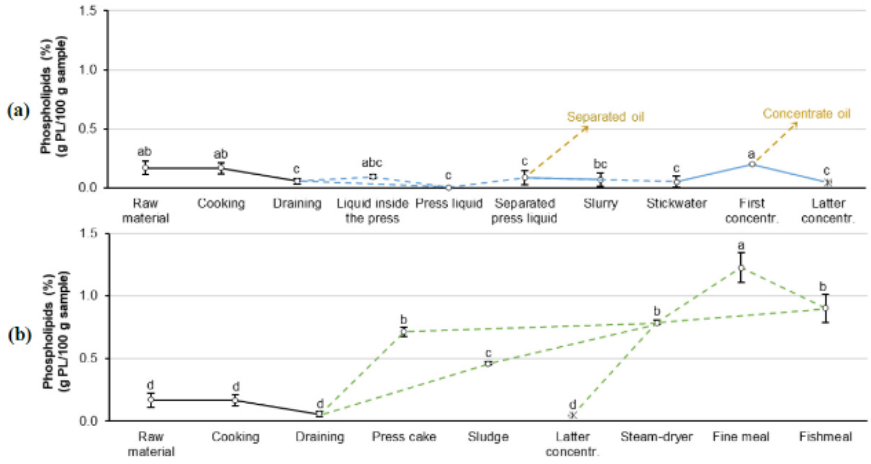


Figure 5. Phospholipid content in liquid streams (**a**) and solid streams (**b**) from the traditional fishmeal and fish oil processing line in Figure 1. Liquid streams are identified by a blue color, solid streams by a green color (**b**), and oil streams by a yellow color. A dashed line indicates where the process breaks up into multiple streams or where they join each other again. Solid lines indicate only one possible gateway. Letters indicate significant differences where $p < 0.05$.

When analyzing the PL content in the solid stream (Figure 5a), a clear increasing trend in PL content was observed after pressing and after the two drying steps. The trend was in agreement with the water removal and potential heat-induced lipid denaturation occurring during these processing

steps, increasing the relative phospholipid concentrations in the fishmeal [35,41]. Interestingly, the PL content of the fine meal in the air-drier was higher than the PL content of the final fishmeal, although the fine meal had a higher water content than the final fishmeal. The PL content changes during drying showed on a lipid basis that the fine meal had a significantly higher PL content (8.9 ± 0.1 g PL/100 g lipid) compared to the final meal (6.2 ± 0.8 g PL/100 g lipid). Since the same trend was seen both on a sample and a lipid basis, this difference cannot be explained by the changes in water content alone, but indicates a difference in the lipid composition of the two meal types. Moreover, the finer particle size of the fine meal would result in a proportionally higher surface area to volume ratio (A/V ratio) of the particles. This increased A/V ratio could increase the availability of microorganisms, oxidizing agents and other degrading factors to the components of the fine meal, compared with the final fishmeal. Hence, the question arises whether the fine meal should be mixed with the final fishmeal or not. Further processing of the solid streams individually (the press cake, sludge and the latter concentrate) might be beneficial to achieve better control of the characteristics of the final products, which in turn would result in both lower energy use and higher quality for each product.

3.1.5. Fatty Acid Composition (FAC) during Standard Processing (90 °C)

The fatty acid composition (FAC) of the samples was analyzed to give an overview of any compositional changes in the lipids during processing (Table 1). The overall FAC profile during processing was generally dominated by monounsaturated fatty acids (MUFA, 38.3–53.7 g/100 g lipid), followed by polyunsaturated fatty acids (PUFA, 13.3–34.6 g/100 g lipid), while containing a fairly low concentration of saturated fatty acids (SFA, 21.4–30.9 g/100 g lipid). The FAC of pelagic fish is highly dependent on the season and the place of the catch [30]. Lower levels of MUFA have been reported in mackerel (32.9 ± 1.4 g MUFA/100 g lipid) [30] compared with the raw material in the current study (42.2 ± 2.6 g MUFA/100 g lipid), while higher values of MUFA have been reported in herring (51.9 ± 0.4 g MUFA/100 g lipid) [32] than in the current study. The same trend was observed with SFA. As the raw material consisted of both herring and mackerel, these results could be expected. However, PUFA values of the raw material in the current study (31.4 ± 2.5 g PUFA/100 g lipid) are reported closer to mackerel (33.8 ± 0.8 g PUFA/100 g lipid) [30] than to herring (22.6 ± 0.6 g PUFA/100 g lipid) [32].

The solid streams entering the drying steps—the press cake, the sludge and the concentrate—differed significantly in FAC. The stickwater and the concentrate shared a similar FAC, which is not surprising as the stickwater is the precursor of the concentrate in the fishmeal processing line. The differences in FFA and PL in the fine meal and fishmeal could not be explained by differences in their fatty acid compositions. However, processing may change the structures or forms of the lipid molecules, as proteins associated with membranes are likely to be affected or influenced in their lipid environment [42], such as changing from the bilayer to the more stable micellar form [26]. Such structural changes could explain the availability of the FFA and PL for analysis in the fine meal compared with the final fishmeal. Such structural changes would, on the other hand, not influence the FAC as such, explaining the similar fatty acids content of the two fishmeal samples.

Both the final fishmeal and oil had PUFA and MUFA contents over 74 g/100 g lipid, including 23–28 g/100 lipid $n-3$ PUFA and a beneficial $n-3/n-6$ ratio of 3.2 ± 0.0 and 4.9 ± 0.1 in the fish oil and fishmeal, respectively. These fatty acid profiles are beneficial for various health effects and may decrease the risk of cardiovascular and coronary vascular diseases [43], prevent the development of breast cancer [44] and prostate cancers [45] and prevent obesity [46]. Although these fatty acid profiles are attractive, the high MUFA and PUFA content makes the products highly susceptible to lipid oxidation [47]. Increased lipid oxidation in food could, in turn, cause toxicity of the lipids as well as the secondary products, as the secondary products are more toxic than hydroperoxides [48].

Table 1. Fatty acid composition of samples from the fishmeal and fish oil processing line presented in Figure 1, with 90 °C temperature in the cooker. Results are presented as g fatty acid/100 g lipid as mean ± SD ($n = 3$) and are compared vertically within each column.

Mackerel and Herring Blend at 90 °C	Lipid Content	SFA	MUFA	PUFA	EPA	EPA/DHA	$n-3$ PUFA	$n-3/n-6$
Raw material	**19.5 ± 2.0** ^{c}	**23.0 ± 0.5** ^{cde}	**42.2 ± 2.55** ^{f}	**31.4 ± 2.5** ^{abc}	*8.7 ± 0.4* ^{a}	**0.75 ± 0.0** ^{abc}	**23.6 ± 1.7** ^{c}	**3.3 ± 0.2** ^{ef}
Pre-heating	14.6 ± 0.6 ^{cd}	23.1 ± 0.4 ^{cde}	42.4 ± 1.0 ^{f}	31.6 ± 0.5 ^{abc}	8.4 ± 0.6 ^{a}	0.7 ± 0.0 ^{cd}	23.9 ± 0.7 ^{bc}	3.5 ± 0.2 ^{cdef}
Cooking	14.7 ± 0.7 ^{cd}	22.4 ± 0.3 ^{cde}	42.2 ± 0.7 ^{f}	31.8 ± 0.6 ^{abc}	8.3 ± 0.1 ^{ab}	0.7 ± 0.0 ^{d}	23.9 ± 0.5 ^{bc}	3.4 ± 0.0 ^{def}
Draining	11.4 ± 0.5 ^{de}	21.5 ± 0.1 ^{de}	46.7 ± 0.4 ^{cd}	28.9 ± 0.3 ^{c}	7.6 ± 0.1 ^{ab}	0.7 ± 0.0 ^{d}	21.9 ± 0.2 ^{c}	3.4 ± 0.0 ^{def}
Liquid inside the press	35.2 ± 8.6 ^{b}	22.6 ± 0.1 ^{cde}	43.2 ± 0.4 ^{ef}	30.2 ± 0.4 ^{bc}	7.6 ± 0.1 ^{ab}	0.6 ± 0.0 ^{ef}	23.6 ± 0.25 ^{c}	4.2 ± 0.0 ^{b}
Separated press liquid	15.5 ± 0.9 ^{cd}	22.0 ± 0.2 ^{cde}	44.3 ± 0.4 ^{def}	30.1 ± 0.5 ^{bc}	8.2 ± 0.2 ^{ab}	0.8 ± 0.0 ^{ab}	22.4 ± 0.4 ^{c}	3.2 ± 0.0 ^{f}
Press cake	8.8 ± 0.6 ^{def}	28.2 ± 1.8 ^{b}	49.0 ± 1.1 ^{bc}	19.5 ± 3.1 ^{d}	4.6 ± 0.8 ^{c}	0.6 ± 0.0 ^{efg}	14.9 ± 2.7 ^{d}	3.8 ± 0.2 ^{bcd}
Sludge	4.7 ± 0.2 ^{ef}	30.9 ± 0.3 ^{a}	53.7 ± 0.4 ^{a}	13.3 ± 0.7 ^{e}	2.8 ± 0.2 ^{d}	0.6 ± 0.0 ^{fg}	9.6 ± 0.6 ^{e}	3.3 ± 0.3 ^{f}
Stickwater	2.1 ± 1.0 ^{f}	23.8 ± 0.0 ^{c}	45.1 ± 0.9 ^{def}	28.3 ± 1.7 ^{c}	7.1 ± 0.7 ^{b}	0.6 ± 0.0 ^{ef}	22.0 ± 1.5 ^{c}	3.8 ± 0.1 ^{bcde}
Latter concentrate	3.9 ± 0.1 ^{f}	21.4 ± 0.1 ^{e}	45.6 ± 0.1 ^{de}	28.9 ± 0.2 ^{c}	7.7 ± 0.0 ^{ab}	0.7 ± 0.0 ^{bcd}	21.6 ± 0.2 ^{c}	3.4 ± 0.0 ^{def}
Steam-dryer	8.9 ± 0.2 ^{def}	28.0 ± 1.2 ^{b}	50.3 ± 2.0 ^{b}	19.3 ± 2.9 ^{d}	4.6 ± 0.8 ^{c}	0.6 ± 0.0 ^{e}	16.8 ± 2.9 ^{d}	3.9 ± 0.3 ^{bc}
Fine meal	12.3 ± 0.8 ^{d}	23.4 ± 0.2 ^{c}	38.8 ± 0.7 ^{g}	34.1 ± 0.6 ^{ab}	8.4 ± 0.1 ^{a}	0.5 ± 0.0 ^{g}	27.6 ± 0.6 ^{ab}	4.8 ± 0.1 ^{a}
Fishmeal	**14.3 ± 0.2** ^{cd}	**23.3 ± 0.3** ^{cd}	**38.3 ± 0.6** ^{g}	**34.6 ± 0.7** ^{a}	**8.6 ± 0.1** ^{a}	**0.5 ± 0.0** ^{g}	**28.0 ± 0.7** ^{a}	**4.9 ± 0.1** ^{a}
Final oil	**99.7 ± 0.1** ^{a}	**22.0 ± 0.1** ^{cde}	**43.7 ± 0.2** ^{def}	**31.3 ± 0.4** ^{abc}	**8.6 ± 0.1** ^{a}	**0.8 ± 0.0** ^{a}	**23.2 ± 0.3** ^{c}	**3.2 ± 0.0** ^{f}

Abbreviations: SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), $n-3$ PUFA (omega-3 PUFA) and $n-3/n-6$ (ratio between omega-3 and omega-6 fatty acids).$^{a-i}$ Letter indicates a significant difference between vertical results, where $p < 0.05$.

3.2. *Effect of Different Cooking Temperatures*

The analysis of the standard 90 °C process indicated that the current fishmeal processing line requires optimization, as shown in earlier sections. As a result of the inefficient breakdown of the raw material during the initial steps of the processing line, the final fishmeal was too high in lipid content. As muscle denaturation and degradation is highly dependent on the heat treatment chosen [14], it can be suggested that optimizations of the temperature in the cooker might obtain better separation between the lipid content and dry matter. Moreover, optimal cooking conditions have been questioned, where a minimum of 20 min above 70 °C for wild fish has been recommended [49], or 20 min at 75 °C [1] for optimal results, questioning the 95–100 °C cooking temperatures recommended by the FAO [5]. However, these temperatures aim primarily for the inactivation of parasites, viruses and bacteria and do not necessarily take separation of lipids and proteins into the equation. The second objective of the study was therefore to investigate the effect of different cooking temperatures (85 °C, 90 °C and 95 °C) on the water, lipid, FFA and PL composition on chosen sampling points throughout processing (Figure 6).

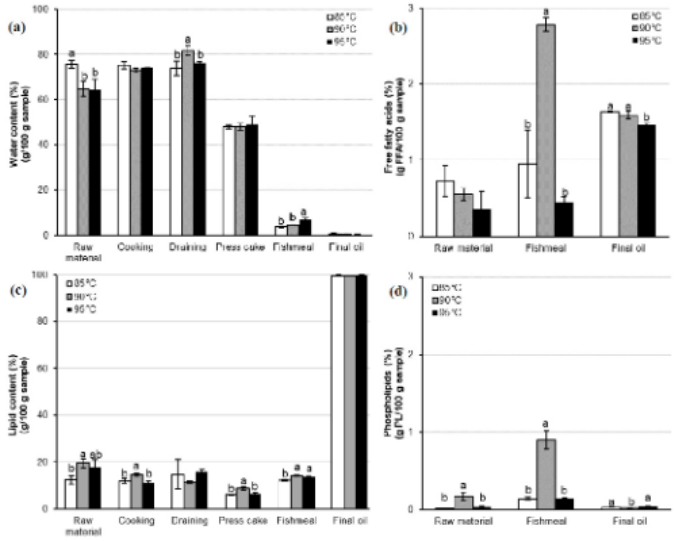


Figure 6. Measurements of water content (**a**); free fatty acids (**b**); lipid content (**c**); and phospholipids (**d**) from a traditional fishmeal and fish oil production line, presented in Figure 1 with a different temperature applied in the cooker (85 °C, 90 °C and 95 °C). All data is presented as a g/100 g sample as mean ± SD (n = 3).

Variations in the chemical composition of the raw material were observed between the three temperature runs, indicating that the raw material was highly heterogeneous. However, no significant differences were observed in the water content, and the variations in the lipid content decreased after cooking. After draining, the samples that underwent the 90 °C cooking were significantly higher in water content, although this was not reflected through the rest of the process. No systematic changes were seen in the water, lipid or FFA content through processing in relation to the observed variation in the chemical content of the raw material, indicating that any observed changes were indeed an effect of the processing treatments.

The water content of the fishmeal samples cooked at 85 °C and 90 °C were significantly lower than the fishmeal treated with 95 °C cooking. Furthermore, the fishmeal cooked at 85 °C was lower

in lipid content compared with the fishmeal samples treated at the other temperatures. Since low water and lipid content is beneficial for the stability of fishmeal, a processing temperature of 85 °C is recommended. No significant differences were observed in the water or lipid composition of the final oil between the three heat treatments.

When looking further at FFA and PL, the FFA concentrations were significantly higher in the fishmeal heated to 90 °C, while no significant differences in FFA and PL were seen in the final fishmeal or the fish oil at 85 °C and 95 °C treatments. Although slightly lower FFA were observed in the final oil at 95 °C, compared to the other heat treatments, this difference is too small to justify a recommendation of applying 95 °C heating.

Overall, the best results were obtained by lowering the temperature to 85 °C, resulting in a fishmeal of low water and lipid content, as well as low FFA and PL content. Lower PL content indicates a more efficient breakdown of the raw material. Higher temperature treatments are likely to denature proteins to a greater extent [13], decreasing their quality and, therefore, also their application possibilities for human consumption. Analysis of the protein quality changes during processing is, however, a matter for a later study.

4. Conclusions

Analysis of the Atlantic mackerel raw material indicated that, although up to three days had passed from catch to processing, the raw material was at a good lipid quality. Large variations in raw material characteristics may, though, make processing problematic and less homogeneous, and long delays between catch and processing may increase such raw material quality variations. It might, therefore, be beneficial for the processing companies to shorten any processing delays to open the possibility of producing higher quality fishmeal and fish oil products. Currently, several companies own trawlers that process the fishmeal onboard directly from catching [1], which could eliminate the processing delay.

One of the main problems of pelagic fishmeal production lies in the high lipid content of the raw material and problems in lipid removal from the fishmeal. Detailed analysis of the chemical changes during processing revealed that the solid streams entering drying have different chemical compositions. Hence, different processing is suggested depending on the characteristics of each stream, such as different drying times for the press cake (50% water) and the latter concentrate (80% water). Moreover, all the solid streams entering the dryers were too high in lipid content, meaning that the initial breakdown of the raw material was not sufficient. Furthermore, the standard process at 90 °C revealed poor effectiveness of water removal during draining, as well as an increase of FFA and PL during the steam and air drying steps of the fishmeal, indicating that the process required optimization.

During the analysis of different cooking temperatures in the mackerel and herring blend, it was evident that the cooking steps had a highly important role in the lipid removal from the fishmeal processing. By lowering the temperature in the heater to 85 °C, the water and lipid content of the fishmeal was lowered, as well as contributing to lower the FFA and PL values, indicating the production of a more stable product at 85 °C compared with the standard 90 °C. Moreover, the PL values were lower at 85 °C, indicating a more efficient breakdown of the raw material. In addition to a higher quality fishmeal, energy costs can be decreased, as fishmeal and fish oil factories are operating with a cooker at temperatures up to 95–100 °C. Moreover, performing a life cycle assessment (LCA) is suggested to investigate the environmental impact of the processing. Lowering the temperature in the heater to 85 °C can therefore be recommended.

Further recommendations include investigation of ways to break down the raw material more efficiently during the first steps in the production line, which could be applied in commercial fishmeal and fish oil factories. As the diversity of the fish protein is high, as well as the volume, a possible solution for homogenizing the raw material is applying enzymatic technology, but fish protein hydrolysates are currently being produced industrially [50]. Drying affected the FFA concentrations and, hence,

optimizing the drying of the different solid streams is recommended to receive the highest value possible and open up the possibility of producing products intended for human consumption.

Author Contributions: Conceptualization, G.S.H., Ó.O., S.A., and M.G.; methodology, G.S.H., S.A., and M.G.; software, G.S.H.; validation, G.S.H.; formal analysis, G.S.H.; investigation, G.S.H; resources, S.A. and M.G.; data curation, G.S.H.; writing—original draft preparation, G.S.H.; writing—review and editing, G.S.H., Ó.O., S.A., and M.G.; visualization, G.S.H., Ó.O., S.A., and M.G.; supervision, Ó.O., S.A., and M.G.; project administration, S.A. and M.G.; funding acquisition S.A. and M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the AVS (The Added Value of Seafood) fund of the Ministry of Fisheries and Agriculture in Iceland (grant number: R18 031-18), the Rannís Technology Development Fund (no. 198883-0611), and the University of Iceland research fund.

Acknowledgments: The work was carried out at the University of Iceland and Matís ohf. The authors thank Síldarvinnslan hf. for access to their facilities, assistance, and raw materials.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Einarsson, M.I.; Bæk, A.M.; Eliasen, O.; Flesland, O.; Jacobsen, C.; Jokumsen, A.; Pálsson, J.; Pedersen, S.A.; Samuelsen, T.A. Nordic center of excellence network in fishmeal and fish oil. *Matís ohf Reykjavík* **2019**. ISSN 1670-7192. [CrossRef]
2. FAO. *The State of World Fisheries and Aquaculture 2020: Sustainability in Action*; FAO: Rome, Italy, 2020.
3. Kose, S. On-board fish processing. In *Fish. Processing*; Hall, G.M., Ed.; Wiley-Blackwell: Oxford, UK, 2010; pp. 167–206.
4. Bimbo, A.P.; Crowther, J.B. Fish meal and oil: Current uses. *J. Am. Oil Chem. Soc.* **1992**, *69*, 221–227. [CrossRef]
5. FAO. *The Production of Fish Meal and Oil*; Fisheries Technical Paper–142; FAO: Rome, Italy, 1986; ISBN 92-5-102464-2. Available online: http://www.fao.org/3/X6899e/X6899E00.HTM (accessed on 17 December 2019).
6. Hall, G.M. Fishmeal production and sustainability. In *Fish. Processing*; Hall, G.M., Ed.; Wiley-Blackwell: Oxford, UK, 2010; pp. 207–235.
7. Oterhals, Å.; Vogt, G. Impact of extraction, refining and concentration stages on the stability of fish oil. In *Food Enrichment with Omega-3 Fatty Acids*; Jacobsen, C., Nielsen, N.S., Horn, A.F., Sørensen, A.-D.M., Eds.; Woodhead Publishing: Cambridge, UK, 2013; pp. 111–129.
8. Bao, H.N.D.; Arason, S.; Þórarinsdóttir, K.A. Effects of dry ice and superchilling on quality and shelf life of arctic charr (Salvelinus alpinus) fillets. *Int. J. Food Eng.* **2007**, 3. [CrossRef]
9. Windsor, M.L. Fish Protein Concentrate. FAO Fisheries and Aquaculture Department 2001. Available online: http://www.fao.org/3/x5917e00.htm#Contents (accessed on 7 February 2020).
10. IFFO. Representing and promoting the fishmeal and fish oil industry worldwide. IFFO–The Marine Ingredients Organisation, 2020. Available online: https://www.iffo.com/about-us (accessed on 6 September 2020).
11. Lee, Y.-H. Food-processing approaches to altering allergenic potential of milk-based formula. *J. Pediatrics* **1992**, *121*, S47–S50. [CrossRef]
12. Davis, P.; Williams, C. Protein modification by thermal processing. *Allergy* **1998**, *53*, 102–105. [CrossRef]
13. Hastings, R.J.; Rodger, G.W.; Park, R.; Matthews, A.D.; Anderson, E.M. Differential scanning calorimetry of fish muscle: The effect of processing and species variation. *J. Food Sci.* **1985**, *50*, 503–506. [CrossRef]
14. Fernandez-Segovia, I.; Camacho, M.M.; Martinez-Navarrete, N.; Escriche, I.; Chiralt, A. Structure and color changes due to thermal treatments in desalted cod. *J. Food Process. Preserv.* **2003**, *27*, 465–474. [CrossRef]
15. ICES. *Report of the Working Group on Widely Distributed Stocks (WGWIDE)*; ICES Headquarters: Copenhagen, Denmark, 2011; ICES CM 2011/ACOM:15.
16. Statistics Iceland. Hagstofa Íslands, 2020. Available online: https://px.hagstofa.is/pxis/pxweb/is/Atvinnuvegir/Atvinnuvegir__sjavarutvegur__aflatolur__londunartegundir/SJA09032.px/chart/chartViewLine/?rxid=fdd9c170-2fa8-4e84-9160-9add4325660d (accessed on 20 January 2020).
17. Eysteinsson, S.T.; Gudjónsdóttir, M.; Jónasdóttir, S.H.; Arason, S. Review of the composition and current utilization of Calanus finmarchicus–Possibilities for human consumption. *Trends Food Sci. Technol.* **2018**, *79*, 10–18. [CrossRef]

18. Prokopchuk, I.; Sentyabov, E. Diets of herring, mackerel, and blue whiting in the Norwegian sea in relation to Calanus finmarchicus distribution and temperature conditions. *ICES J. Mar. Sci.* **2006**, *63*, 117–127. [CrossRef]
19. Ocaño-Higuera, V.M. Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. *Food Chem.* **2011**, *125*, 49–54. [CrossRef]
20. Standal, I.B.; Mozuraityte, R.; Rustad, T.; Alinasabhematabadi, L.; Carlsson, N.-G.; Undeland, I. Quality of filleted Atlantic mackerel (Scomber scombrus) during chilled and frozen storage: Changes in lipids, vitamin D, proteins, and small metabolites, including biogenic amines. *J. Aquat. Food Prod. Technol.* **2018**, *27*, 38–357. [CrossRef]
21. ISO. *Animal Feeding Stuffs*; International Organization for Standardization: Geneva, Switzerland, 1999; Available online: https://www.iso.org/obp/ui/#iso:std:iso:6496:ed-2:v1:en (accessed on 10 March 2020).
22. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917. [CrossRef] [PubMed]
23. Lowry, R.R.; Tinsley, I.J. Rapid colorimetric determination of free fatty acids. *J. Am. Oil Chem. Soc.* **1976**, *53*, 470–472. [CrossRef]
24. Bernárdez, M.; Pastoriza, L.; Sampedro, G.; Herrera, J.J.R.; Cabo, M.L. Modified method for the analysis of free fatty acids in fish. *J. Agric. Food Chem.* **2005**, *53*, 1903–1906. [CrossRef] [PubMed]
25. Stewart, J.C.M. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Anal. Biochem.* **1980**, *104*, 10–14. [CrossRef]
26. Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *The Lipid Bilayer. Molecular Biology of the Cell*, 4th ed; Garland Science: New York, NY, USA, 2002.
27. AOCS. *Official Method Ce 1b-89. Official Method and Recommended Practices of the AOC*, 7th ed.; American Oil Chemists' Society: Champaign, IL, USA, 1998.
28. Rodriguez, A.C. Effects of food processing on disease agents. In *Foodborne Infections and Intoxications*, 4th ed.; Morris, J.G., Potter, M.E., Eds.; Academic Press: San Diego, CA, USA, 2013; pp. 455–478.
29. Roos, Y.H. Water activity. Effect on food stability. In *Encyclopedia of Food Sciences and Nutrition*, 2nd ed.; Caballero, B., Ed.; Academic Press: Oxford, UK, 2013; pp. 6094–6101.
30. Romotowska, P.E.; Karlsdóttir, M.G.; Gudjónsdóttir, M.; Kristinsson, H.G.; Arason, S. Seasonal and geographical variation in chemical composition and lipid stability of Atlantic mackerel (Scomber scombrus) caught in Icelandic waters. *J. Food Compos. Anal.* **2016**, *49*, 9–18. [CrossRef]
31. Romotowska, P.E.; Karlsdóttir, M.G.; Gudjónsdóttir, M.; Kristinsson, H.G.; Arason, S. Influence of feeding state and frozen storage temperature on the lipid stability of Atlantic mackerel (Scomber scombrus). *Int. J. Food Sci. Technol.* **2016**, *51*, 1711–1720. [CrossRef]
32. Dang, H.T.T.; Gudjónsdóttir, M.; Karlsdóttir, M.G.; Van Nguyen, M.; Romotowska, P.E.; Tómasson, T.; Arason, S. Influence of temperature stress on lipid stability of Atlantic herring (Clupea harengus) muscle during frozen storage. *J. Am. Oil Chem Soc.* **2017**, *94*, 1439–1449. [CrossRef]
33. Hinchcliffe, J.; Carlsson, N.G.; Jönsson, E.; Sundell, K.; Undeland, I. Aquafeed ingredient production from herring (Clupea harengus) by-products using pH-shift processing: Effect from by-product combinations, protein solubilization-pH and centrifugation force. *Anim. Feed Sci. Technol.* **2019**, *247*, 273–284. [CrossRef]
34. de Koning, A.J. Quantitative quality tests for fish meal. II. An investigation of the quality of South African fish meals and the validity of a number of chemical quality indices. *Int. J. Food Prop.* **2002**, *5*, 495–507. [CrossRef]
35. de Koning, A.J. Properties of South African fish oils: A review. *Int. J. Food Prop.* **1999**, *2*, 205–216. [CrossRef]
36. Fellows, P. *Food Processing Technology: Principles and Practice*, 3rd ed.; Ellis Horwood: Chichester, UK; VHC: Weinheim, Germany, 1988.
37. Jacobsen, C. Enrichment of foods with omega-3 fatty acids: A multidisciplinary challenge. *Ann. N. Y. NY Acad. Sci.* **2010**, *1190*, 141–150. [CrossRef] [PubMed]
38. de Koning, A.J.; Milkovitch, S.; Fick, M.; Wessels, J.P.H. The free fatty acid content of fish oil. An analysis of anchovy lipids at different stages in the manufacture of anchovy meal and oil. *Fette Seifen Anstrichm.* **1986**, *88*, 404–406. [CrossRef]
39. Hwang, K.T.; Regenstein, J.M. Characteristics of mackerel mince lipid hydrolysis. *J. Food Sci.* **1993**, *58*, 79–83. [CrossRef]

40. EFSA. Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. *EFSA J.* **2010**, *8*, 1874. [CrossRef]
41. Šližytė, R.; Rustad, T.; Storrø, I. Enzymatic hydrolysis of cod (Gadus morhua) by-products: Optimization of yield and properties of lipid and protein fractions. *Process Biochem.* **2005**, *40*, 3680–3692. [CrossRef]
42. Jenski, L.J. Omega-3 fatty acids and the expression of membrane proteins: Emphasis on molecules of immunologic importance. In *Current Organic Chemistry*; Bentham Science Publishers: Sharjah, UAE, 2000; pp. 1185–1200.
43. Larsen, R.; Eilertsen, K.-E.; Elvevoll, E.O. Health benefits of marine foods and ingredients. *Biotechnol. Adv.* **2011**, *29*, 508–518. [CrossRef]
44. Zheng, J.-S.; Hu, X.-J.; Zhao, Y.-M.; Yang, J.; Li, D. Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: Meta-analysis of data from 21 independent prospective cohort studies. *BMJ* **2013**, *346*, f3706. [CrossRef]
45. Berquin, I.M.; Min, Y.; Wu, R.; Wu, J.; Perry, D.; Cline, J.M.; Thomas, M.J.; Thornburg, T.; Kulik, G.; Smith, A.; et al. Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. *J. Clin. Investig.* **2007**, *117*, 1866–1875. [CrossRef]
46. Gunnarsdottir, I.; Tomasson, H.; Kiely, M.; Martinéz, J.A.; Bandarra, N.M.; Morais, M.G.; Thorsdottir, I. Inclusion of fish or fish oil in weight-loss diets for young adults: Effects on blood lipids. *Int. J. Obes.* **2008**, *32*, 1105–1112. [CrossRef]
47. Jacobsen, C. Some strategies for the stabilization of long chain n-3 PUFA-enriched foods: A review. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 1853–1866. [CrossRef]
48. Erickson, M.C. Lipid oxidation: Flavor and nutritional quality deterioration in frozen foods. In *Quality in Frozen Food*; Erickson, M.C., Hung, Y.-C., Eds.; Springer: Boston, MA, USA, 1997; pp. 141–173.
49. Nygaard, H. Standard Norwegian fishmeal- and fishoil process. In *Heat Treatment Requirements*; Nofima Marine AS: Tromsø, Norway, 2010.
50. Thorkelsson, G.; Slizyte, R.; Gildberg, A.; Kristinsson, H.G. Fish proteins and peptides. Processing methods, quality and functionality. In *Marine Functional Foods*; Academic Publishers: Wageningen, The Netherlands, 2009.

Paper III

processes

MDPI

Article

Changes in Protein and Non-Protein Nitrogen Compounds during Fishmeal Processing—Identification of Unoptimized Processing Steps

Hang Thi Nguyen [1,2,*], Gudrún Svana Hilmarsdóttir [1], Tumi Tómasson [3], Sigurjón Arason [1,4] and María Gudjónsdóttir [1,4]

[1] Faculty of Food Science and Nutrition, University of Iceland, Aragata 14, 102 Reykjavik, Iceland; gsh9@hi.is (G.S.H.); sigurjar@hi.is (S.A.); mariagu@hi.is (M.G.)
[2] Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu, Nha Trang 650000, Vietnam
[3] UNESCO GRÓ—Fisheries Training Programme, Fornubudum 5, 220 Hafnarfjordur, Iceland; tumi@groftp.is
[4] Matis Ltd., Company, Icelandic Food and Biotech R&D, Vinlandsleid 12, 113 Reykjavik, Iceland
* Correspondence: thn10@hi.is

Abstract: Quality changes of protein and non-protein nitrogen compounds during industrial fishmeal processing of fatty pelagic species (mackerel/herring rest material blend, MHB) and lean fish (whole blue whiting, BW) were studied to identify processing steps that require optimization to allow production of products for human consumption. Samples from protein-rich processing streams throughout the fishmeal production were analyzed for proximate composition, salt soluble protein content (SSP), biogenic amines (BA), total volatile basic nitrogen (TVB-N), trimethylamine (TMA), and dimethylamine (DMA). Mass flows throughout processing were balanced based on the total mass and proximate composition data. The quality of the final fishmeal products was highly dependent on the fish species being processed, indicating that the processes require optimization towards each raw material. The chemical composition changed in each processing step, resulting in different properties in each stream. Most of the non-protein nitrogen compounds (including BA, TVB-N, TMA, and DMA) followed the liquid streams. However, the concentrate contributed less than 20% to the produced fishmeal quantity. Mixing of this stream into the fishmeal processing again, as currently carried out, should thus be avoided. Furthermore, the cooking, separating, and drying steps should be optimized to improve the water and lipid separation and avoid the formation of undesired nitrogen compounds to produce higher-value products intended for human consumption.

Keywords: fishmeal; protein; biogenic amines; trimethylamine; dimethylamine; TVB-N

Citation: Nguyen, H.T.; Hilmarsdóttir, G.S.; Tómasson, T.; Arason, S.; Gudjónsdóttir, M. Changes in Protein and Non-Protein Nitrogen Compounds during Fishmeal Processing—Identification of Unoptimized Processing Steps. *Processes* **2022**, *10*, 621. https://doi.org/10.3390/pr10040621

Academic Editor: Reza Tahergorabi

Received: 4 February 2022
Accepted: 18 March 2022
Published: 22 March 2022

1. Introduction

Fish is a nutrient-dense food containing high-quality proteins with a well-balanced amino acid composition, long-chain polyunsaturated fatty acids (LC PUFA), and micronutrients [1,2]. In 2015, fish contributed to about 17% of the human intake of animal proteins and 7% of the world's total protein consumption [3]. Global fish production reached about 179 million tons in 2018, of which approximately 88% went to human consumption [3]. Nevertheless, fish is a limited resource, and the depletion of marine fisheries resources and growing environmental challenges are global issues that require action [3]. At the same time, the growing global human population will increase the need for fish and fishery products [1,4]. It is predicted that more than 10% of the world's human population could face micronutrient and fatty acids deficiencies due to the reduced availability of fish over the coming decades [1]. Many underutilized fish species and protein-rich rest materials are used for low-value fishmeal intended for aquaculture or other animal feed. Most small fish species caught on an industrial scale are used for fishmeal and fish oil production rather than for direct human consumption [3,5,6]. The Food and Agriculture Organization of the

United Nations (FAO) suggests that more attention should be paid to utilizing low-value nutrient-rich fish species, such as small pelagic fish, for human food. Decreasing the use of wild fish for fishmeal and fish oil production and redirecting fish reduction facilities towards the processing of fish protein for human consumption can be a valuable step in meeting the future human protein demand [4–6]. Small-sized fish species, which have a valuable source of essential micronutrients and high-quality protein [7], can be used for human consumption or as a start feed in aquaculture or agriculture if the processes are updated and optimized. Fish protein products from underutilized species can also be used in functional food or ready-to-eat products, which has encouraged food manufacturers to develop new methods to process fish protein powders [8,9].

Fish muscle is, however, highly perishable and susceptible to degradation during handling, processing, and storage. Protein stability is one of the most important characteristics of processed fish products, affecting the nutritional, digestive, and sensory quality. Protein changes can lead to the loss of essential amino acids, an overall decrease in nutritional value, and protein functionality and digestibility [10–13]. Several studies have shown a loss of salt soluble proteins (SSP) in refrigerated, frozen, and salted fish during processing and storage [10–12,14]. However, there is little information available on protein changes driven by heat and drying treatment during fishmeal processing.

Non-protein nitrogen compounds formed during the degradation of proteins play a significant role in determining the taste and smell of fishery products. Free amino acids, peptides, purine bases, urea, and trimethylamine oxide (TMAO) are the main components of this chemical group [15]. Post-catch handling and processing may cause changes in proteins due to the formation of undesirable non-protein nitrogen compounds such as biogenic amines, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), dimethylamine (DMA), and ammonia [13]. Therefore, it is important to control and minimize the formation of these compounds if the products are intended for human consumption.

Globally, 65–75% of the fishmeal and fish oil is produced from small pelagic fish [3,6,16]. Pelagic fish species constitute a large part of the captured fish in Iceland and made up 51% of the total catch in 2020. Blue whiting (*Micromesistius poutassou*), Atlantic mackerel (*Scomber scombrus*), and Atlantic herring (*Clupea harengus*) are the three dominant pelagic species, accounting for 42% of the total catch. However, these species only accounted for about 13% of the total value [17]. Therefore, much can be gained from developing higher-value products from these species. In Iceland, most of the herring and mackerel catches are processed for human consumption as frozen, headed, and gutted or filleted fish. The side streams (cut-offs, heads, guts, viscera, backbone, etc.) are collected and used for fishmeal and fish oil production, along with any bycatch [18,19]. The mackerel and herring seasons overlap, and these species are thus often processed into fishmeal and oil simultaneously. Mackerel and herring are histidine-rich species [20,21], and fish guts are rich in a wide variety of enzymes and bacteria. Biogenic amines are generated from the decarboxylation of free amino acids by endogenous enzymes of raw material or by bacterial activities. Biogenic amines, such as histamine, tyramine, putrescine, and cadaverine, are a potential health risk because of their toxic characteristics [22,23]. It is thus of high importance to limit the formation of biogenic amines during production. This is one of the main challenges when producers want to optimize the utilization of side streams from mackerel and herring fishmeal processing for human consumption into the development of other high-value-added products.

Blue whiting made up about 50% of the pelagic catch around Iceland in 2020 [17]. This species is used primarily for fish meal production and is generally not considered tasty enough for direct human consumption. Blue whiting is a lean fish of the gadoid family, with high trimethylamine oxide (TMAO) and TMAOase levels [24]. During post-catch handling and processing, TMAO may be broken down by spoilage bacteria into trimethylamine (TMA), generating pungent and undesirable fishy flavours and odours. Moreover, TMAO may be split into dimethylamine (DMA) and formaldehyde (FA) under TMAOase catalysis.

It has been shown that TMAO decomposition plays an important role in the total volatile base nitrogen (TVB-N) production in this species [24].

Traditional fishmeal/oil products have been processed with the same technology for decades, forming low-quality products of relatively low economic value. These production processes are primarily purposed toward water removal. Meanwhile, the protein quality, lipid removal, and separation of unwanted non-protein nitrogen compounds have not been considered in detail [16,25]. Furthermore, the processes are not optimized towards variations in the raw materials or the processing of different species.

This study therefore aimed to indicate how proteins and unwanted non-protein nitrogen compounds change and/or separate during processing from the initial raw materials to the final products during processing of different pelagic species. Evaluating changes in protein characteristics during each step of the current fishmeal processes is crucial in order to systematically change these processes towards producing high-quality, protein-rich products for human consumption. The changes in protein and non-protein nitrogen compounds during traditional processing of fatty pelagic fish species and leaner fish were investigated to identify necessary improvements towards the production of protein products for human consumption, as affected by species and raw material characteristics.

2. Material and Methods

2.1. Raw Material and Sampling

2.1.1. Raw Materials

Raw materials were collected on two occasions to compare the efficiency of a fishmeal factory during the processing of a fatty (a mackerel/herring blend, MHB) raw material and lean raw material (blue whiting, BW).

The Atlantic mackerel/herring was caught from 3 September to 7 September 2017, off the southeast coast of Iceland, by midwater trawling. The mackerel and herring were mechanically headed and gutted (Baader 221: Automatic Pelagic Processing Line) upon arrival to the processing facility. The cut-offs were collected along with the bycatch, and the material was pumped into the fishmeal processing facilities, where it was stored in a receiver tank and kept at 3 ± 1.5 °C until processed 1–3 days upon arrival to shore. The raw materials for the MHB fishmeal production contained 58% of Atlantic mackerel (*Scomber scombrus*) cut-offs, 37% of Atlantic herring (*Clupea harengus*) cut-offs, 4.5% of blue whiting (*Micromesistius poutassou*), and about 0.5% of bycatch species.

The blue whiting was caught on 30 April 2019, south of the Faroe Islands, by midwater trawls. The BW was refrigerated at 2 ± 2 °C on board for 24 h before being transferred to the fishmeal processing facility, where it was processed in the same way as the MHB as described in the following section. More detailed information about the raw materials and their handling were described by Hilmarsdottir et al. [16].

2.1.2. Sampling during Industrial Fishmeal and Oil Processing

A detailed flow chart of the industrial fishmeal and oil production processes is presented in Figure 1. Upon arrival at the factory, the raw material was preheated for 20 min at 55 °C. Next, the mixture entered a cooking step at 85–95 °C for 20 min before being drained and pressed to remove excess water. Then, the press liquid and drained liquid were transferred to a decanter, forming a liquid mixture called ***separated press liquid***. The separated press liquid entered centrifuges and evaporators to separate the fish oil from the solid processing streams. The liquid streams were led through two evaporators to produce a ***concentrate***, which was combined with the ***press cake*** and ***sludge*** before drying.

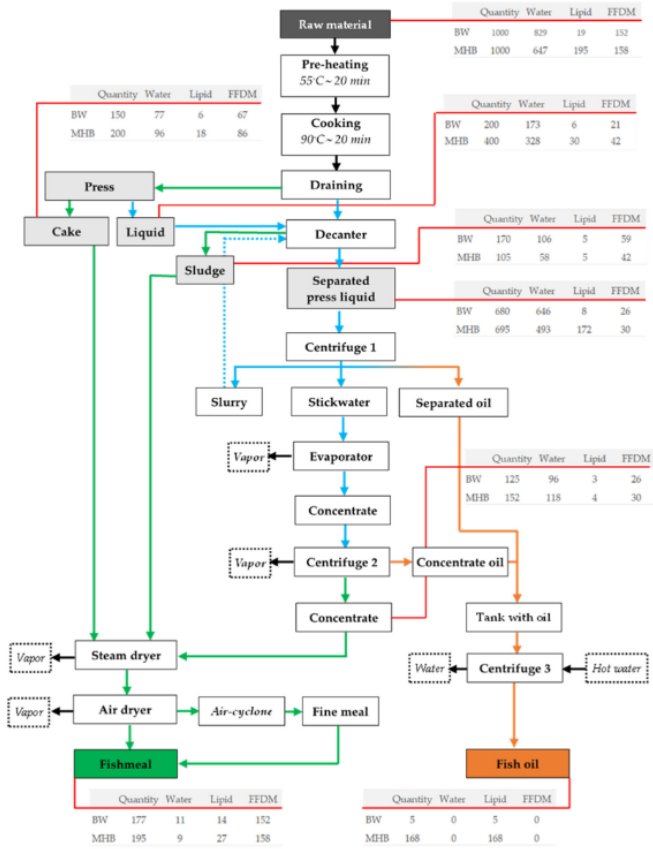


Figure 1. Industrial fishmeal and fish oil production process. The green colour indicates the solid streams throughout the process, the blue represents the liquid streams, and the yellow colour expresses the oil streams. Mass balance from 1000 kg of raw material was calculated for blue whiting (BW) and mackerel/herring blend (MHB), shown by red lines. The quantity of each processing stream and the amounts of water, lipid, and fat-free dry matters (FFDM) in each stream are shown in kg. The flow chart was adapted from Hilmarsdottir et al. [18].

The first drying step was performed in a rotary disc steam dryer for 30 ± 5 min (steam temperature of 160 °C, drying temperature of 95 °C), reducing the moisture content of the material to approximately 40–50%. The material underwent a second drying step in a Hetland air dryer for 16 ± 2 min (maximum input air temperature 450 °C, drying temperature 150 °C at the middle of the dryer, wet bulb temperature of about 65 °C). Some fine particle meal (***fine meal***) was blown out through the air duct during the air drying.

This meal was recovered and combined with the rest of the dried meal, forming the final ***fishmeal***, which had a moisture content of 5–10%.

Samples were collected at key locations throughout the processing, as indicated in Figure 1. After collecting, the samples were cooled overnight to 0 ± 2 °C and transported the following morning to the laboratory. The samples were then stored at −25 °C until analysis, which took up to six months for the MHB and three months for the BW. Prior to analysis, samples were left to thaw at 0–4 °C for 12–36 h. Three samples (n = 3) were collected at each location, and chemical analyses were performed in duplicate for each individual sample. In order to assess the effectiveness and quality changes occurring during processing, a combination of well-known and/or accredited analytical methods, which are commonly applied during food production, were applied. The same analytical methods were furthermore applied to both raw materials, as that allows quantitative and qualitative comparisons of processing of the two raw materials. The applied analytical methods are described in detail in Sections 2.2–2.4.

2.1.3. Chemicals

All chemicals used in the study were of analytical grade and purchased from the Sigma-Aldrich Company (Missouri, TX, USA).

2.2. *Proximate Composition Changes during Processing*

Water content was measured according to ISO 6496:1999. About 5.0 g of sample was weighed and placed in a small porcelain bowl. The bowls were left to dry for 4 h at 104 ± 2 °C and allowed to cool to ambient temperature in a desiccator for 30 min before being weighed again.

Crude protein content of the samples was measured according to ISO 5983-2 (2009). About 2 g of homogenized sample was digested in 17.5 mL concentrated H_2SO_4 in the presence of two Kjeldahl tablets (each tablet contains 0.4 g $CuSO_4$ and 3.5 g K_2SO_4) as an oxidative catalyst at approximately 420 °C for 2.5 h. The digested sample was made alkaline by adding NaOH, and the nitrogen distilled off as NH_3. The NH_3 was then "trapped" in a 1% boric acid solution. The amount of ammonia nitrogen in the solution was quantified by titration with a standardized H_2SO_4 solution. The nitrogen content was multiplied by 6.25 to obtain the ratio of crude protein.

Lipids were extracted from 25 g samples with 50 mL of chloroform, 50 mL of methanol, and 25 mL or 0.88% KCl according to the Bligh and Dyer method [26]. After homogenizing for 4 min, the mixture was centrifuged at 2500 rpm for 20 min at 4 °C. The lower chloroform phase, containing the lipid fraction, was collected and filtrated on a glass microfiber filter paper under vacuum suction. The extracts were then removed from the upper phase and filled with chloroform to reach a volume of 50 mL. Exactly 2 mL of the chloroform phase was pipetted in a glass tube and blown by a nitrogen jet at 55 °C to remove the solvent. The remaining solution was weighed to determine the total lipid content.

Ash was defined as the remaining components of the dry matter. The ash content was calculated as the total wet weight (100%) after removing the water, lipid, and crude protein contents. Fat-fee dry matter (FFDM) was calculated as the total wet weight (100%) minus the water and the lipid contents. The water, crude protein, lipid, and ash content were expressed as a percentage of wet weight.

2.3. *Mass Balances during Processing*

Material balances are essential for effective process development in the food industry [27] and aid in assessment of the quantity of products and side streams.

As the fishmeal and fish oil production was assessed under steady-state conditions, the mass of the raw materials entering the process facilities equalled the mass of the products and other exiting processing streams [28]. The raw materials are composed of three major components: solids (FFDM), lipids, and water. The primary purpose of the fishmeal process lies in the separation of these major components [13]. The mass balances were established

throughout the production, and the quantity of side streams was estimated based on changes in these components after each processing step. The overall mass balances were calculated based on an input of *1000 kg of raw materials*. When fitting the mass balance between operation steps, average values were used on an FFDM base.

2.4. Protein Changes during Processing

2.4.1. Salt Soluble Protein Content (SSP)

Salt soluble proteins (SSP) were extracted from the samples with a NaCl buffer (1 M NaCl and 0.02 Na_2CO_3, pH 7.0) according to the method described by Kelleher and Hultin [29]. Exactly 190 mL of buffer solution was added to 10 g sample, and the mixture was homogenized in an Ultra-Turrax homogenizer (Ika Laborttechnik, T25 basic, Staufen, Germany) for 1 min. The mixture was incubated on ice for an hour before being centrifuged at 4 °C for 15 min at 10,000 rpm (Avanti Centrifuge J.10, Beckmann Coulter, Fullerton, CA, USA). The SSP were measured by quantifying the amount of solubilized protein in the supernatant based on the Bradford method [30]. The diluted supernatant and the Bradford reactive solution were placed in a 96-well microplate, and the absorbance read at 595 nm (Sunrise Microplate Reader, Tecan GmbH, A-5082 Grodig, Austria). The SSP were calculated based on a calibration curve made with bovine serum albumin with concentrations ranging between 0.1–1.4 mg/mL. Results were expressed as a percentage of the wet weight.

2.4.2. Biogenic Amines (BA)

Samples were tested for biogenic amines, including tyramine, putrescine, cadaverine, and histamine, using a method developed by Olajos [31]. About 5 g of the sample was homogenized with 45 mL of 0.6 M perchloric acid using an Ultra-Turrax homogenizer for 1 min. The homogenate was then filtered through a Whatman pleated filter paper 113 V. The filtrate was pressed using a disposable syringe assembled into a membrane filter (pore size 0.45 µm). This extract was then used for the measurement by using liquid chromatography (LC-30/20 AD with two low-pressure pumps high-performance liquid chromatography (HPLC) system) (Shimadzu, Kyoto, Japan). The BA were separated on a reversed-phase column (Zorbax Eclipse Plus C 18 4.6 × 250 mm, 5 µm), and after online derivatization (post-column derivatization) using ortho-phthalaldehyde, they were measured by fluorescence detection. A standard curve was made using a mixture of standard solutions, including tyramine hydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, and histamine dihydrochloride solutions, spanning a range of 2.5–100 mg/L. The BA contents were calculated and expressed as g/kg wet weight (ww).

2.4.3. Total Volatile Basic Nitrogen (TVB-N), Trimethylamine (TMA) and Dimethylamine (DMA)

TVB-N was determined using the steam distillation method described by Malle and Poumeyrol [32]. Approximately 50 g of sample was homogenized with 100 mL of 7.5% aqueous trichloroacetic acid solution. The blend was filtrated through a Whatman pleated filter paper 113 V. Then, 25 mL of the extract was transferred into a distillation flask with 6 mL of 10% NaOH solution. Steam distillation was then performed using a Kjeldahl-type distillatory, and the TVB-N was collected under a condenser into a beaker containing 10 mL solution of 4% boric acid and indicators (0.04 mL of methyl red and bromocresol green), which turned green when alkalinized by the TVB-N. The alkalized mixture was titrated with a standardized H_2SO_4 (0.037 N) solution using a 0.05 mL graduated burette. Complete neutralization was achieved when the colour turned pink on addition of a further drop of sulphuric acid solution.

The TMA and DMA were measured according to the liquid chromatography–mass spectrometry method described by Baliño-Zuazo and Barranco [33]. About 2.5 g of sample was homogenized with 50 mL of 10 mM acetic acid solution and centrifuged at 13,400 rpm at 4 °C. Twenty µL of the supernatant of the extract was mixed with 480 µL of tetraethy-

lammonium chloride hydrate 3.2 µg/mL in acetonitrile/water (6:4), 20 µL of 0.5 M bicarbonate buffer, and 1 mL of tert-butyl bromoacetate (5 mg/mL in acetonitrile). The mixture was incubated in a water bath for 1 h at 60 °C for a derivatization reaction. The derivatized samples were analyzed using a Luna HILIC column (150 × 2 mm I.D., 3 µm) (Phenomenex Torrance, CA, USA) in a Dionex Ultimate 3000 HPLC (Thermo Fisher Scientific, Waltham, MA, USA) coupled to a TSQ Quantiva mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The TVB-N, TMA, and DMA were calculated and indicated as mg N/100 g ww.

2.5. Statistical Analysis

All data summaries and statistical analyses were performed using the IBM SPSS Statistics software (Version 22, IBM, 1 New Orchard Road, Armonk, New York, NC 10504-1722, USA) and Microsoft Office Excel 2013 (Microsoft Inc., Redmond, WA, USA). One-way analysis of variance (ANOVA), Tukey's HSD tests, and Student t-tests were performed on means of the variables. Significant difference was set at the 5% level ($p < 0.05$) for all statistical analyses.

3. Results and Discussion

3.1. Changes in Proximate Composition during Processing

The water content of the blue whiting (82.9 ± 0.6%) was significantly higher than in the mackerel/herring blend (64.6 ± 3.3%) ($p < 0.05$). By contrast, the MHB had a significantly higher lipid content (19.5 ± 2.0%) than the BW (1.8 ± 0.1%). The difference in water and lipid contents between the different raw materials agrees with the species-dependent lipid content, as water and lipid content have an inverse linear relationship in fish muscle [34,35]. The BW had slightly higher water and lower crude protein content than the blue whiting reported by Egerton et al. [34], which had lipid, crude protein, and water contents ranging between 3–5%, 16–18%, and 75–80%, respectively. These differences can mainly be explained by different location and time of fishing [36].

Water content decreased significantly during pressing to 51.5 ± 2.3% and 47.9 ± 1.6% in the BW and MHB press cakes, respectively (Figure 2). The water content of the sludge was 62.4 ± 0.6% and 56.3 ± 0.9% in the BW and MHB, respectively, which was significantly lower than the water content in the separated press liquid (91.1 ± 0.0% and 71.2 ± 2.1%). These results confirm that the press and decanter play an important role in water removal. Water content is an important quality parameter of fishmeal. Low water content can inhibit protein browning and bacterial-caused deterioration. However, too low water activity increases the risk of lipid oxidation and loss of protein solubility [37]. Therefore, a water content of 5–12% is generally recommended for fishmeal [13,25]. The final BW and MHB fishmeal had water contents close to the suggested range (6.4 ± 0.1% and 4.6 ± 0.2%, respectively) and are comparable to earlier published results for these species [38,39].

Most of the lipids followed the liquid processing streams after the separation steps, resulting in increased crude protein and decreased lipid content in the press cake and sludge (Figures 1 and 2), more so in the MHB processing due to the higher lipid content in the raw material. The MHB press cake had a crude protein content of 37.5 ± 2.4% and a lipid content of 8.8 ± 0.6%, compared to 12.0 ± 0.3% crude protein and 19.5 ± 2.0% lipid content in the raw material. Similarly, after passing the decanter, the sludge contained 34.6 ± 0.7% protein and 4.7 ± 0.2% lipid, compared to 10.0 ± 0.2% protein and 18.2 ± 3.3% lipid in the separated press liquid. The crude protein content significantly increased while the lipid content decreased in the final MHB fishmeal (65.2 ± 0.3% protein and 14.3 ± 0.2% lipid) compared to the raw material (Figure 2b). Both the proportional protein and lipid contents were significantly higher in the final BW fishmeal than in the raw material, mainly due to water removal. The BW fishmeal had a similar crude protein content to the BW fishmeal in earlier studies (68–70.5%) [38,40]. The protein contents of the fishmeal were comparable to fishmeal made from other pelagic species reported in the literature [38,41,42] and higher than the protein content in fishmeal made from both cod and saithe (61.9%) [39] and

tuna cut-offs (56.2–59.1%) [43]. The lipid content of fishmeal from pelagic fish is typically between 6–10% [39,41]. The MHB fishmeal had a considerably higher lipid content and lower protein content than the BW fishmeal, reflecting the different composition of the raw materials used [13]. Lipid separation is thus of special importance during processing of fatty fish species. However, both the BW and MHB fishmeal had a high lipid content and were thus classified as type C fishmeal and should not be used for human food under current processing conditions [13,18,25]. The high lipid content indicates inefficiency in lipid separation and removal during processing of both species. The processes thus require optimization with regards to the lipid separation if the fishmeal is intended for human consumption. However, the optimal processing changes might be different while processing the two different species. Hilmarsdottir et al. [18] suggested that optimization of early processing steps, including the heating steps, would improve lipid separation during fishmeal processing of a mackerel–herring blend. Furthermore, their study suggested that drying the press cake, the sludge, and the latter concentrate individually could result in more flexibility in processing and process control, ultimately leading to the production of higher-quality products.

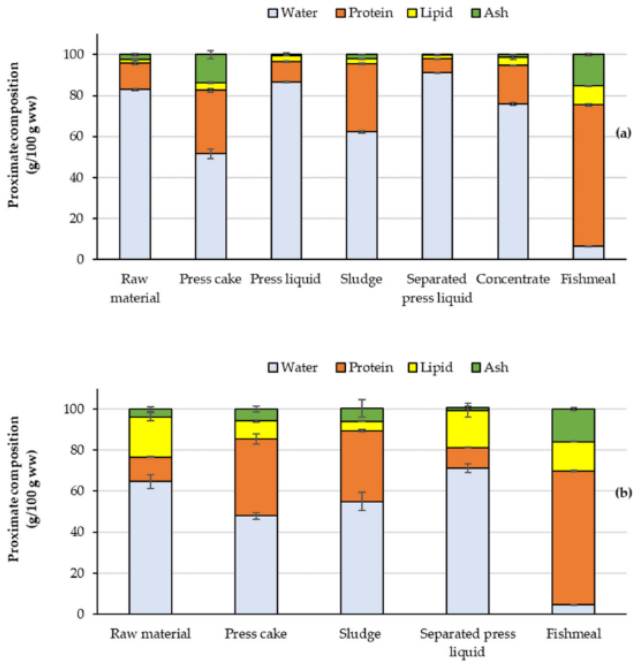


Figure 2. Proximate composition (%, g/100 g ww) in BW (**a**) and the MHB (**b**) fishmeal products during industrial processing.

The press cake of both raw materials contained higher amounts of ash than other intermediate stages of processing or 13.7 ± 1.9% and 5.7 ± 1.5% in the BW and MHB, respectively. The high ash content of the press cake reflects that the bones of the fish were

mainly left in the press cake. This was especially evident in the BW processing. After entering the decanter, the remaining ash content remained in the sludge, resulting in a lower ash content in the separated press liquid (0.3 ± 0.0% in BW and 1.3 ± 0.8% in MHB). The water and lipid removal led to a relative increase of ash content in both the BW and MHB fishmeal (15.6 ± 0.4% and 15.9 ± 0.7%, respectively), of which results are comparable with values observed in anchovy fishmeal (15.0%) [41] but lower than those seen in fishmeal made from cod and saithe off-cuts (22.4%) [39].

3.2. Mass Balances during Processing

The factory produces fishmeal and fish oil from around 1200 tons of raw material per hour when operating at full capacity [18]. However, to ease the assessment of yield and size of processing streams, a basis of 1000 kg of raw materials was set for the mass balance calculations. The different raw materials resulted in very different proportions of mass flow through the press cake, sludge, and concentrate, as well as differences in the yield of fishmeal and oil (Figure 1), but processing yields are highly dependent on the chemical composition of the raw materials [13]. The ratios between the press cake and separated press liquid were approximately 3:4 and 1:2 for the BW and MHB, respectively, indicating that the chemical composition had a high impact on the balance between the liquid and solid streams during processing, and thus the effectiveness of the process. The obtained ratios between the press cake and press liquid furthermore indicate that the pressing was more efficient in the MHB than in the BW. In agreement with this, the MHB, which had higher FFDM and lipid content in the raw material, resulted in a higher production yield of both fishmeal and oil than the BW (Figure 1).

Although most of the lipids followed the separated press liquid, a significant amount of lipids remained in the press cake (approximately 4% and 9% in the BW and MHB, respectively) and sludge (3% and 5% in the BW and MHB, respectively). After evaporation of the press liquid, the concentrate was mixed with the sludge and press cake prior to entering the drying steps. The press cake contributed the biggest proportion (44% and 54%) of the total mass in the combined solid stream, followed by the sludge (39% and 27%), and the smallest proportion originated from the concentrate (17% and 19%) during the BW and MHB fishmeal productions, respectively. The press cake was the highest contributor of lipids (39% in BW and 67% in MHB) in the fishmeal. In BW, the highest contributor to water was the sludge (38%), whereas the concentrate was the highest contributor of water to the MHB fishmeal (44%). The different contributions of the solid streams towards the composition of the final fishmeal during processing of the two different raw materials are of high concern and highlight the importance of adjusting the processing towards the optimal efficiency and quality of each raw material. The high water content in the BW production indicates that the decanter did not operate properly, potentially due to overload, leaving a higher water proportion in the BW sludge (38%) compared to the MHB sludge (21%) (Figure 1). Overload of the decanter should thus be avoided. Assessment of the fishmeal composition, furthermore, identified the high importance of efficient lipid separation, both from the BW sludge and the MHB press cake, indicating that the initial processing steps require optimization for the removal of lipids during processing of both species.

Most of the lipids present in the separated press liquid were effectively extracted with the two centrifuges, forming the final fish oils and decreasing the lipid content of the fishmeal. However, only 26% of the lipid content of the BW raw material was extracted to form the BW fish oil, whereas 86% of the MHB lipid content in the MHB raw material was extracted to form the MHB fish oil. This could partially be dependent on differences in the total lipid content as well as the lipid composition and availability of lipid classes between the species [16]. Improving the lipid separation from the solid streams and directing them toward the liquid streams would therefore not only increase the fishmeal quality but also increase the oil yield. The different efficiency of the processing steps due to the variation in chemical compositions of the different raw materials indicate that further optimizations of the processing of each species are necessary.

The BW and MBH fishmeal could both be classified as type C fish protein concentrate (FPC), according to their lipid content. For a type A FPC, the lipid content should be lower than 0.75% [13,25]. Substantial changes are thus required during processing of both species to obtain a type A FPC classification of the products. However, as the solid streams differ in proximate composition (Figure 1), a suitable end product needs to be aligned with the properties of each raw material and each processing stream, including the quality of the proteins, which are discussed in the following section.

3.3. Protein Quality Changes during Processing

3.3.1. Salt Soluble Protein Content (SSP)

Salt soluble protein content (SSP) decreased significantly during processing, from 5.7 ± 0.7% to 0.9 ± 0.0% in the BW and from 6.1 ± 0.5% to 0.9 ± 0.0% in the MHB (Figure 3a,b), indicating substantial protein denaturation and associated loss of protein solubility during the processing of both species. Generally, the total protein content in fish muscle ranges from 11–24% wet weight, in which SSP account for 85–90% [2,44]. Low SSP content was expected in this study due to the high content of connective tissues (stroma protein) in the raw material [45]. Moreover, the low SSP in both BW and MHB raw materials may be due to protein denaturation during cold storage before processing and the frozen storage of the samples until they were analyzed. The SSP content in the BW was slightly higher than in the blue whiting studied by Derkach et al. [46], which had an SSP content of 5.2%.

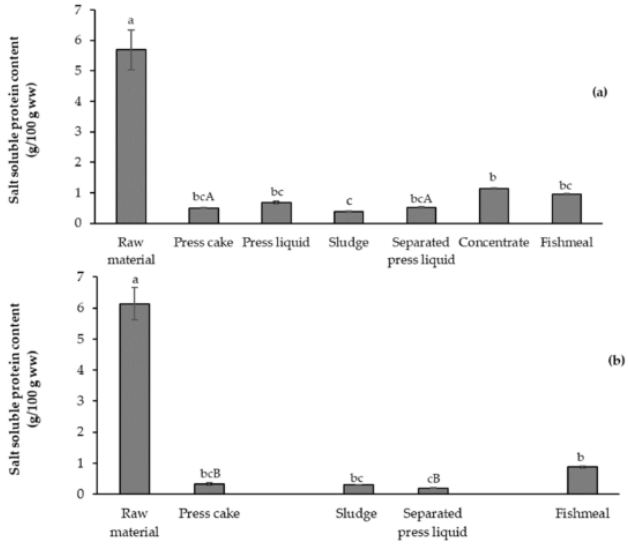


Figure 3. Salt soluble proteins (%, g SSP/100 g ww) in chosen BW (**a**) and MHB processing samples during industrial fishmeal production (**b**). Lowercase letters indicate significant differences in SSP between sampling locations, while uppercase letters indicate significant differences between two types of raw materials at the same processing step. Statistical significance levels were set to $p < 0.05$ for all analyses.

The SSP significantly decreased right after the cooking steps and remained stable during the following steps, reflecting that the proteins were already mostly denatured during cooking. This is in agreement with earlier studies, which have shown that protein denaturation occurs mainly during the cooking step as cell membranes break down, and the fat depots rupture, separating the oil and water from the fish muscle [13]. Heating thus causes irreversible changes to the protein structure, such as protein unfolding, exposing previously hidden hydrophobic groups, or heat-induced aggregation, resulting in decreased content of salt soluble proteins [45,47]. The very low SSP content after the cooking step indicates that most myofibrillar proteins denatured, and sarcoplasmic proteins precipitated during the heat treatment. These protein changes can furthermore decrease protein digestibility in vitro [48]. Actin, myosin, and sarcoplasmic proteins account for 85–95% of total fish proteins [44]. Fish myosin begins to denature at around 35 °C; sarcoplasmic proteins are denatured at around 44 °C, while actin is denatured in the temperature range of 58–68 °C [49–51]. Most fish proteins have thus denatured at temperatures around 75 °C [13]. Significant decreases in protein solubility due to heat processing have also been reported earlier [52,53]. A proportional increase was observed in SSP in the final fishmeal compared to the solid streams entering the dryers. Although some further protein denaturation is expected to occur during the drying step, this increase in SSP can mainly be explained by the removal of water and lipids during drying.

SSP content is related to protein solubility, which is considered the first functional characteristic during the development and testing of a new protein ingredient. Protein solubility is the primary property of proteins used in liquid foods [45]. The low SSP in the fishmeal hence limit their practical uses. The heat treatment applied in the current fishmeal processing appears to be too rough. For the products to be fit for human consumption, the heating step thus requires changing. Adding a suitable amount of polyphosphate or sucrose could potentially protect the proteins from denaturation during drying, as suggested by Shaviklo [8]. In addition, potential alternative processing solutions could involve the reduction of the temperature but extending the heating step duration both during cooking and drying or use tailored enzymes (proteases) to facilitate more effective protein breakdown without losing the SSP. The use of enzymes may decrease the processing time, lower the energy input, and increase the economic effectiveness as shown in various industrial food processing, such as fish protein concentrate or hydrolysate production [54]. Protein hydrolysates are good nutritional supplements since they have high bioavailability and can be utilized for various metabolic activities [55]. The enzymatic process could be performed at a temperature range from 45–60 °C [56,57] for an extended time. However, these temperature conditions can also promote microbial, biochemical, and chemical spoilage during processing [58–60] and should thus be applied with care. The products derived from an enzymatic protein hydrolysis can have a bitter taste, which is one of the key issues that limits its application in food products [57]. However, this showcases the wide potential that lies in pelagic fish processing and high-quality product development and innovation.

3.3.2. Biogenic Amines (BA)

The four BA, tyramine, putrescine, cadaverine, and histamine, decreased during processing and were more strongly indicated in the MHB than in the BW processing (Figure 4a,b). Cadaverine was the most abundant biogenic amine in all sampling locations during processing of both BW and MHB. In the BW process, histamine was only detected in the raw material (0.3 $\pm$ 0.1 g/kg ww) and the liquid streams (press liquid and separated press liquid, each with the content of 0.01 g/ kg ww). No histamine was detected in the BW fishmeal, indicating that the histamine was successfully removed during the processing. The histamine level in the initial MHB raw material was 3.5 $\pm$ 0.2 g/kg ww and decreased to 0.8 $\pm$ 0.1 g/kg ww in the final fishmeal. However, higher histamine levels were detected in all processing streams in the MHB processing than what is acceptable for human food (<0.2 g/kg ww), as established by the European Commission Regulation No 2073/2005. The histamine in the raw materials was higher than the acceptable level for

human consumption but was at acceptable levels for fishmeal in BW (<1 g/kg ww) [61]. High BA levels in the BW and MHB raw materials may have resulted from bacterial activity during the delay between catching and processing [13,18]. Since both mackerel and herring are histidine-rich species, and the raw material used for fishmeal processing contained a large ratio of viscera and dark muscle, the risk of bacterial growth is high [62]. Furthermore, the generation of BA in mackerel and herring has been shown to occur even when stored at low temperatures, such as 2 °C [21].

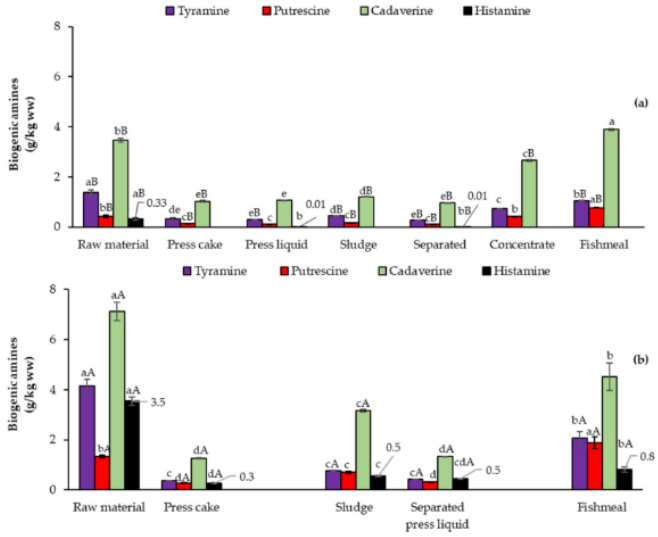


Figure 4. Biogenic amines (BA) (g/kg ww) obtained during fishmeal and oil processing from BW (**a**) and MHB (**b**). Within each BA type, lowercase letters indicate a significant difference between sampling locations, while uppercase letters show significant differences between raw materials at the same processing step. Statistical significance levels were set to $p < 0.05$ for all analyses.

The BA content was significantly higher in the MHB than the BW raw material, and the same trend was seen in the corresponding fishmeal. This indicates that the BA formation is species specific [63,64]. Histidine, the precursor to histamine, exists in abundance in the dark muscle of fish. Thus, dark-muscle-rich fish species generally contain more histidine than leaner species [59,63]. Furthermore, there is a positive correlation between the amino acid histidine and the amount of histamine formed [65]. Tuna and mackerel, which belong to the scombroid family, thus often have high histidine levels [60,64], resulting in high histamine contents in products from these species. This is in good agreement with the different BA levels in the species in the current study, but no histamine was found in the BW fishmeal, while a histamine content of 0.8 g/kg ww was obtained in the MHB.

Several previous studies have indicated that BA are thermally stable even during boiling [66,67] and are thus not likely to be primarily affected by the thermal steps. However, in this study, significant decreases were observed in the BA after the cooking and pressing steps, during which the total content of the four studied BA went from 5.6 g/kg ww in the raw material to 1.5 g/kg ww in both the press cake and press liquid of the BW and from 16.2 g/kg ww in the raw material to 2.2 g/kg ww in the press cake for the MHB.

Overall, about 82% and 89% of the total BA content in the raw materials was removed during the BW and MHB processing, respectively (Figures 1 and 4). These overall decreases in BA content after processing could possibly be explained by their complex decomposition into other volatile compounds under heating [67]. BA are of low molecular weight and mostly water-soluble [60,68] and are thus released into the liquid streams rather than the oil and solid streams. This results in a higher BA amount in the press liquid than press cake (in the BW) and higher BA in the separated press liquid than the sludge (BW and MHB). Mixing the liquid streams back into the fishmeal processing, as is currently carried out, could thus cause problems when processing raw materials with high BA content and should be avoided, at least for histamine-rich species such as mackerel. However, the effect of the BA levels of other species, such as BW, should not be neglected since high levels of individual BAs (such as cadaverine) can cause problems when adapting the processes towards human consumption. The relative increase in total BA observed in the concentrate (BW) and both fishmeal products (BW and MHB) may then be due to the water and lipid removal during processing, as discussed above.

3.3.3. Total Volatile Basic Nitrogen (TVB-N), Trimethylamine, and Dimethylamine

TVB-N in fish and fishery products primarily includes ammonia, TMA, and DMA [13]. TVB-N levels are often used as a quality criterion for the freshness of raw materials destined for fishmeal processing. Threshold values are set to not exceed 60 mg N/100 g in the raw material if the products are intended for human consumption (European Commission Implementing Regulation No 2019/627) [13] and should be less than 80 mg N/100 g in the raw material for fishmeal production [13]. High TVB-N and TMA levels were observed in the raw materials (with TVB-N contents of 83.9 ± 0.6 and 68.1 ± 3.4 mg N/100 g ww in the BW and MHB, respectively, and TMA content of 60.3 ± 5.3 and 35.8 ± 4.6 mg N/100 g ww in the BW and the MHB, respectively. These values indicate spoilage in the raw material during the delay between catch and processing, in agreement with the observation of the BA formation in the raw material prior to processing. Furthermore, the raw materials contain viscera, which have a high content of bacteria and enzymes that can promote spoilage during the transport and storage of the fish on board the fishing vessel before entering the fishmeal processing [13,24]. The TVB-N level of the BW was higher than the recommended level for the raw material and substantially higher than reported values in the light muscle of blue whiting, even after six days of storage on ice at 0 °C (22.7 ± 1.6 mg N/100 g ww) [69]. The DMA level of the BW (11.9 ± 1.1 mg N/100 g ww) was higher than in the light muscle of blue whiting as studied by Rey-Mansilla et al. [24], who detected DMA levels of only 4 mg N/100 g ww after seven days of iced storage. The TVB-N, TMA, and DMA were significantly higher in the BW raw material than in the MHB. This may be due to the BW being a gadoid fish, which has high level TMAO and TMAase enzyme [24,70]. According to the study by Mizuguchi et al. [70] DMA is formed faster in dark muscle than light gadoid muscle, and that DMA formation was triggered by two main factors, i.e., nonheme iron and taurine levels, which are both abundant in gadoid dark muscle. DMA formation is therefore of special concern during processing of BW cut-offs, which contain a high proportion of dark muscle. Furthermore, the TMAO may already have partially decomposed into DMA in the raw material during the delay between catch and processing, explaining high DMA levels in the BW raw material, as discussed earlier.

The TVB-N levels decreased during the fishmeal processing of both the BW and MHB before the drying steps (Figure 5). The water removal during drying may have resulted in a relative increase in the TVB-N content in both final products, in a similar manner as seen in the BA and SSP results. About 81% and 62% of the TVB-N in the raw material evaporated during BW and MHB processing, respectively (Figures 1 and 5). TMA levels showed similar trends as the TVB-N levels during processing (Figure 5), indicating that TMA is a dominant component of the TVB-N, as shown by Howgate [71]. Since TMA is a volatile amine [72], a part of the TMA content that existed in the raw material may have evaporated during the cooking and pressing steps, resulting in lower TMA content

in both the press cake and press liquid (in BW). More than 90% of the TMA in the raw materials was removed during both BW and MHB processing (Figures 1 and 5). However, the same trend was not as clearly indicated in the DMA changes. The DMA content was stable before entering the centrifugation step. After evaporation and centrifugation, the removal of water and oil led to significantly higher levels of DMA, TMA, and TVB-N in the concentrate than the separated press liquid in BW (Figure 5a). TVB-N, TMA, and DMA are water-soluble compounds; thus, they are mainly dispersed into the liquid phases during processing, resulting in a significantly higher amount in the liquid streams (BW press liquid and BW- and MHB-separated press liquid) than in the solid ones (BW press cake and BW and MHB sludge, respectively). TMAO is decomposed into TMA, DMA, and formaldehyde (FA) during thermal processing [73]. Therefore, the remaining TMA and DMA in the products may result from two concurrent processes, the generation from TMAO decomposition and loss due to volatilization. Rapid DMA non-enzymatic formation was observed in fish muscle dried at 90 °C by Spinelli and Koury [74]. This may explain why the DMA was not lost during the fishmeal processing in this study in the same manner as the TVB-N and TMA (Figure 5). DMA levels increased significantly during evaporation in the BW fishmeal processing, from 11.0 ± 0.5 mg N/kg ww in the separated press liquid to 30.2 ± 0.6 mg N/kg ww in the concentrate. During the drying step, a large part of the water was removed, which could lead to a relative increase in the TMA such as other dry matter components. However, the TMA in the final fishmeal products was lower than during processing (press cake, sludge, and concentrate). This indicates that the TMA was removed in the drying steps, probably mainly due to the removal of water.

Although the TVB-N contents in the raw material and intermediate processing streams were generally higher in the BW than the corresponding MHB samples, the MHB fishmeal had a significantly higher TVB-N level than the BW fishmeal. Meanwhile, the TMA and DMA were higher in the BW than in the MHB fishmeal. This could be due to potentially higher ammonia formation during the pre-processing delay of the MHB by-product blend than in the BW due to a higher proportion of viscera in the MHB raw materials. Viscera, which are rich in enzymes and bacteria, can promote protein changes and spoilage, forming amino acids and ammonia [72], resulting in the formation of undesirable odours and flavours. Ammonia generation during thermal degradation of protein and amino acids has also been observed in earlier studies [75,76].

The fact that the non-protein nitrogen compounds followed the liquid streams, resulting in lower values in the solid streams (the press cake and sludge), indicates that processing these streams individually could lead to lower volatile nitrous compounds in the final products, especially if the BA-rich liquid streams are not redirected into the process. This is in agreement with the observations of Hilmarsdottir et al. [18], who identified inefficient water removal during the draining and concentration steps and that the lipid separation from the fishmeal was insufficient for the production of high-quality products, such as for human consumption or even fish feed. Hilmarsdottir et al. [18] thus recommended that the main streams entered to final fishmeal (press cake, sludge, and latter concentrate) should be processed separately. This would allow production of higher-quality protein products from the press cake, while the sludge and concentrate could contribute to lower value products. The current observations on BA, TVB-N, and TMA content support this notion as well. However, the sludge had a high protein ratio (88 g/100 g dry matter (DM) in BW and 78 g/100 g DM in MHB) and a low lipid and ash proportion, comparable with the proximate composition of fish protein hydrolysates [9]. This stream, therefore, could potentially be used to produce high-value products, such as special feeds, animal feed enrichments, or nutritional supplements and healthy foods for human consumption, such as fish protein hydrolysates or fish protein concentrates [8,9]. These potential uses may bring more economic value than traditional fishmeal production. However, to develop high-quality products for humans, other quality properties of this part, such as TVB-N and biogenic amines of the sludge, need to comply with safety requirements. Processing should thus primarily be optimized to reduce these unwanted non-protein nitrogen compounds.

Adding membrane filtration at appropriate settings to the processing could potentially provide a solution to this problem.

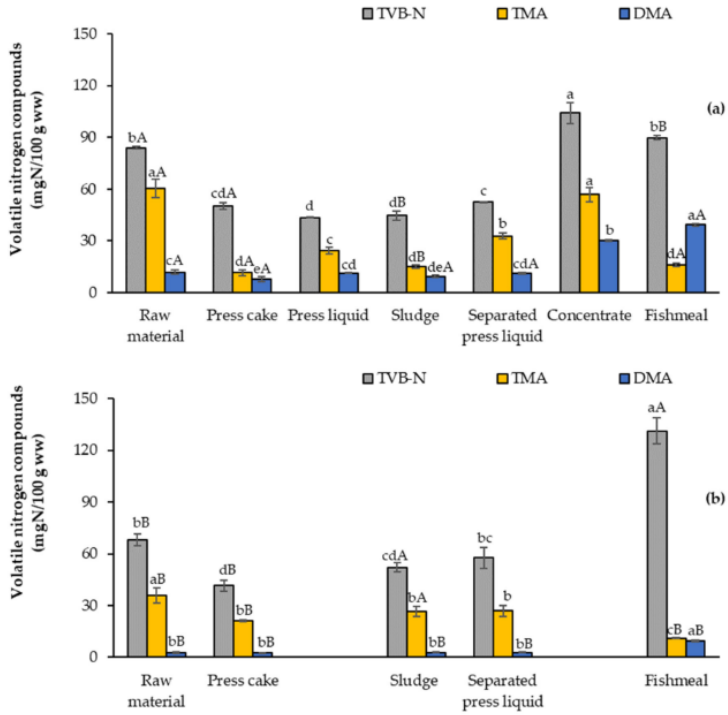


Figure 5. Volatile nitrogen content (mg N/100 g ww) in streams from the industrial BW fishmeal production (**a**) and MHB fishmeal production (**b**). Within each parameter, lowercase letters investigate significant differences between sampling locations, uppercase letters show significant differences between raw materials at the sample processing step. Statistical significance levels were set to $p < 0.05$ for all analyses.

4. Conclusions

Chemical characteristics of the protein-rich processing streams in two industrial fishmeal processes of BW and MHB were evaluated in this study. The raw materials and processing samples were collected at the fishmeal factory. With an input of 1200 tons of raw materials per day, the quality of raw materials entering the factory can be highly variable. The fishmeal production processes were conducted three days post-catch to ensure enough raw materials to fulfil the capacity criteria of the factory. However, this pre-processing delay resulted in considerable heterogeneity and quality degradation in the raw material. In addition, the analysis showed that processing conditions at each step could fluctuate significantly, and the processing efficiency was highly dependent on the species being processed. These variations furthermore influenced the chemical properties of the samples and the quality of the resulting fishmeal products.

Large amounts of non-protein nitrogen compounds were observed in the raw materials, probably due to the three-day pending time from catch to entering the fishmeal processing. Removing the viscera and proper collecting, handling, stable cooling, and storing of the raw materials before processing would improve the safety and quality of the final protein products. This can widen the utilization of the final protein products, potentially even for human consumption, and simultaneously bring more economic benefits of the production.

The BW fishmeal had a higher protein content (69.1 $\pm$ 0.5%) than typical fishmeal (64–67%), and BA and TVB-N levels were within acceptable thresholds for fishmeal. The MHB fishmeal had a protein content of 65.2 $\pm$ 0.3% and a histamine content below 1 g/kg, currently making it acceptable for animal feed. However, the histamine (0.8 $\pm$ 0.1 g/kg) and TVB-N (131.4 $\pm$ 7.3 mg N/100 g) in the fishmeal were higher than acceptable for human consumption. These two products can thus be graded as type C fishmeal with lipid contents above 3%.

Soluble protein content and non-protein nitrogen compounds were readily released into the liquid processing streams together with most of the water and lipids, while high-molecular-weight proteins were retained in the solid streams. Most undesirable non-protein nitrogen compounds were removed during the processing of both species, especially during the drying step. The lipid quality was also highly affected by heating in this step, as described earlier by Hilmarsdottir et al. [18]. This indicates that the drying step requires optimization. Spray drying of the processing streams could potentially provide milder drying and higher quality. Other unoptimized processing steps, such as pressing and concentration, were also identified, which need to be improved in order to produce high-quality products in the future. The testing of alternative processing is left for future studies. Comparison between the two species, moreover, showed that the processes need to be adapted to each raw material for higher-value product production.

In both industrial fishmeal processes (BW and MHB), the press cake had high protein contents and low contents of non-protein nitrogen compounds, making the press cake a promising material for the development of higher-value products. Furthermore, separate processing of the solid streams (press cake, sludge, concentrate) thus shows promising potential for production of a wider range of products, including high-value products for human consumption. However, to be used as human food, the products from the optimized processes should be studied further regarding amino acid profiles, digestibility, and sensory attributes. It would also be of interest to study applications of the optimized products as ingredients for the development of other value-added products included for human consumption.

Author Contributions: Conceptualization, H.T.N., G.S.H., T.T., S.A. and M.G.; methodology, H.T.N., G.S.H., S.A. and M.G.; software, H.T.N.; validation, H.T.N.; formal analysis, H.T.N. and G.S.H.; investigation, H.T.N.; resources, S.A. and M.G.; data curation, H.T.N. and G.S.H.; writing—original draft preparation, H.T.N.; writing—review and editing, H.T.N., G.S.H., T.T., S.A. and M.G.; visualization, H.T.N., G.S.H., T.T., S.A. and M.G.; supervision, S.A. and M.G.; project administration, S.A. and M.G.; funding acquisition, S.A. and M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the AVS (the Added Value of Seafood) fund of the Ministry of Fisheries and Agriculture in Iceland (grant number: R18 031-18). The PhD scholarship was funded by the UNESCO affiliated GRÓ Fisheries Training Programme.

Acknowledgments: The authors thank Sildarvinnslan ohf. (Iceland) for access to their facilities, assistance, and raw materials for the study and the staff at Matís ohf, Food and Biotech R&D, for their assistance with analytical measurements.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Golden, C.D.; Allison, E.H.; Cheung, W.W.L.; Dey, M.M.; Halpern, B.S.; McCauley, D.J.; Smith, M.; Vaitla, B.; Zeller, D.; Myers, S.S. Nutrition: Fall in fish catch threatens human health. *Nature* **2016**, *534*, 317–320. [CrossRef]

2. Sikorski, Z.E.; Bonnie, S.P.; Fereidoon, S. *Seafood Proteins*; Springer: New York, NY, USA, 1995.
3. FAO. *The State of World Fisheries and Aquaculture 2020: Sustainability in Action*; FAO: Rome, Italy, 2020.
4. Merino, G.; Barange, M.; Blanchard, J.L.; Harle, J.; Holmes, R.; Allen, I.; Allison, E.H.; Badjeck, M.C.; Dulvy, N.K.; Holt, J.; et al. Can marine fisheries and aquaculture meet fish demand from a growing human population in a changing climate? *Glob. Environ. Change* **2012**, *22*, 795–806. [CrossRef]
5. Béné, C.; Barange, M.; Subasinghe, R.; Pinstrup-Andersen, P.; Merino, G.; Hemre, G.I.; Williams, M. Feeding 9 billion by 2050—Putting fish back on the menu. *Food Secur.* **2015**, *7*, 261–274. [CrossRef]
6. Cashion, T.; Le Manach, F.; Zeller, D.; Pauly, D. Most fish destined for fishmeal production are food-grade fish. *Fish Fish.* **2017**, *18*, 837–844. [CrossRef]
7. Abbey, L.; Glover-Amengor, M.; Atikpo, M.O.; Atter, A.; Toppe, J. Nutrient content of fish powder from low value fish and fish byproducts. *Food Sci. Nutr.* **2016**, *5*, 374–379. [CrossRef]
8. Shaviklo, A.R. Development of fish protein powder as an ingredient for food applications: A review. *J. Food Sci. Technol.* **2015**, *52*, 648–661. [CrossRef]
9. Chalamaiah, M.; Dinesh kumar, B.; Hemalatha, R.; Jyothirmayi, T. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chem.* **2012**, *135*, 3020–3038. [CrossRef]
10. Barraza, F.A.A.; León, R.A.Q.; Álvarez, P.X.L. Kinetics of protein and textural changes in Atlantic salmon under frozen storage. *Food Chem.* **2015**, *182*, 120–127. [CrossRef]
11. Soladoye, O.P.; Juárez, M.L.; Aalhus, J.L.; Shand, P.; Estévez, M. Protein oxidation in processed meat: Mechanisms and potential implications on human health. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14*, 106–122. [CrossRef]
12. Sikorski, Z.E.; Kolakowska, A. Changes in protein in frozen stored fish. In *Seafood Proteins*; Springer: New York, NY, USA, 1994; pp. 99–112.
13. FAO. *The Production of Fishmeal and Oil*; FAO: Rome, Italy, 1986.
14. Nguyen, M.V.; Thorarinsdottir, K.A.; Gudmundsdottir, A.; Thorkelsson, G.; Arason, S. The effects of salt concentration on conformational changes in cod (*Gadus morhua*) proteins during brine salting. *Food Chem.* **2011**, *125*, 1013–1019. [CrossRef]
15. Lozano, G.; Hardisson, A. Fish | Fish as Food. In *Encyclopedia of Food Sciences and Nutrition*, 2nd ed.; Caballero, B., Ed.; Academic Press: Oxford, UK, 2003; pp. 2417–2423. [CrossRef]
16. Hilmarsdottir, G.S.; Ogmundarson, Ó.; Arason, S.; Gudjónsdóttir, M. Efficiency of fishmeal and fish oil processing of different pelagic fish species: Identification of processing steps for potential optimization toward protein production for human consumption. *J. Food Process. Preserv.* **2021**, *45*, e15294. [CrossRef]
17. Statistics Iceland. *Profitability in Fishing and Fish Processing 2020*; Statistics Iceland: Reykjavik, Iceland, 2021.
18. Hilmarsdottir, G.S.; Ogmundarson, O.; Arason, S.; Gudjónsdóttir, M. The effects of varying heat treatments on lipid composition during pelagic fishmeal production. *Processes* **2020**, *8*, 1142. [CrossRef]
19. Sveinsdóttir, H.I.; Karlsdóttir, M.G.; Arason, S.; Stefánsson, G.; Sone, I.; Skåra, T.; Rustad, T.; Larsson, K.; Undeland, I.; Gudjonsdottir, M. Effect of antioxidants on the sensory quality and physicochemical stability of Atlantic mackerel (*Scomber scombrus*) fillets during frozen storage. *Food Chem.* **2020**, *321*, 126744. [CrossRef]
20. Karovičová, J.; Kohajdová, Z. Biogenic amines in food. *Chem. Pap.* **2005**, *59*, 70–79.
21. Klausen, N.K.; Lund, E. Formation of biogenic amines in herring and mackerel. *Z. Lebensm. Unters. Forsch.* **1986**, *182*, 459–463. [CrossRef]
22. Ruiz-Capillas, C.; Herrero, A.M. Impact of biogenic amines on food quality and safety. *Foods* **2019**, *8*, 62. [CrossRef]
23. Visciano, P.; Schirone, M.; Paparella, A. An overview of histamine and other biogenic amines in fish and fish products. *Foods* **2020**, *9*, 1795. [CrossRef]
24. Rey-Mansilla, M.; Sotelo, C.G.; Gallardo, J.M. Decomposition of trimethylamine oxide during iced storage of blue whiting (*Micromesistius poutassou*). *Z. Lebensm. Unters. Forsch.* **1999**, *208*, 267–269. [CrossRef]
25. Einarsson, M.I.; Jokumsen, A.; Bæk, A.; Jacobsen, C.; Pedersen, S.A.; Samuelsen, T.A.; Pálsson, J.; Eliasen, O.; Flesland, O. *Nordic Centre of Excellence Network in Fishmeal and Fish Oil, 1670–7192*; Matís Ohf/Matis—Food Research, Innovation & Safety: Reykjavik, Iceland, 2019.
26. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917. [CrossRef]
27. Valentas, K.J.; Rotstein, E.; Singh, R.P. *Handbook of Food Engineering Practice*; CRC Press: New York, NY, USA, 1997.
28. Fellows, P.J. *Food Processing Technology: Principles and Practice*; Elsevier: Amsterdam, The Netherlands, 2009.
29. Kelleher, S.D.; Hultin, H.O. Lithium chloride as a preferred extractant of fish muscle proteins. *J. Food Sci.* **1991**, *56*, 315–317. [CrossRef]
30. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem* **1976**, *72*, 248–254. [CrossRef]
31. Olajos, I. Implementation and Verification of an Analytical Method for the Quantification of Biogenic Amines in Seafood Products. Master's Thesis, University of Iceland, Reykjavik, Iceland, 2015.
32. Malle, P.; Poumeyrol, M. A new chemical criterion for the quality control of fish: Trimethylamine/total volatile basic nitrogen (%). *J. Food Prot.* **1989**, *52*, 419–423. [CrossRef] [PubMed]

33. Baliño-Zuazo, L.; Barranco, A. A novel liquid chromatography–mass spectrometric method for the simultaneous determination of trimethylamine, dimethylamine and methylamine in fishery products. *Food Chem.* **2016**, *196*, 1207–1214. [CrossRef] [PubMed]
34. Egerton, S.; Mannion, D.; Culloty, S.; Whooley, J.; Stanton, C.; Ross, R.P. The proximate composition of three marine pelagic fish: Blue whiting (*Micromesistius poutassou*), boarfish (*Capros aper*) and Atlantic herring (*Clupea harengus*). *Irish J. Agric. Food Res.* **2020**, 185–200. [CrossRef]
35. Yeannes, M.I.; Almandos, M.E. Estimation of fish proximate composition starting from water content. *J. Food Compos. Anal.* **2003**, *16*, 81–92. [CrossRef]
36. Romotowska, P.E.; Karlsdóttir, M.G.; Gudjónsdóttir, M.; Kristinsson, H.G.; Arason, S. Seasonal and geographical variation in chemical composition and lipid stability of Atlantic mackerel (*Scomber scombrus*) caught in Icelandic waters. *J. Food Compos. Anal.* **2016**, *49*, 9–18. [CrossRef]
37. Sun, Q.; Senecal, A.; Chinachoti, P.; Faustman, C. Effect of water activity on lipid oxidation and protein solubility in freeze-dried beef during storage. *J. Food Sci.* **2002**, *67*, 2512–2516. [CrossRef]
38. Ariyawansa, S. *The Evaluation of Functional Properties of Fish Meal*; United Nations University, Fisheries Training Programme: Reykjavik, Iceland, 2000.
39. Ween, O.; Stangeland, J.K.; Fylling, T.S.; Aas, G.H. Nutritional and functional properties of fishmeal produced from fresh by-products of cod (*Gadus morhua* L.) and saithe (*Pollachius virens*). *Heliyon* **2017**, *3*, e00343. [CrossRef]
40. Cozzolino, D.; Chree, A.; Scaife, J.R.; Murray, I. Usefulness of near-infrared reflectance (NIR) spectroscopy and chemometrics to discriminate fishmeal batches made with different fish species. *J. Agric. Food Chem.* **2005**, *53*, 4459–4463. [CrossRef]
41. Cho, J.H.; Kim, I.H. Fish meal–nutritive value. *J. Anim. Physiol. Anim. Nutr.* **2011**, *95*, 685–692. [CrossRef]
42. de Koning, A.J. Quantitative quality tests for fish meal. II. An investigation of the quality of South African fish meals and the validity of a number of chemical quality indices. *Int. J. Food Prop.* **2002**, *5*, 495–507. [CrossRef]
43. Kasmiran, B.; Suvveb, N.V.; Jónsson, A. *Comparison and Evaluation of the Quality of Fish Oil and Fishmeal Extracted from the Heads of Yellowfin Tuna (Thunnus albacares) and Albacore Tuna (Thunnus alalunga)*; Final Project; Nations University Fisheries Training Programme: Reykjavik, Iceland, 2016.
44. Ustunol, Z. *Applied Food Protein Chemistry*; John Wiley & Sons: Hoboken, NJ, USA, 2014.
45. Zayas, J.F. *Functionality of Proteins in Food*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2012.
46. Derkach, S.R.; Grokhovsky, V.A.; Kuranova, L.K.; Volchenko, V.I. Nutrient analysis of underutilized fish species for the production of protein food. *Foods Raw Mater.* **2017**, *5*, 15–23. [CrossRef]
47. Yada, R.Y. *Proteins in Food Processing*; Woodhead Publishing: Cambridge, UK, 2004.
48. Gulati, P.; Li, A.; Holding, D.; Santra, D.; Zhang, Y.; Rose, D.J. Heating reduces proso millet protein digestibility via formation of hydrophobic aggregates. *J. Agric. Food Chem.* **2017**, *65*, 1952–1959. [CrossRef]
49. Skipnes, D.; Van der Plancken, I.; Van Loey, A.; Hendrickx, M.E. Kinetics of heat denaturation of proteins from farmed Atlantic cod (*Gadus morhua*). *J. Food Eng.* **2008**, *85*, 51–58. [CrossRef]
50. Yu, X.; Llave, Y.; Fukuoka, M.; Sakai, N. Estimation of color changes in fish surface at the beginning of grilling based on the degree of protein denaturation. *J. Food Eng.* **2014**, *129*, 12–20. [CrossRef]
51. Vieira, S.; Franco, A.R.; Fernandes, E.M.; Amorim, S.; Ferreira, H.; Pires, R.A.; Reis, R.L.; Martins, A.; Neves, N.M. Fish sarcoplasmic proteins as a high value marine material for wound dressing applications. *Colloids Surf. B Biointerfaces* **2018**, *167*, 310–317. [CrossRef]
52. Ghelichpour, M.; Shabanpour, B. The investigation of proximate composition and protein solubility in processed mullet fillets. *Int. Food Res. J.* **2011**, *18*, 1343.
53. Xu, Y.; Obielodan, M.; Sismour, E.; Arnett, A.; Alzahrani, S.; Zhang, B. Physicochemical, functional, thermal and structural properties of isolated Kabuli chickpea proteins as affected by processing approaches. *Int. J. Food Sci.* **2017**, *52*, 1147–1154. [CrossRef]
54. Singh, R.; Kumar, M.; Mittal, A.; Mehta, P.K. Microbial enzymes: Industrial progress in 21st century. *3 Biotech* **2016**, *6*, 1–15. [CrossRef]
55. Clemente, A. Enzymatic protein hydrolysates in human nutrition. *Trends Food Sci. Technol.* **2000**, *11*, 254–262. [CrossRef]
56. Fernandes, P. Enzymes in fish and seafood processing. *Front. Bioeng. Biotechnol.* **2016**, *4*, 59. [CrossRef]
57. Nilsang, S.; Lertsiri, S.; Suphantharika, M.; Assavanig, A. Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. *J. Food Eng.* **2005**, *70*, 571–578. [CrossRef]
58. Sikorski, Z.E.; Kotakowski, E. Endogenous enzyme activity and seafood quality: Influence of chilling, freezing, and other environmental factors. In *Seafood Enzymes Utilization and Influence on Postharvest Seafood Quality*; Marcel Dekker: New York, NY, USA, 2000; pp. 451–488.
59. Bulushi, I.A.; Poole, S.; Deeth, H.C.; Dykes, G.A. Biogenic amines in fish: Roles in intoxication, spoilage, and nitrosamine formation—A review. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 369–377. [CrossRef] [PubMed]
60. Halász, A.; Baráth, A.; Simon-Sarkadi, L.; Holzapfel, W. Biogenic amines and their production by microorganisms in food. *Trends Food Sci. Technol.* **1994**, *5*, 42–49. [CrossRef]
61. Pike, I.H.; Hardy, R.W. Standards for assessing quality of feed ingredients. In *Crustacean Nutrition—Advances in World Aquaculture*; D'Abramo, L., Conclin, D., Akiyama, D., Eds.; World Aquaculture Society: Sorrento, LA, USA, 1997; Volume 6, pp. 473–492.

62. Wu, T.H.; Nigg, J.D.; Stine, J.J.; Bechtel, P.J. Nutritional and chemical composition of by-product fractions produced from wet reduction of individual red salmon (*Oncorhynchus nerka*) heads and viscera. *J. Aquat. Food Prod. Technol.* **2011**, *20*, 183–195. [CrossRef]
63. Prester, L. Biogenic amines in fish, fish products and shellfish: A review. *Food Addit. Contam.* **2011**, *28*, 1547–1560. [CrossRef] [PubMed]
64. Santos, M.H.S. Biogenic amines: Their importance in foods. *Int. J. Food Microbiol.* **1996**, *29*, 213–231. [CrossRef]
65. Ruiz-Capillas, C.; Moral, A. Free amino acids and biogenic amines in red and white muscle of tuna stored in controlled atmospheres. *Amino Acids* **2004**, *26*, 125–132. [CrossRef]
66. Naila, A.; Flint, S.; Fletcher, G.; Bremer, P.; Meerdink, G. Control of biogenic amines in food—Existing and emerging approaches. *J. Food Sci.* **2010**, *75*, 139–150. [CrossRef]
67. Köse, S.; Quantick, P.; Hall, G. Changes in the levels of histamine during processing and storage of fish meal. *Anim. Feed Sci. Technol.* **2003**, *107*, 161–172. [CrossRef]
68. Mih, H.; Lacherai, A. Nutritional properties of fish meal produced from fresh by-products of *Sardina pilchardus*. *J. Fish. Environ.* **2020**, *44*, 16–23.
69. Aubourg, S.P.; Medina, I.; Gallardo, J.M. Quality assessment of blue whiting (*Micromesistius poutassou*) during chilled storage by monitoring lipid damages. *J. Agric. Food Chem.* **1998**, *46*, 3662–3666. [CrossRef]
70. Mizuguchi, T.; Kumazawa, K.; Yamashita, S.; Safey, J. Factors that accelerate dimethylamine formation in dark muscle of three gadoid species during frozen storage. *Fish Sci.* **2011**, *77*, 143–149. [CrossRef]
71. Howgate, P. A critical review of total volatile bases and trimethylamine as indices of freshness of fish. Part 2. Formation of the bases, and application in quality assurance. *Electron. J. Environ. Agric. Food Chem.* **2010**, *9*, 58–88.
72. Wu, T.H.; Bechtel, P.J. Ammonia, dimethylamine, trimethylamine, and trimethylamine oxide from raw and processed fish by-products. *J. Aquat. Food Prod. Technol.* **2008**, *17*, 27–38. [CrossRef]
73. Lin, J.K.; Hurng, D.C. Thermal conversion of trimethylamine-N-oxide to trimethylamine and dimethylamine in squids. *Food Chem. Toxicol.* **1985**, *23*, 579–583. [CrossRef]
74. Spinelli, J.; Koury, B. Nonenzymic formation of dimethylamine in dried fishery products. *J. Agric. Food Chem.* **1979**, *27*, 1104–1108. [CrossRef]
75. Sohn, M.; Ho, C.T. Ammonia generation during thermal degradation of amino acids. *J. Agric. Food Chem.* **1995**, *43*, 3001–3003. [CrossRef]
76. Biswal, B.K.; Huang, H.; Dai, J.; Chen, G.H.; Wu, D. Impact of low-thermal pretreatment on physicochemical properties of saline waste activated sludge, hydrolysis of organics and methane yield in anaerobic digestion. *Bioresour. Technol.* **2020**, *297*, 122423. [CrossRef]

Paper IV

Journal of Cleaner Production 343 (2022) 130880

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Identification of environmental hotspots in fishmeal and fish oil production towards the optimization of energy-related processes

Gudrun Svana Hilmarsdóttir [a,*], Ólafur Ögmundarson [a], Sigurjón Arason [a,b], María Gudjónsdóttir [a,b]

[a] University of Iceland, Faculty of Food Science and Nutrition, Aragata 14, 102, Reykjavík, Iceland
[b] Matís Ohf, Icelandic Food and Biotech R&D, Vínlandsleið 12, 113, Reykjavík, Iceland

ARTICLE INFO

Handling Editor: Bin Chen

Keywords:
Cleaner production
Cooking temperature
Fish oil
Fishmeal
Life-cycle assessment
Heavy fuel oil
Hydropower

ABSTRACT

This study assessed the environmental impacts of a pelagic fishmeal and fish oil production plant in Iceland with the life cycle assessment methodology. The study focused on assessing the effects of different energy sources for utility production due to the high energy intensity of fishmeal and fish oil production, as quality improved with lower cooking temperature. The environmental hotspots of three different processing scenarios were assessed, where the factory was run on hydropower (*Scenario 0*), heavy fuel (*Scenario 1*) and a composition of both (*Scenario 2*), from cradle-to-factory gate. Midpoint results showed that the raw material acquisition contributed the most to the environmental impact when the fishmeal factory was operating on hydropower. However, drying had the highest impact when heavy fuel oil was used for utility production. This study also demonstrated that lowering the cooking temperature from 90 to 85 °C, led to improved quality and simultaneously reduced environmental impacts during processing. This indicated that a small energy adjustment in the production can have an environmental gain, demonstrating the necessity to optimize each processing step in the fishmeal and fish oil production process both for increased product quality and minimizing environmental impacts.

1. Introduction

Fishmeal and fish oils are considered the most nutritious and digestible ingredients for farmed fish and are increasingly being used in specific production stages of aquaculture (FAO, 2020). Cut-offs and small pelagic species used for fishmeal and fish oil production intended for feed and generally have lower quality and value than fish for direct human consumption (FAO, 2020). Moreover, as the fishmeal and fish oil production process is energy-intensive (Smárason et al., 2017), opportunities remain of producing higher-value products with less energy and lower environmental impact. In Iceland, the most commonly processed pelagic species are capelin (*Mallotus villosus*) and blue whiting (*Micromesistius poutassou*) (Statistics Iceland, 2019), which are generally processed directly to fishmeal and fish oil, along with cut-offs from Atlantic mackerel (*Scomber scombrus*) and Atlantic herring (*Clupea harengus*) fillet production (Statistics Iceland, 2019). Furthermore, fishmeal production may also include other small pelagic species and by-catch (FAO, 2020), causing high variations and heterogeneity of the final product. Although the high variation of the raw material can result in processing challenges, few improvements have been made to the fishmeal and -oil processes throughout the decades (FAO, 1986) but the market demand is changing, calling for improved knowledge and optimized processing methods in the fishmeal industry, both for higher nutritional value and economic value (Einarsson et al., 2019).

Current trends in market demand for higher quality fishmeal and fish oil, both for aquaculture feed and for direct human consumption (FAO, 2020), which calls for an increase in quality from optimized fishmeal and fish oil production processes. High temperatures applied in different processing steps have shown to result in negative quality effects due to protein and lipid denaturation (Þorkelsson et al., 2009). In response to this, Hilmarsdottir et al. (2020) showed that lowering the cooking temperature from 90 °C to 85 °C led to improvements in fishmeal and oil quality. While there is a need for higher quality fishmeal products, there is also increased demand for environmentally sustainable products to minimize their environmental impacts. In fishmeal and fish oil production, the highest energy use occurs during cooking and drying (Smárason et al., 2017), which indicates that optimizing these processing steps could reduce the environmental impact of fishmeal and oil

* Corresponding author.
E-mail address: gsh9@hi.is (G.S. Hilmarsdóttir).

https://doi.org/10.1016/j.jclepro.2022.130880
Received 25 September 2021; Received in revised form 28 January 2022; Accepted 8 February 2022
Available online 16 February 2022

processing. Thus, assessing the environmental impact of process optimizations are an important step towards increased sustainability of the production.

When exploring literature assessing the environmental impacts of fishmeal and fish oil production, few studies can be found. The most relevant study assesses the environmental sustainability of fishmeal and fish oil factories in Peru with life-cycle assessment (LCA). Fréon et al. (2017)) concluded that to decrease the environmental impact of the Peruvian fishmeal industry, which included the use of natural gas instead of heavy fuel oil, modernization of the oldest processing plants, and production of higher quality fishmeal was necessary. Moreover, recommendations included an assessment of different energy sources used for operating the fishmeal factories (Fréon et al., 2017). The different energy sources affect the high global warming impacts connected to unsustainable energy sources and most other impact categories. Furthermore, the energy used to operate the fishmeal plants had the highest environmental contribution when comparing the usage, construction, and maintenance of the fishmeal and fish oil factories (Fréon et al., 2017).

Improving the primary production phase and feed sourcing practices have been reported to return the highest environmental improvement in the supply chain of exported, frozen tilapia products (Pelletier and Tyedmers, 2010). Hence, the supply chain, starting from raw material acquisition to all steps of the production process of fishmeal and fish oil, was investigated in the current study. Analyses of the literature revealed that assessment of environmental sustainability of fishmeal and fish oil production are generally assessed as part of other product supply chains, such as aquaculture feed. (Samuel-Fitwi et al., 2013) compared the impacts of different fishmeals in aquafeed, and the study showed that replacing trout feed with soy or rapeseed meal reduced global warming by 40% and acidification by 25%. This is among others due to the high energy intensity of fishmeal production. Crop-derived feed inputs are then reported to be less impactful than fish-based inputs (Pelletier and Tyedmers, 2010). Other potential means to reduce the environmental impact of aquafeed include increasing the feed efficiency, such as using fishmeal from by-products from other processes instead of using fish directly caught for aquafeed applications (Papatryphon et al., 2004). Furthermore, recycling nutrients has been reported as one of the key roles in improving environmental performance in aquaculture (Pelletier and Tyedmers, 2010), lowering stressing the importance of using fish industry side-streams and by-products for fishmeal and fish oil production.

Energy usage within the fishmeal and fish oil factories has not yet been studied thoroughly, although rough fuel consumption estimates during fishmeal production (FAO, 1986) and raw material acquisition in Norway (Schau et al., 2009) are available. As quality is the driving force of each product, process adjustment resulting in a higher value product, such as lowering the cooking temperature (Hilmarsdottir et al., 2020), could benefit the environment. In addition to lower heat-treatment during fishmeal and fish oil processing, the usage of green energy sources and fossil-based energy sources has not yet been compared, although there is a potential for a sustainable solution and hence, cleaner production.

In Europe, most fishmeal factories operate partially or totally on heavy fuel oil, while in Iceland, most factories are fuelled with greener energy sources, such as hydropower, or a mix of fossil-based and green energy (Table 1). Given the fact that fishmeal production is energy-intensive, opportunities lie in changing their energy source for environmental gains. Due to the strong influence of the energy sources chosen during the operational time of the fishmeal and fish oil plants, decreasing the cooking temperature by 5 °C, in addition to hotspot analysis, would give a clearer view of the processing steps needing optimization (Hilmarsdottir et al., 2020). As the fishmeal and fish oil production process is energy-intensive (Smárason et al., 2017), from 265 to 576 kg CO_2 eq per 1 tonne fishmeal (Fréon et al., 2017), a relatively small adjustment within the energy usage of the factory could result in a significant environmental gain, even with simple solutions that require small investments (Thrane et al., 2009). Moreover, before changing the production process drastically, it is necessary to identify the future optimization potential of the fishmeal plants. As the fishmeal production process needs optimization, investigating the changes at an early stage of the development by applying a life cycle assessment (LCA) is highly recommended (Ögmundarson et al., 2020), prioritizing the processes that require optimization. A concurrent increase in product quality needs to be secured, making this an iterative process where product quality and environmental sustainability go hand in hand.

Table 1
Fuel types used in the fishmeal and fish oil industry in 2019, depending on the country. Factories can operate on more than one fuel type. The table is adapted and updated from (EUfishmeal, 2019).

Country	Factories	Electricity	Oil	Gas	Other
Iceland	10	5	5		
Norway	6		4	3	1 (LPG)
Denmark	3			3	1 (Coal)
UK	3		1	2	
France	2				2 (External Power)
Faroe Island	2		2		
Germany	1			1	
Ireland	1		1		
Finland	1		1		
Total	**29**	**5**	**14**	**9**	**4**

The main goal of this study was to assess the environmental impacts of fishmeal and fish oil production from the cradle-to-factory gate. This research identifies the environmental benefits of energy adjustment during production processes in a fishmeal and oil factory in Iceland. A hotspot analysis was conducted on this base case to identify the future optimization potential of the analyzed fishmeal and fish oil production process. The potential environmental benefits of changing the energy sources from heavy fuel oil to hydropower were also investigated, as most European countries still operate on heavy fuel oil (Table 1) (EUfishmeal, 2019).

2. Materials and methods

The framework followed in the current study was in accordance to International Standard Organization (ISO) 14040 (ISO, 2006, p. 14040) and 14044 (ISO, 2006, p. 14044) and addressed the four mandatory steps when conducting an LCA study; the goal and scope definition phase, the inventory analysis phase, the impact assessment phase, and the interpretation phase (Hauschild et al., 2017). In addition, calculations of the mass and energy flow of the production process gave estimations of the energy and power consumption, where the samples used to build the mass balance were collected first-hand.

2.1. Research objectives

This study aimed to:

1) assess the environmental impacts of fishmeal and fish oil production from cradle-to-gate with a focus on energy sources,
2) identify the environmental benefits of energy adjustments during processing steps while increasing product quality,
3) identify future optimization potentials of the fishmeal and fish oil production process by using hotspot analysis, and
4) provide a detailed life cycle inventory (LCI), which aims to link all unit processes required to produce the fishmeal and fish oil.

The functional unit assessed was "the production of 1000 kg of pelagic fishmeal including fish oil from cradle to factory gate, produced in Iceland in 2018" and included three scenarios depending on theoretical application of different energy sources during the production of

fishmeal and fish oil.

2.2. The fishmeal and fish oil process description

An overview of a traditional pelagic fishmeal and fish oil process with an average of 1200 tonnes of raw material entering the production each day can be seen in Fig. 1. The raw materials entered a preheating step (55 °C for 20 min), which used excess energy from the steam-drier and the evaporators to lower the energy cost (Einarsson et al., 2019). Then, the raw materials were cooked (at 90 °C for 20 min) and drained for water removal. The resulting liquid stream was treated with a decanter to remove the remaining solids and concentrated further. Oil was recovered from the liquid stream through three centrifuges, and the solid streams, which were obtained from the press, decanter, and evaporators, were mixed during the initial drying steps. The steam-drying included a rotary disc steam dryer lowering the water content to 40%, by applying a steam temperature of 160 °C and a drying temperature of 95 °C for 25–35 min. The air dryer used was a Hetland air dryer, which decreased the water content to 5–10% water, with input air at 450 °C, although having 150 °C in the middle of the dryer.

Samples were collected during a steady-state of the production line and cooled overnight at 0 ± 2 °C, followed by transport to the laboratory where it was kept at −25 °C until further analysis. Analyzing the samples took up to seven months after collection, and all samples were measured in triplicates.

2.3. Data collection and system modeling

2.3.1. Energy use during raw material acquisition

The energy usage at sea during fishing of the studied pelagic species was estimated as the average energy use for all trips from one trawler during the capelin fishing season in 2018. The ratio between sailing towards the catching ground, fishing and chilling, and sailing back to shore was compared and aligned with the annual energy consumption. The fishing vessel assessed was one of the younger fishing vessels in the Icelandic pelagic fishing fleet (from 2014). Hence, the energy usage during the raw material acquisition at sea might be underestimated in the current study as the average fishing vessel age in the Icelandic fishing fleet is currently around 21 years old (The Directorate of Fisheries, 2020).

2.3.2. Mass and energy balances during fishmeal and fish oil processing

Samples from each processing step in the fishmeal and fish oil production were collected and analyzed for water and lipid content, and the remaining material expressed as fat-free dry matter (FFDM) during the production of fishmeal and fish oil from capelin (C), a blend of mackerel and herring (MHB) and blue whiting (BW), respectively. However, during the blue whiting (BW) production, data included only first-hand chemical composition results from the raw material, press (liquid and cake), separate press liquid, sludge, concentrate, fishmeal and fish oil. Other sampling locations were modeled according to the capelin production, as the BW production was expected to perform in a similar way due to similar lipid content of capelin and blue whiting.

The mass balances during the fishmeal and oil productions were set up and modeled through gathered data on the total mass, water, lipid and fat-free dry matter (FFDM) composition at each sampling location for the three different pelagic species (Hilmarsdottir et al., 2021), and at different cooking temperatures (Hilmarsdottir et al., 2020). A functional production unit of 1000 kg fishmeal and fish oil was assumed in each scenario. The quantity of each processing stream was modeled during production of C and MHB, as well as at a few key sampling places during production of BW as mentioned earlier. The energy consumption of each processing step was calculated based on the mass flow and balances along with known heat transfer equations explained in both Fellows (2000) and Geankoplis (1993) during the fishmeal and fish oil (kW) production from the different species and at the different cooking temperatures. The time during each processing step was then included in the assessment of the power consumption (kWh) of each processing step. All calculated values were aligned with documented energy and power usage from the company's open green reports for 2018 (Síldarvinnslan, 2018). The obtained modeled power values were used in the following LCA calculations.

The preheating and draining steps were not considered in the LCA calculations as excess energy from the steam dryer and the evaporators were used for the preheating, and draining does not require energy as it is a sieving process.

The annual use in 2018 of chemical agents, materials and energy per functional production unit (1000 kg fishmeal) are open to the public by the Environment Agency of Iceland stated (Síldarvinnslan, 2018). Staff members from the fishmeal and fish oil plant studied estimated annual reparations of the fishing gear used during capelin fishing, used in the raw material acquisition.

2.4. LCA system boundaries

For a successful life cycle assessment, it was necessary to define the system boundaries and which variables were included in the assessment (Fig. 2). In the current study, the system boundaries were defined to include the catching of the capelin raw material, emissions, and energy use on the trawlers (sailing towards the catching ground, during catching and superchilling, and sailing towards shore), as well as the mass of raw materials entering the fishmeal and fish oil production process on land (Raw material acquisition). Fishing gear and other material usages on board were inside the system boundaries and included the use of nylon, hydraulic fracturing fluid, lubricating oil, and fuel (heavy fuel oil) (see *Appendix*, Table A.1).

As the assessed fishmeal and fish oil plant studied is almost 50 years old, and it is estimated that around 1.2 million tonnes of fishmeal have been produced during its lifetime to date, it was assumed that including the plant itself was negligible. Hence, the construction of the fishmeal and fish oil facilities were considered outside the system boundaries. Cleaning, waste and maintenance were assessed within the system boundary.

The backup energy generator was assumed to power the whole fishmeal and fish oil production process for short periods (Síldarvinnslan, 2018), e.g. during bad weather conditions. However, if the energy

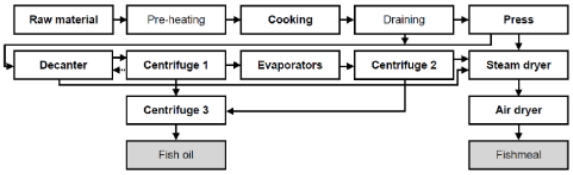


Fig. 1. A traditional fishmeal and fish oil production process. (Hilmarsdottir et al., 2020).

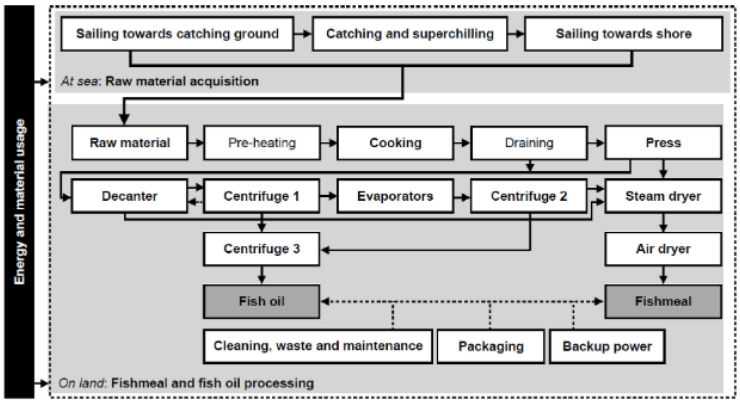


Fig. 2. Process flow of the production system, identifying the system boundaries of the life cycle assessment during production of 1000 kg of pelagic fishmeal, including fish oil from cradle to factory gate, in Iceland in 2018.

consumption of the generator was distributed evenly during processing, the heavy fuel oil impact would be lost, and was hence assigned as a separate process step.

2.5. Life cycle impact assessment (LCIA)

The Life Cycle Assessment (LCA) calculations were modeled with the SimaPro version 9.1.0.8 software (PReConsultants, Amersfoort, Netherlands) in connection to the ecoinvent 3.6 life cycle inventory. SimaPro modeled from the average energy of the functional unit, products and chemicals used at each step within the system boundary (Fig. 2). In the current study, the impact assessment method used was midpoint level ReCiPe 2016. The following impact categories were included in the assessment: global warming, stratospheric ozone depletion, ionizing radiation, ozone formation, human toxicity, fine particulate matter impacts, tropospheric ozone formation, acidification, eutrophication, ecotoxicity, land use, resources depletion, and water consumption. Midpoint impact categories were used to identify environmental hotspots across different life cycle stages and identifying the most contributing mass and energy inputs and outputs at each production step (Ögmundarson et al., 2020), which has effectively been applied earlier to evaluate the impact of variating protein sources in aquafeed (Samuel-Fitwi et al., 2013).

2.6. Analyzed energy source scenarios

Three energy source scenarios were modeled and analyzed in this study. For all scenarios, the same raw material acquisition was included. The energy source in Scenario 0 was 100% hydropower. As the assessed fishmeal and fish oil production factory was in 2018 the only known fishmeal plant operating on 100% hydropower worldwide, two different scenarios were also set up to assess the effects of fuel choice on the environmental impact of the production process. Hence, two scenarios were added to assess the most common energy source in European fishmeal and fish oil factories (Table 1), and the average energy source combination of Icelandic fishmeal and fish oil factories according to the Icelandic Union of Fishmeal factories (see Appendix, Table A.1). Scenario 1 thus only ran on heavy fuel oil, and Scenario 2 with a combination of 24.6% heavy fuel oil and 75.4% hydropower. The energy was measured in kWh in all three scenarios.

2.7. Uncertainty and sensitivity

The uncertainty of the assessment results was calculated by performing a Monte Carlo simulation using a Pedigree matrix approach (Hauschild et al., 2017). The predefined uncertainty factories in ecoinvent 3.6 were used, except for acetic acid, which was manually added as it was missing in the ecoinvent 3.6 databases (see Appendix, Fig. A.1). The number of simulations performed in the Monte Carlo simulation was 5000 runs to assess the 2.5%–97.5% confidence intervals of the results.

Five sensitivity scenarios were identified to evaluate the sensitivity of the results to changes in the system modeling and settings, as summarised in Table 2. The sensitivity level included various potentially influencing factors during raw material acquisition and fishmeal and fish oil production.

Oil usage during raw material acquisition differs on various factors such as the vessel's age, time spent on the ocean, different captains,

Table 2
Sensitivity scenarios at different processing steps when producing 1000 kg of fishmeal, including fish oil.

Sensitivity scenario	Scenario description
Raw material acquisition The fishmeal and fish oil processing	• Different fishing gear applied, weather conditions, catch size, time spent on the ocean, captain • Depending on the freshness of the raw material, water content could fluctuate (depending on catch and species), and hence the drying steps differ in time and energy • During cooking, the raw material can be heterogeneous and differ in freshness, and the breakdown of the raw material can hence be affected. If the raw material is too fresh, it can be difficult to process • During evaporation, the number of solid particles in the evaporation can differ, affecting the viscosity of the streams • Cleaning agents might be difficult to monitor and different depending on fishmeal factories. Moreover, other cleaning agents might be used in different scenarios.

catch size, trawling time, and weather conditions (Table 2). Hence, the average oil usage during seven fishing trips from one fishing vessel was applied in the calculations. During these fishing trips the fishing vessel was operated with only two captains and during the same season to keep the variations at minimum.

The raw material entering the fishmeal and fish oil production plant can variate in various factors of the raw materials, including variations in water, lipids and other chemical composition factors (Hilmarsdottir et al., 2020), including seasonal variation (Romotowska et al., 2016) (Table 3). Those factors affect the efficiency of the processing steps, e.g., the drying can differ in time and energy depending on the water content. According to the staff members operating the studied fishmeal factory, processes oriented towards homogenizing the material depend on the freshness of the raw material, where too fresh raw material can be more viscous than older raw material and hence, difficult to handle. Evaporation could also increase viscosity due to variate amounts of solid particles in the stream (Hall, 2010). Cleaning agents can variate between years and processing plants, but the amount of chemicals used is not closely monitored. Moreover, it is assumed that the fishmeal and fish oil production plants do not save chemical cleaning agents when it comes to cleaning due to the strict regulations regarding hygienic standards.

2.8. Statistical analysis

Statistical analyses were performed in Microsoft Office 365 with Excel (Microsoft, Redmond, WA, USA). Results were shown as mean values ± standard deviation (SD), and the significance level was set to $p < 0.05$ to prove with 95% certainty if the theory being investigated was significant or not due to change. This level of significance is commonly used for the assessment of biological processes such as those encountered during food and feed production.

3. Results and discussion

3.1. Midpoint analysis of the raw material acquisition

The midpoint analysis results of the raw material acquisition can be seen in Table 4. This life cycle stage was divided into three different substages, as is commonly done by the industry to assess monetary costs related to fishing. The substages were divided into i) sailing towards catching ground, ii) catching and superchilling of the catch, and iii) sailing towards shore. The raw material acquisition was identical for all three scenarios studied (see section 2.6). A hotspot analysis identified which of the life cycle stages of the raw material acquisition contributed most to each impact category. The highest contributing process was burning heavy fuel oil (diesel) to motor the fishing vessel, where sailing towards the shore was the most energy-intensive part of the raw material acquisition.

During the raw material acquisition at sea (Fig. 2), most of the fuel was spent sailing towards shore with the catch, or on average, 44 ± 13% of the total fuel usage (Table 4). A significant difference in fuel usage was also identified when analyzing the different fishing gear used when catching and superchilling capelin. Using a purse seiner resulted in lower average fuel use (17 ± 7% of the total fuel usage) than trawling (31 ± 4% of the total fuel usage). However, sailing towards shore did not result in a significant difference, despite the resistance of the water during trawling. For the fishing trips, see details in Appendix, Table A.3.

The effect of different fishing gear and fuel usage has been studied before, but high variations can be seen in fuel usage, according to the chosen fishing gear, origin of catch and species caught Trawling is for example generally considered more energy-intensive compared to purse seiner (Schau et al., 2009). Cashion et al. (2017) estimated a carbon dioxide equivalent release of 1.34 $CO_{2\ eq}$ per 1000 kg of capelin (Mallotus villosus) caught with a pelagic trawl. In the current study, the total impact results on global warming were 3.2×10^2 $CO_{2\ eq}$ during catching of capelin, producing 1000 kg of fishmeal and fish oil, which was higher than expected. However, indications towards the purse seiner having a lower carbon footprint than the trawl were observed in both studies (Cashion et al., 2017). Moreover, the fuel use differed significantly between fishing gear in the current study (Table 5) and can be seen in detail in Appendix, Table A.2).

Table 3
Sensitivity scenarios at different processing steps when producing 1 tonne of fishmeal, including fish oil.

Sensitivity scenario	Scenario description
Raw material acquisition	Different fishing gear applied, weather conditions, catch size, time spent on the ocean, captain
The fishmeal and fish oil processing	Depending on the freshness of the raw material, water content could fluctuate (depending on catch and species), and hence the drying steps differ in time and energy During cooking, the raw material can be heterogeneous and differ in freshness, and the breakdown of the raw material can hence be affected. If the raw material is too fresh, it can be difficult to process During evaporation, the number of solid particles in the evaporation can differ, affecting the viscosity of the streams Cleaning agents might be difficult to monitor and different depending on fishmeal factories. Moreover, other cleaning agents might be used in different scenarios.

3.2. Power usage analysis of the fishmeal and fish oil processing

The mass and energy flow was obtained from chemical composition results from all processing step during the capelin and mackerel/herring blend fishmeal productions, and from key processing locations in the blue whiting fishmeal production. The mass and energy flow from the draining, slurry, stickwater, separated press oil, centrifuged oil, and the latter concentrate were modeled for the blue whiting. The quantity of each process stream was modeled to fit the functional production unit of 1000 kg of fishmeal in each process (modeled values are expressed in italic font in Fig. 3).

The energy usage was calculated from the mass balance, and as different inputs of raw material entered the process, the energy differs between the species processed. The capacity of the steam separator connected to the evaporator was 4.5 tonnes per hour, and hence the energy was calculated on an hourly basis. Next, the annual power consumption per 1000 kg fishmeal and fish oil, from the studied company (Síldarvinnslan, 2018) was aligned with the calculated values where the production was estimated to run for 3 h on average per day. The power to heat the raw material from preheating (50 °C) to 85 °C was compared to the effects of the 90 °C heating during cooking, which affected the power ratio of each processing step (Table 6). The preheating step was not included in the power and energy calculations of the functional unit, as excess steam from the evaporators and the steam dryer was recycled to heat the raw materials from an ambient temperature to approximately 50 °C. The capacity of the steam separator in the evaporator and the amount of raw materials hence, variated.

The processing steps are displayed in Table 6. The drying steps consumed the most energy (see Appendix, Table A.4), followed by evaporation, pressing, decanters, cookers, and centrifuges. During the production of fishmeal and fish oil, the power difference using 85 °C in the cookers instead of 90 °C was lowered by 11–12% during cooking, resulting in different energy distribution among the processing steps overall (Table 6). The power consumption in each step in kWh is summarised in Appendix, Table A.5.

The average overall fishmeal yield in the fishmeal and fish oil production company studied was 18.5% and 5.7%, respectively, in 2018 according to calculations from the green accounting reports and ranged from 16 to 20%, while the fish oil yield ranged between 0.5 and 17%, depending on the season and catch (Table 6).

Table 4
Midpoint analysis of the energy use during raw material acquisition at sea, and their contribution to each impact category. Darker colours represent a higher environmental impact.

Raw material acquisition	Total energy use (2.5^{th}-97.5^{th}%)	Unit	Sailing towards catching ground	Cathing and superchilling	Sailing towards shore
Global warming	3.2×10^{1} (3.1×10^{0}–3.4×10^{2})	kg CO_2 eq	$9.7\times10^{0}\pm3.0\times10^{0}$	$8.1\times10^{1}\pm3.0\times10^{0}$	$1.4\times10^{0}\pm4.3\times10^{0}$
Stratospheric ozone depletion	9.5×10^{-6} (6.0×10^{-6}–1.7×10^{-4})	kg CFC11 eq	$2.9\times10^{-6}\pm8.8\times10^{-6}$	$2.4\times10^{-5}\pm8.9\times10^{-6}$	$4.2\times10^{-6}\pm1.3\times10^{-5}$
Ionizing radiation	2.9 (1.2–6.5)	kBq Co-60 eq	$8.8\times10^{-1}\pm2.7\times10^{-1}$	$7.4\times10^{-1}\pm2.7\times10^{-1}$	$1.3\pm3.9\times10^{-1}$
Ozone formation, Human health	7.0 (4.7–1×10^{1})	kg NO_x eq	$2.1\pm6.5\times10^{-1}$	$1.8\pm6.6\times10^{-1}$	$3.1\pm9.5\times10^{-1}$
Fine particulate matter formation	2.3 (2.0-2.6)	kg PM2.5 eq	$6.8\times10^{-1}\pm2.1\times10^{-1}$	$5.7\times10^{-1}\pm2.1\times10^{-1}$	$1.0\pm3.0\times10^{-1}$
Ozone formation, Terrestrial ecosystems	7.1 (4.8-1×10^{1})	kg NO_x eq	$2.1\pm6.5\times10^{-1}$	$1.8\pm6.6\times10^{-1}$	$3.1\pm9.5\times10^{-1}$
Terrestrial acidification	7.2 (6.3-8.3)	kg SO_2 eq	$2.2\pm6.6\times10^{-1}$	$1.8\pm6.7\times10^{-1}$	$3.2\pm9.6\times10^{-1}$
Freshwater eutrophication	4.0×10^{-3} (1.8×10^{-3}–9.1×10^{-3})	kg P eq	$1.2\times10^{-3}\pm3.7\times10^{-4}$	$1.0\times10^{-3}\pm3.7\times10^{-4}$	$1.8\times10^{-3}\pm5.4\times10^{-4}$
Marine eutrophication	3.6×10^{-4} (2.8×10^{-4}-5.0×10^{-4})	kg N eq	$1.1\times10^{-4}\pm3.3\times10^{-5}$	$9.1\times10^{-5}\pm3.4\times10^{-5}$	$1.6\times10^{-4}\pm4.9\times10^{-5}$
Terrestrial ecotoxicity	2.8×10^{2} (1.6×10^{2}-5.5×10^{2})	kg 1,4-DCB	$8.4\times10^{1}\pm2.6\times10^{1}$	$7.0\times10^{1}\pm2.6\times10^{1}$	$1.2\times10^{2}\pm3.7\times10^{1}$
Freshwater ecotoxicity	7.0×10^{-1} (5.1×10^{-1}-1.0)	kg 1,4-DCB	$2.1\times10^{-1}\pm6.5\times10^{-2}$	$1.8\times10^{-1}\pm6.5\times10^{-2}$	$3.1\times10^{-1}\pm9.4\times10^{-2}$
Marine ecotoxicity	1.2 (9.2×10^{-1}-1.8)	kg 1,4-DCB	$3.7\times10^{-1}\pm1.1\times10^{-1}$	$3.1\times10^{-1}\pm1.1\times10^{-1}$	$5.5\times10^{-1}\pm1.7\times10^{-1}$
Human carcinogenic toxicity	1.4 (9.1×10^{-1}-2.4)	kg 1,4-DCB	$4.2\times10^{-1}\pm1.3\times10^{-1}$	$3.5\times10^{-1}\pm1.3\times10^{-1}$	$6.2\times10^{-1}\pm1.9\times10^{-1}$
Human non-carcinogenic toxicity	2.0×10^{1} (1.3×10^{1}-3.3×10^{1})	kg 1,4-DCB	6.0±1.8	5.0±1.8	8.8±2.7
Land use	5.3×10^{-1} (3.6×10^{-1}-8.4×10^{-1})	m^2a crop eq	$1.6\times10^{-1}\pm4.9\times10^{-2}$	$1.3\times10^{-1}\pm5.0\times10^{-2}$	$2.4\times10^{-1}\pm7.1\times10^{-2}$
Mineral resource scarcity	1.0×10^{-1} (6.9×10^{-2}-1.7×10^{-1})	kg Cu eq	$3.2\times10^{-2}\pm9.6\times10^{-3}$	$2.6\times10^{-2}\pm9.7\times10^{-3}$	$4.6\times10^{-2}\pm1.4\times10^{-2}$
Fossil resource scarcity	1.0×10^{2} (6.8×10^{1}-1.2×10^{2})	kg oil eq	$3.1\times10^{1}\pm9.4$	$2.6\times10^{1}\pm9.5$	$4.5\times10^{1}\pm1.4\times10^{1}$
Water consumption	1.5×10^{1} (7.3-2.1×10^{1})	m^3	4.5±1.4	3.8±1.4	6.6±2.0

Abbreviations: CO_2=carbon dioxide, Eq=equivalent, CFC11=trichlorofluoromethane or freon-11, Co-60=cobalt isotope ^{60}Co, NO_x=nitrogen oxide, PM2.5=fine particulate matter less than 2.5 micrometers, SO_2=sulfur dioxide, P=phosphorus, N=nitrogen, 1,4-DCB=1,4 dichnolorbenzene, Cu=copper

Table 5
Results from fuel usage and time spent on the ocean when catching capelin in 2018 using two different fishing gears.

Dates from capelin catching from one vessel in 2018	Fuel usage			Time		
	Sailing towards catching ground	Catching and superchilling	Sailing towards shore	Sailing towards catching ground	Catching and superchilling	Sailing towards shore
Average fuel and time with trawl	30 ± 3%	31 ± 4%[a]	39 ± 6%	19 ± 7%	69 ± 5%	11 ± 2%
Average fuel and time with purse seiner	31 ± 15%	17 ± 7%[b]	52 ± 16%	25 ± 11%	60 ± 13%	15 ± 14%
Overal average fuel and time	30 ± 9%	25 ± 9%	44 ± 13%	22 ± 9%	65 ± 10%	13 ± 8%

Similar power usage has been reported by Hall (2010) during fish-meal and fish oil production. The power usage during cooking, evapo-ration and drying were compared between the studies, as information on other steps were lacking (Hall, 2010). The comparison indicated higher power usage during evaporation, or 52% of the total energy, whereas, in the current study, evaporation accounted for 16–21% of the total power consumption. Cooking accounted for 4.5–7% of the total energy in the current finding, while cooking accounted for 10% in the study by (Hall, 2010). Moreover, drying accounted for 46–55% in the current study, while 38% was reported by (Hall, 2010). However, the current energy usage was estimated into power due to the different operating times of each operation, followed by an estimation of operating time per day. The power usage for other processing steps (excluding cookers, evaporators and dryers) during March 2018 was 564 kWh. However, when power estimations (kWh) obtained from green accounting reports of annual usage were aligned with the mass flow, the factory was estimated to operate for 6 h per day on average in 2018.

3.3. Midpoint analysis of different energy source scenarios

Different trends were noticed in the midpoint analysis results on which processing steps affect the different impact categories depending on the energy source in each of the Scenarios (Fig. 3). Energy-related processes affected the impact categories less in Scenario 0 compared to the other scenarios. Therefore, cleaning agents, waste and mainte-nance resulted in the highest environmental impact in most of the impact categories in Scenario 0. Furthermore, the ratio of cleaning agents, waste and maintenance was higher than all other processing steps combined in stratospheric ozone depletion, ionizing radiation, fine particulate matter formation, terrestrial acidification, freshwater- and marine eutrophication, freshwater- and marine ecotoxicity, human non-carcinogenic toxicity, land use, and fossil resource scarcity.

Analysis of the heavy fuel oil-driven fishmeal and fish oil production process (Scenario 1) showed that cleaning, waste, and maintenance remained highest in freshwater- and marine eutrophication, freshwater ecotoxicity, and water consumption, emphasizing the environmental gain of operating the fishmeal and fish oil factory on hydropower instead of fossil fuels (Fig. 3). Combined drying steps accounted for the highest 54%, evaporation highest 16%, cooking up to 5%, and other processes combined up to 7% of the total environmental impact in each impact category. Similar to Scenario 0, combined drying steps accounted for 54% of global warming, followed by evaporation (16%), while cleaning, waste, and maintenance accounted for 2%.

In Scenario 2, analysis of the fishmeal and fish oil process operated partially on hydropower (75.4%) and partially on heavy fuel oil (24.6%) resulted in similar trends as in Scenario 0 and Scenario 1 (Fig. 4). Freshwater- and marine eutrophication, and freshwater ecotoxicity remained the highest environmental contributors during cleaning, waste and maintenance as in the other Scenarios, where the drying steps remained the environmental contributors in global warming (51%) followed by evaporation (15%), while 5% resulted from cleaning, waste, and maintenance, as in Scenario 1.

3.3.1. Optimisation of the cooking step during fishmeal and fish oil processing

The environmental benefits of lowering the cooking temperature

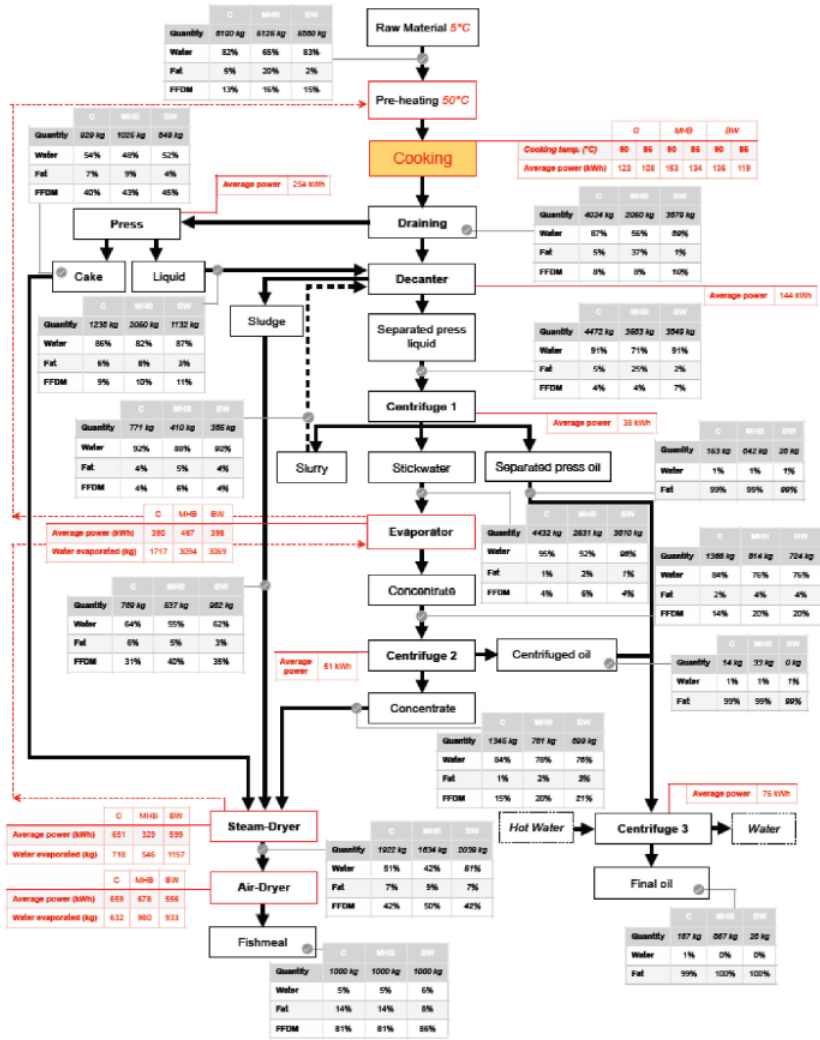


Fig. 3. Flowchart of the investigated traditional fishmeal and fish oil production process, including mass and energy balances of 1000 kg of fishmeal and fish oil production from capelin (C), a blend of mackerel and herring cut offs (MHB), and blue whiting (BW). Measured values were water, lipids and fat free dry matter (FFDM) and applied power (kWh) and water evaporated (kg) modeled for 90 °C cooking temperature and presented with red color. Dashed lines indicate reused stream and italic letters modeled values.

Table 6
Power usage at each processing step of the total power consumption during fishmeal and fish oil production at 90 °C and 85 °C cooking temperature, respectively. Fishmeal and fish oil yield percentages were calculated.

Power usage of processing steps (% of total energy)	Capelin		Mackerel/ herring blend		Blue whiting	
	90 °C	85 °C	90 °C	85 °C	90 °C	85 °C
Cookers	5.1	4.5	7.0	6.2	6.0	5.3
Presses	10.7		11.6–11.7		11.3–11.4	
Decanters	6.0–6.1		6.6		6.4	
Centrifuges 1	1.6		1.7–1.8		1.7	
Evaporator	16.3–16.5		21.3–21.5		17.7–17.8	
Centrifuges 2	2.1		2.3		2.3	
Steam dryer	27.3–27.4		15.0–15.1		26.6–26.8	
Air dryer	27.8		31.2		24.8	
Centrifuges 3	3.2		3.5		3.4	
Fishmeal yield	16%		20%		18%	
Fish oil yield	3%		17%		0.5%	

from 90 °C to 85 °C were analyzed based on a higher quality of the fishmeal obtained at 85 °C (Hilmarsdottir et al., 2020). While the increased physicochemical quality in the fishmeal was tested and presented in Hilmarsdottir et al. (2020), no studies have estimated the environmental impact of changing the cooking temperature during fishmeal and oil processing.

The overall gain by decreasing the cooking temperature from 90 °C to 85 °C, in each Scenario can be seen in Table 7. The highest gain over all the impact categories was observed in Scenario 1 (heavy fuel oil), where 4.6 kg CO_2 eq was saved by the temperature reduction, or 0.6% of global warming impact the production. If capelin caught in Iceland in 2018 accounted for 186 000 tonnes (The Marine and Freshwater Research Institute, 2019), 205 kg CO_2 eq would thus be saved annually if all fishmeal and fish oil factories in Iceland would decrease their cooking temperature from 90 °C to 85 °C during the capelin season (Scenario 2).

Reducing the cooking temperature resulted in an overall environmental gain in all energy sources studied, and higher quality fishmeal and fish oil (Hilmarsdottir et al., 2020). Therefore, a cooking temperature of 85 °C instead of 90 °C is proposed for all studied species and energy source scenarios. Until recently, common practice has been 90–95 °C (FAO, 1986). However, heating above 75 °C has not resulted in a higher fat separation (FAO, 1986), and moreover, improved fat separation in capelin has been reported at 70–80 °C (Nygaard, 2010). Parallel to environmental gain with lower temperatures, less overheating is assumed, resulting in higher quality products. A reduction in the use of cleaning agents can also be assumed as the energy efficiency during cooking can be increased if product build-up on processing equipment surfaces due to overheating can be minimized (Hall, 2010). Therefore, an investigation into fishmeal and fish oil quality and their environmental impact at lower temperatures could be subject for a follow-up study.

3.4. Midpoint analysis of the effect of cleaning, waste and maintenance

Midpoint analysis on the production processes showed that the evaporation, drying steps, and cleaning, waste and maintenance, had the highest environmental impacts across all energy sources studied. Fuel use has been reported to dominate climate change (87%) during fishmeal and fish oil production (Fréon et al., 2017), while higher emphasis should also be on chemical agents.

The overall highest impact from the cleaning agents, waste and maintenance, came from the use of acetic acid, followed by sodium hydroxide usage in all impact categories, except for nitric acid having the highest impact on the stratospheric ozone depletion (80%), and municipal solid waste on the marine eutrophication (Table 8). Other processes included iron scrap, formaldehyde, hydrochloric acid, and sodium hypochlorite, which impact was below 6% in all impact categories. Cleaning, waste and maintenance remained the same in all Scenarios.

3.5. Hotspot analysis of different fuel source scenarios

Analysis of midpoint evaluation in Scenario 0 (Table 9), fishmeal and fish oil production operated entirely on hydropower, showed that most environmental impacts originated from the raw material acquisition, followed by the fishmeal and fish oil processing, and the usage of cleaning agents, waste and maintenance. Packaging materials and the backup power (operated on heavy fuel oil) did not appear as hotspots for any of the assessed impact categories. However, packaging provides a significant part of the total environmental burden when producing canned mackerel and herring (Thrane et al., 2009). The hotspot for global warming impact lies in the raw material acquisition, causing 69% of the total global warming impacts across the assessed life cycle stage (Table 9). For terrestrial acidification, ozone formation, and fine particulate matter formation, more than 98% of each of the impact categories came from the raw material acquisition (Table 9). This was not surprising since the assessed fishing vessels operate entirely on heavy fuel oil, and uses high quantities of oil lubricant and other fossil-based materials. Moreover, 90% of the fossil resource scarcity originated from the raw material acquisition.

Results from the midpoint evaluation of Scenario 1 (Table 9), where fishmeal and fish oil production entirely operate on heavy fuel oil, most environmental hotspots shifted to the processing life cycle stage. Packaging material and backup power remained negligible (causing less than 2% of the impacts per assessed impact category), and the proportional contribution of cleaning agents, waste and maintenance, became a smaller part of the environmental impact compared to Scenario 0. While water consumption was highest in the processing life cycle stage in Scenario 0, water consumed was the highest in the raw material acquisition in Scenario 1, or 97% of the total impact, due to the different energy sources. Environmental hotspots related to the burning of fossil fuels in Scenario 1 were the highest in the processing, such as terrestrial ecotoxicity (91%) and global warming (68%), of the total impact per impact category.

Scenario 2 showed similar trends as Scenario 0 (Table 9), as a high proportion of green energy sources was portrayed in Scenario 2. The division of environmental hotspots across impact categories, between processing, raw material acquisition, and cleaning, waste and maintenance, followed the same trends as in Scenario 0. Packaging and backup power remained close to 0% of the total impacts in most impact categories. Terrestrial acidification, ozone formation, fine particulate matter formation, and water consumption remained highest during the raw material acquisition (>76%), related to the burning of fossil fuels, and remained the same as in Scenario 0 and Scenario 1. Categories as terrestrial ecotoxicity remained highest in processing (71%), which resulted in a similar distribution of environmental hotspots as in Scenario 1.

In all Scenarios, the highest environmental impact of terrestrial ecotoxicity, freshwater and marine eutrophication originated from the cleaning waste and maintenance life cycle stage. Furthermore, the highest impact on ozone formation in terrestrial ecosystems and human health originated from the raw material life cycle stage across all the Scenarios.

3.6. Identification of other potential optimization steps based on hotspots analysis

Results from the hotspot analysis indicated where to focus future optimizations of the fishmeal and fish oil production on minimizing its environmental impact. However, the results do not summarize what can be changed to improve the environmental impact in each process. Hence, the sensitivity scenarios will identify those improvement points

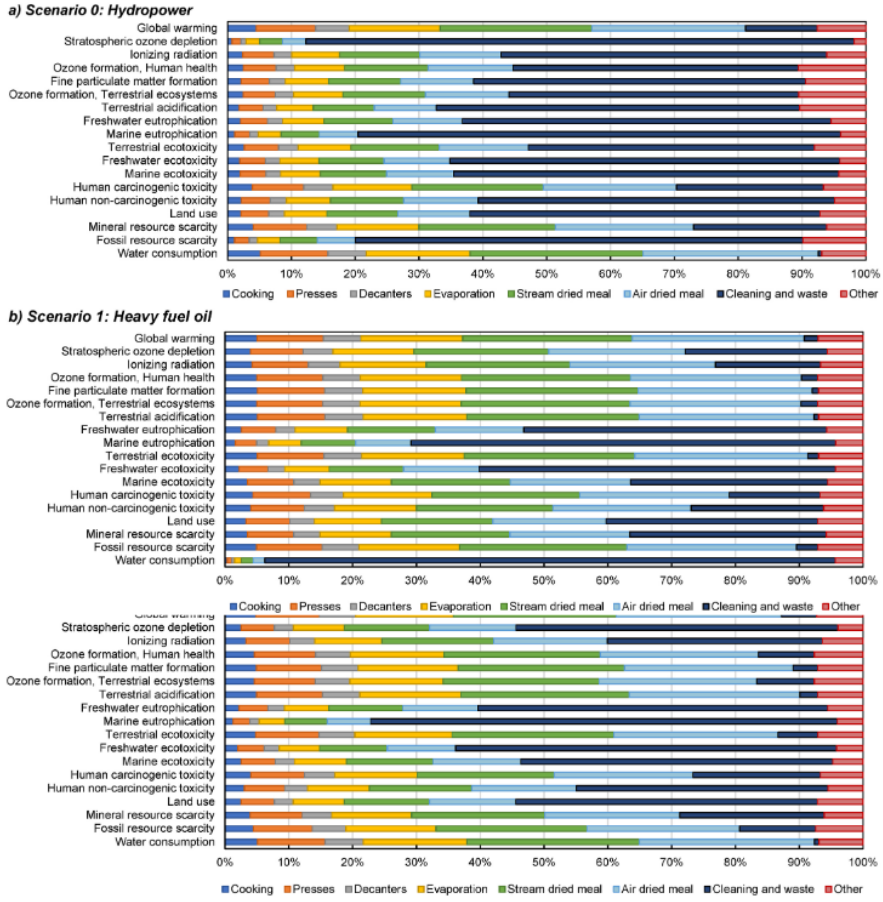


Fig. 4. Midpoint analysis of capelin fishmeal and fish oil production processes operating with 90 °C cooking temperature on different energy sources. Processing steps marked as "Other" summarize centrifuges, packaging material, and backup power.

(see Table 10).

The recommended energy source producing fishmeal and fish oil is hydropower (or other green energy sources), as operating the factory on heavy fuel oil is estimated to have more than double the impact on global warming, stratospheric ozone depletion, ionizing radiation, fine particulate matter formation, terrestrial acidification and human non-carcinogenic toxicity. Furthermore, heavy fuel oil was estimated to be three times higher in fossil resource scarcity and over nine times higher in terrestrial ecotoxicity. Hence, changing the energy source to hydropower would lower the environmental impact significantly. It is also estimated that changing to other more sustainable energy sources, where hydropower is not available, would potentially lower the environmental impacts of fishmeal and fish oil production but requires further investigation.

The raw material acquisition turned out to have a high impact on the environmental impact categories and was affected by several factors. Land-based connection to electricity at the harbor has for example become more frequent, which is estimated to save around 300 000 L of oil annually (Morgunbladid, 2021). This indicates that a combination of actions can be taken to decrease the environmental impact of the raw material acquisition. Along with altering the energy source, the fishing gear can affect the environmental impact, where purse seiner resulted in

Table 7
Overall gain of environmental impact categories estimated at 85 °C and 90 °C cooking temperature, respectively. The environmental impact was multiplied by an estimation of annual production of capelin fishmeal (18.5% fishmeal yield) in 2018 in Iceland in each Scenario.

Impact categories	Gain from lowering the cooking temperature from 90 °C to 85 °C			Unit	Annual gain from lowering the cooking temperature from 90 °C to 85 °C based on annual capelin fishmeal production in Iceland (2018		
	Scenario 0	Scenario 1	Scenario 2		Scenario 0	Scenario 1	Scenario 2
Global warming	0.6%	0.6%	0.6%	kg CO_2 eq	194	217	205
Stratospheric ozone depletion	0.1%	0.5%	0.3%	kg CFC11 eq	29	172	107
Ionizing radiation	0.3%	0.5%	0.4%	kBq Co-60 eq	102	183	141
Ozone formation, Human health	0.3%	0.6%	0.6%	kg NO_x eq	107	216	195
Fine particulate matter formation	0.3%	0.6%	0.6%	kg PM2.5 eq	92	220	208
Ozone formation, Terrestrial ecosystems	0.3%	0.6%	0.6%	kg NO_x eq	105	215	195
Terrestrial acidification	0.2%	0.6%	0.6%	kg SO_2 eq	78	220	210
Freshwater eutrophication	0.3%	0.3%	0.3%	kg P eq	88	112	94
Marine eutrophication	0.1%	0.2%	0.2%	kg N eq	49	70	54
Terrestrial ecotoxicity	0.3%	0.6%	0.6%	kg 1,4-DCB	112	218	202
Freshwater ecotoxicity	0.2%	0.3%	0.2%	kg 1,4-DCB	83	95	86
Marine ecotoxicity	0.2%	0.4%	0.3%	kg 1,4-DCB	84	152	109
Human carcinogenic toxicity	0.5%	0.5%	0.5%	kg 1,4-DCB	168	189	173
Human non-carcinogenic toxicity	0.3%	0.5%	0.4%	kg 1,4-DCB	94	174	129
Land use	0.3%	0.4%	0.3%	m^2a crop eq	91	142	108
Mineral resource scarcity	0.5%	0.4%	0.5%	kg Cu eq	174	151	169
Fossil resource scarcity	0.1%	0.6%	0.5%	kg oil eq	48	214	188
Water consumption	0.6%	0.0%	0.6%	m^3	221	15	220

Abbreviations: CO_2 = carbon dioxide, Eq = equivalent, CFC11 = trichlorofluoromethane or freon-11, Co-60 = cobalt isotope ^{60}Co, NO_x = nitrogen oxide, PM2.5 = fine particulate matter less than 2.5 μm, SO_2 = sulfur dioxide, P = phosphorus, N = nitrogen, 1,4-DCB = 1,4 dichnolorbenzene, Cu = copper.

Table 8
Impact categories of the cleaning, waste, and maintenance processing step. Total results and the effect of each component on the impact category are presented. Darker colours represent a higher environmental impact.

Impact categories	Total results (2.5th-97.5th%)	Unit	Acetic acid	Cleaning and consumables	Nitric acid	Sodium hydroxide	Municipal solid waste	Other processes
Global warming	1.6×10^{1} (1.4×10^{1}–1.8×10^{1})	kg CO_2 eq	72%	3%	8%	10%	3%	4%
Stratospheric ozone depletion	4.7×10^{-6} (3.3×10^{-6}–6.8×10^{-6})	kg CFC11 eq	13%	2%	80%	4%	0%	1%
Ionizing radiation	1.2 (2.0×10^{-1}–5.0)	kBq Co-60 eq	71%	2%	1%	16%	0%	10%
Ozone formation, Human health	3.8×10^{-2} (3.3×10^{-2}–4.6×10^{-2})	kg NO_x eq	74%	3%	7%	11%	0%	4%
Fine particulate matter formation	2.7×10^{-2} (2.3×10^{-2}-3.3×10^{-2})	kg PM2.5 eq	74%	4%	4%	14%	0%	4%
Ozone formation, Terrestrial ecosystems	4.0×10^{-2} (3.5×10^{-2}×10^{1})	kg NO_x eq	75%	3%	7%	11%	0%	4%
Terrestrial acidification	5.9×10^{-2} (5.0×10^{-2}-8.3)	kg SO_2 eq	72%	4%	8%	11%	0%	5%
Freshwater eutrophication	5.4×10^{-3} (2.5×10^{-3}-9.1×10^{-3})	kg P eq	72%	3%	2%	16%	0%	7%
Marine eutrophication	9.7×10^{-4} (7.2×10^{-4}-5.0×10^{-4})	kg N eq	26%	17%	0%	9%	45%	3%
Terrestrial ecotoxicity	5.7×10^{1} (3.0×10^{1}-1.2×10^{2})	kg 1,4-DCB	73%	4%	4%	12%	0%	7%
Freshwater ecotoxicity	1.2 (7.1×10^{-1}-2.0)	kg 1,4-DCB	59%	3%	3%	10%	19%	6%
Marine ecotoxicity	1.6 (9.2×10^{-1}-2.6)	kg 1,4-DCB	59%	3%	3%	10%	19%	6%
Human carcinogenic toxicity	5.4×10^{-1} (2.3×10^{-1}-1.5)	kg 1,4-DCB	72%	3%	2%	15%	2%	6%
Human non-carcinogenic toxicity	2.2×10^{1} (1.2×10^{1}-3.9×10^{1})	kg 1,4-DCB	55%	3%	4%	10%	22%	5%
Land use	3.7×10^{-1} (2.9×10^{-1}-5.2×10^{-1})	m^2a crop eq	56%	26%	1%	10%	0%	6%
Mineral resource scarcity	6.1×10^{-2} (3.7×10^{-2}-1.1×10^{-1})	kg Cu eq	89%	5%	9%	11%	0%	6%
Fossil resource scarcity	7.6 (6.4-9.2)	kg oil eq	89%	2%	1%	5%	0%	2%
Water consumption	3.8×10^{1} (-7.8-7.1)	m^3	78%	5%	2%	11%	0%	4%

NO_x=nitrogen oxide, PM2.5=fine particulate matter less than 2.5 micrometers, SO_2=sulfur dioxide, P=phosphorus, N=nitrogen, 1,4-DCB=1,4 dichnolorbenzene, Cu=copper

a lower environmental impact compared to trawling due to lesser fuel usage.

In the production process, the drying and evaporation steps together accounted for 48–54% of the total environmental impact (not depending on the energy source) of global warming. Hence, optimizing these steps might result in a great environmental gain despite the energy source. A small change as lowering the cooking temperature by 5 °C resulted in a visible environmental gain in the production process, even though cooking accounted for <5% in the impact categories. The optimization of drying and evaporation steps will hence be of great beneficial environmental impact. As thermal decrease has resulted in higher quality fishmeal (Hilmarsdottir et al., 2020), optimized drying techniques could lead to higher quality fishmeal.

During the production process, cleaning agents are used excessively, and not necessarily with any restrictions. There are no suggested nor standardized amounts for the usage of cleaning agents. Exchanging cleaning agents with environmentally friendly cleaning agents would positively influence the environment.

4. Conclusions

The current work identified the environmental impacts of producing fishmeal and fish oil and suggested possible solutions that entail changing the energy source in the raw material acquisition.

Although the raw material acquisition resulted as the highest environmental contributor when using green energy sources, the fishmeal and fish oil accounted for the highest environmental impact using fossil fuel-based energy. It is clear that drying and evaporation contributed to a large part of the fishmeal and fish oil processing production, and with minor changes, such as changing the cooking temperature during the production, positive impacts on the environment may be achieved. Lowering the cooking temperature from 90 °C to 85 °C impacted all

Table 9
Hotspot analysis of the Scenarios during capelin fishmeal and fish oil production at 90 °C cooking temperature. Darker color indicates a higher environmental impact. Backup power was 1% in terrestrial ecotoxicity in Scenario 0, but 0% in other categories in all Scenarios.

***Scenario 0*: Hydropower**	**Total results** (2.5^{th}-97.5^{th}%)	**Unit**	**Raw material**	**Processing**	**Packaging**	**Cleaning, waste and maintenance**
Global warming	4.5×10^{2} (4.3×10^{2}-4.8×10^{2})	kg CO_2 eq	70%	26%	0%	3%
Stratospheric ozone depletion	1.5×10^{-4} (1.1×10^{-4}-2.2×10^{-4})	kg CFC11 eq	64%	5%	0%	31%
Ionizing radiation	5.1 (1.7-1.5×10^{1})	kBq Co-60 eq	57%	19%	1%	23%
Ozone formation, Human health	7.1 (4.8-1.0×10^{1})	kg NOx eq	99%	1%	0%	1%
Fine particulate matter formation	2.3 (2.0-2.7)	kg PM2.5 eq	98%	1%	0%	1%
Ozone formation, Terrestrial ecosystems	7.2 (4.8-1.0×10^{1})	kg NO_x eq	99%	1%	0%	1%
Terrestrial acidification	7.3 (6.4-8.4)	kg SO_2 eq	99%	0%	0%	1%
Freshwater eutrophication	1.3×10^{-2} (6.1×10^{-3}-2.8×10^{-2})	kg P eq	30%	27%	2%	41%
Marine eutrophication	1.6×10^{-3} (1.3×10^{-3}-2.1×10^{-3})	kg N eq	22%	16%	2%	60%
Terrestrial ecotoxicity	4.0×10^{2} (2.5×10^{2}-6.7×10^{2})	kg 1,4-DCB	69%	15%	0%	14%
Freshwater ecotoxicity	2.6 (1.8-4.0)	kg 1,4-DCB	27%	26%	1%	46%
Marine ecotoxicity	3.8 (2.6-5.5)	kg 1,4-DCB	33%	25%	1%	41%
Human carcinogenic toxicity	3.6 (1.9-7.7)	kg 1,4-DCB	38%	46%	1%	15%
Human non-carcinogenic toxicity	5.8×10^{1} (3.8×10^{1}-9.4×10^{1})	kg 1,4-DCB	34%	27%	1%	38%
Land use	1.2 (9.2×10^{-1}-1.6)	m^2a crop eq	44%	22%	2%	31%
Mineral resource scarcity	3.8×10^{-1} (2.4×10^{-1}-6.3×10^{-1})	kg Cu eq	27%	56%	0%	16%
Fossil resource scarcity	1.1×10^{2} (9.8×10^{1}-1.3×10^{2})	kg oil eq	90%	2%	0%	7%
Water consumption	8.1×10^{1} (-8.4×10^{3}-6.8×10^{3})	m^3	18%	81%	0%	0%
***Scenario 1*: Heavy fuel oil**						
Global warming	1.0×10^{3} (9.7×10^{2}-1.1×10^{3})	kg CO_2 eq	31%	67%	0%	1%
Stratospheric ozone depletion	3.0×10^{-4} (1.8×10^{-4}-5.2×10^{-4})	kg CFC11 eq	32%	52%	0%	15%
Ionizing radiation	9.6×10^{1} (3.6-2.3×10^{1})	kBq Co-60 eq	30%	57%	1%	11%
Ozone formation, Human health	8.5 (5.9-1.2×10^{1})	kg NOx eq	83%	16%	0%	0%
Fine particulate matter formation	4.8 (2.7-1.4×10^{1})	kg PM2.5 eq	47%	53%	0%	1%
Ozone formation, Terrestrial ecosystems	8.5 (6.0-1.2×10^{1})	kg NO_x eq	83%	17%	0%	0%
Terrestrial acidification	1.5×10^{1} (8.0-4.6×10^{1})	kg SO_2 eq	48%	51%	0%	0%
Freshwater eutrophication	1.5×10^{-2} (6.5×10^{-3}-3.4×10^{-2})	kg P eq	26%	36%	2%	34%
Marine eutrophication	1.8×10^{-3} (1.4×10^{-3}-2.4×10^{-3})	kg N eq	20%	24%	2%	52%
Terrestrial ecotoxicity	3.4×10^{3} (2.1×10^{3}-5.6×10^{3})	kg 1,4-DCB	8%	90%	0%	1%
Freshwater ecotoxicity	2.8 (1.9-4.2)	kg 1,4-DCB	25%	31%	1%	41%
Marine ecotoxicity	6.1 (4.4-8.5)	kg 1,4-DCB	20%	53%	1%	24%
Human carcinogenic toxicity	5.0 (3.2-8.8)	kg 1,4-DCB	28%	61%	0%	10%
Human non-carcinogenic toxicity	1.2×10^{2} (7.7×10^{1}-2.0×10^{2})	kg 1,4-DCB	16%	65%	0%	17%
Land use	1.6 (1.1-2.6)	m^2a crop eq	33%	42%	2%	22%
Mineral resource scarcity	2.9×10^{-1} (1.8×10^{-1}-4.8×10^{-1})	kg Cu eq	36%	43%	1%	20%
Fossil resource scarcity	3.2×10^{2} (2.4×10^{2}-4.2×10^{2})	kg oil eq	32%	65%	0%	2%
Water consumption	1.5×10^{1} (-1.0×10^{1}-3.7×10^{1})	m^3	97%	0%	0%	2%
***Scenario 2*: Composition of hydropower (75.4%) and heavy fuel oil (24.6%)**						
Global warming	5.9×10^{2} (5.7×10^{2}-6.2×10^{2})	kg CO_2 eq	54%	43%	0%	3%
Stratospheric ozone depletion	1.9×10^{-4} (1.3×10^{-4}-3.0×10^{-4})	kg CFC11 eq	51%	23%	0%	25%
Ionizing radiation	6.2 (2.3-1.7×10^{1})	kBq Co-60 eq	47%	34%	1%	19%
Ozone formation, Human health	7.5 (5.2-1.1×10^{1})	kg NOx eq	94%	5%	0%	1%
Fine particulate matter formation	2.9 (2.3-5.0)	kg PM2.5 eq	77%	22%	0%	1%
Ozone formation, Terrestrial ecosystems	7.5 (5.2-1.1×10^{1})	kg NO_x eq	94%	5%	0%	1%
Terrestrial acidification	9.1 (7.0-1.6×10^{1})	kg SO_2 eq	78%	21%	0%	1%
Freshwater eutrophication	1.4×10^{-2} (6.1×10^{-3}-2.9×10^{-2})	kg P eq	29%	29%	2%	40%
Marine eutrophication	1.7×10^{-3} (1.3×10^{-3}-2.2×10^{-3})	kg N eq	22%	18%	2%	58%
Terrestrial ecotoxicity	1.1×10^{3} (7.7×10^{2}-1.7×10^{3})	kg 1,4-DCB	24%	70%	0%	5%
Freshwater ecotoxicity	2.7 (1.8-3.9)	kg 1,4-DCB	26%	28%	1%	45%
Marine ecotoxicity	4.3 (3.2-6.1)	kg 1,4-DCB	28%	35%	1%	36%
Human carcinogenic toxicity	4.0 (2.3-7.3)	kg 1,4-DCB	35%	51%	1%	14%
Human non-carcinogenic toxicity	7.4×10^{1} (5.1×10^{1}-1.1×10^{2})	kg 1,4-DCB	27%	42%	1%	30%
Land use	1.3 (1.0-1.8)	m^2a crop eq	41%	28%	2%	29%
Mineral resource scarcity	3.6×10^{-1} (2.3×10^{-1}-5.6×10^{-1})	kg Cu eq	29%	54%	1%	17%
Fossil resource scarcity	1.6×10^{2} (1.4×10^{2}-2.0×10^{2})	kg oil eq	63%	32%	0%	5%
Water consumption	6.5×10^{1} (-6.6×10^{3}-5.1×10^{3})	m^3	23%	76%	0%	1%

Abbreviations: CO_2=carbon dioxide, Eq=equivalent, CFC11=trichlorofluoromethane or freon-11, Co-60=cobalt isotope ^{60}Co, NO_x=nitrogen oxide, PM2.5=fine particulate matter less than 2.5 micrometers, SO_2=sulfur dioxide, P=phosphorus, N=nitrogen, 1,4-DCB=1,4 dichnolorbenzene, Cu=copper

Table 10
Optimization potential from the hotspot analysis, based on sensitivity scenarios.

Sensitivity scenario	Scenario description
Raw material acquisition	• Purse seiner resulted in lower energy usage compared to trawl • Changing energy source on the vessels is proposed
The fishmeal and fish oil processing	• A green energy source is proposed • Optimising the drying and evaporation steps • Exchange cleaning agents for eco-friendly cleaning agents

environmental categories beneficially, although being relatively small gain throughout both the raw material acquisition and the fishmeal and fish oil processing. Drying, evaporating and cleaning resulted in the highest contribution to the environmental impact during the processing. Hence, optimizing the drying and separation techniques might significantly lower the environmental impacts. Limiting the use of cleaning agents or exchanging them for eco-friendly chemicals also had positive effects on the environmental impact. As the fishmeal and fish oil industry moves towards higher sustainability and higher use of green energy in the future, applying the LCA methodology is highly recommended to estimate the effects of the life cycle changes beforehand to contribute positively towards a cleaner production.

Recommended for future research is to investigate alternative energy sources in the raw material acquisition and to change the setup of the traditional fishmeal and fish oil processing to investigate the environmental impact. Optimizing the drying steps and limiting the cleaning agents could not only benefit the environment but perhaps produce a higher quality product.

CRediT authorship contribution statement

Gudrun Svana Hilmarsdóttir: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Ólafur Ögmundarson:** Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Writing – review & editing, Visualization, Supervision. **Sigurjón Arason:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **María Gudjónsdóttir:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the AVS (The Added Value of Seafood) fund of the Ministry of Fisheries and Agriculture in Iceland (Redesign of fishmeal and fish oil factories, grant number: R18 031-18), the Rannís Icelandic Technology Development Fund (Product development from flexible fish processing no. 198883-0611 and BIOZOOSTAIN no. 2021267-0611), ERA-NET BlueBioCofund Call (BIOZOOSTAIN) and the University of Iceland research fund. The work was carried out at the University of Iceland and Matís ohf. The authors thank Síldarvinnslan hf. for access to their facilities, assistance, and raw materials.

Appendix

System boundary

Raw material within the system boundaries per functional unit can be seen in Table A.1.

Table A.1
Raw materials inside the system boundaries per functional unit.

Impact category	Total	Unit	Diesel	Hydraulic fracturing fluid	Lubricating oil	Nylon
Global warming	3.2E+02	kg CO_2 eq	3.2E+02	2.1E-03	5.4E-01	1.6
Stratospheric ozone depletion	9.5E-05	kg CFC11 eq	8.0E-05	1.3E-09	4.9E-07	1.5E-05
Ionizing radiation	2.9	kBq Co-60 eq	2.8	5.3E-05	6.7E-02	3.7E-04
Ozone formation, Human health	7.0	kg NO_x eq	7.0	8.4E-06	3.2E-03	3.3E-03
Fine particulate matter formation	2.3	kg PM2.5 eq	2.2	3.8E-06	1.0E-03	1.3E-03
Ozone formation, Terrestrial ecosystems	7.1	kg NO_x eq	7.1	8.7E-06	4.3E-03	3.4E-03
Terrestrial acidification	7.2	kg SO_2 eq	7.1	9.2E-06	2.8E-03	3.9E-03
Freshwater eutrophication	4.0E-03	kg P eq	3.8E-03	4.8E-07	1.8E-04	3.5E-05
Marine eutrophication	3.6E-04	kg N eq	3.0E-04	8.4E-08	1.2E-05	5.1E-05
Terrestrial ecotoxicity	2.8E+02	kg 1,4-DCB	2.7E+02	1.1E-02	2.7	2.1E-01
Freshwater ecotoxicity	7.0E-01	kg 1,4-DCB	6.6E-01	7.9E-05	3.6E-02	3.6E-03
Marine ecotoxicity	1.2	kg 1,4-DCB	1.2	1.1E-04	5.0E-02	5.3E-03
Human carcinogenic toxicity	1.4	kg 1,4-DCB	1.3	5.9E-05	2.0E-02	2.5E-02
Human non-carcinogenic toxicity	2.0E+01	kg 1,4-DCB	1.9E+01	1.6E-03	6.7E-01	8.1E-02
Land use	5.3E-01	m^2a crop eq	5.2E-01	3.6E-04	1.3E-02	3.7E-04
Mineral resource scarcity	1.0E-01	kg Cu eq	9.9E-02	9.2E-06	4.8E-03	1.1E-04
Fossil resource scarcity	1.0E+02	kg oil eq	1.0E+02	7.2E-04	6.0E-01	4.0E-01
Water consumption	1.5E+01	m^3	1.0E-01	3.2E-05	5.2E-03	1.1E-02

Uncertainty

Uncertainty for acetic acid had to be added manually in the pedigree matrix. To calculate the uncertainty with Monte Carlo analysis, each parameter value must be covered and documented as a statistical parameter (Hauschild et al., 2017). Fig. A.1 demonstrates how the uncertainty value

was obtained and how the reliability, completeness, temporal- and geographical- and further technological correlation was estimated.

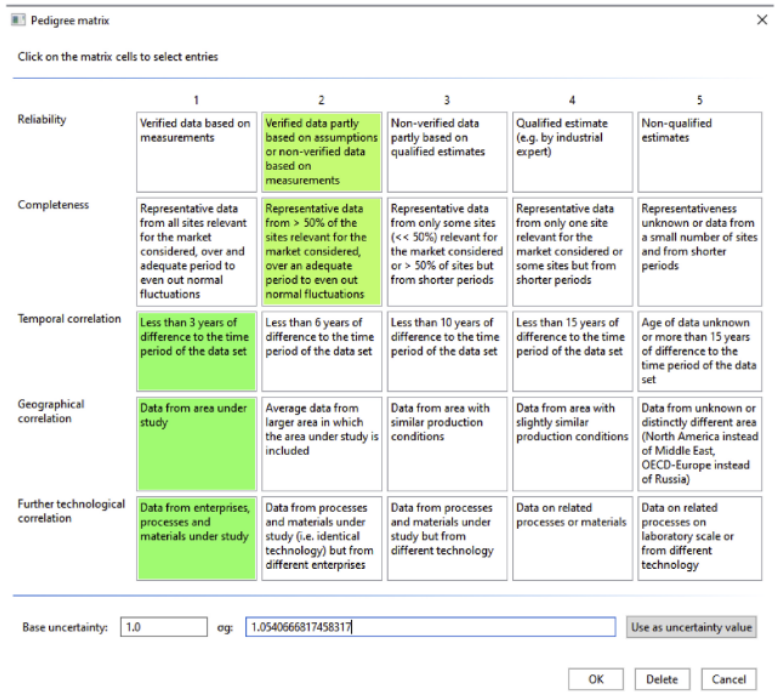


Figure A.1. Uncertainty for acetic acid manually added in SimaPro

Raw material acquisition

The documented annual energy usage from fishmeal and fish oil producers for the years 2017–2019 are summarised in Table A.2, where the division between oil and electricity was calculated for Scenario 2, or 75.4% hydropower and 24.6% heavy fuel oil.

Table A.2
Energy use from different energy resources for the fishmeal and fish oil production in Iceland in 2017–2019 (FIF, 2019).

Energy use from different energy resources for fishmeal and fish oil production in 2017–2019						
Year	Oil		Electricity		Raw material (tonne)	Energy ratio (oil)
	Liters	liters/tonne RM	kWh	kWh/tonne		
2017	8.0E+06	13.7	2.3E+06	401.2	5.8E+05	0.3
2018	7.7E+06	11.7	2.4E+06	368.9	6.6E+05	0.2
2019	3.4E+06	8.3	1.7E+06	427.4	4.1E+05	0.2

The raw material acquisition was estimated from seven fishing trips from one fishing vessel during the capelin season in 2018. Table A.3 demonstrates the fuel usage and time at each trip in 2018 and compared the energy use when using trawl to purse seiner.

Table A.3
Fuel usage and time during raw material acquisition was divided into sailing towards catching ground, catching and superchilling, and sailing towards shore.

Dates from capelin catching from one vessel	Fuel usage			Time		
	Sailing towards catching ground	Catching and superchilling	Sailing towards shore	Sailing towards catching ground	Catching and superchilling	Sailing towards shore
Trip nr. 1) 6.1.-10.1.	34%	30%	35%	19%	70%	11%
Trip nr. 2) 12.1.-16.1.	28%	27%	46%	14%	74%	12%
Trip nr. 3) 17.1.-21.1.	28%	31%	42%	30%	62%	8%
Trip nr. 4) 21.1.-23.1.	30%	38%	32%	15%	72%	14%
Average fuel and time with trawl	30 ± 3%	31 ± 4%	39 ± 6%	19 ± 7%	69 ± 5%	11 ± 2%
Trip nr. 5) 27.2.-2.3.	45%	14%	42%	23%	47%	30%
Trip nr. 6) 5.3.-9.3.	33%	25%	41%	38%	59%	3%
Trip nr. 7) 10.3.-14.3.	14%	12%	73%	15%	73%	12%
Average fuel and time with purse seiner	31 ± 15%	17 ± 7%	52 ± 18%	25 ± 11%	60 ± 13%	15 ± 14%
Overal average fuel and time (trip 1–7)	30 ± 9%	25 ± 9%	44 ± 13%	22 ± 9%	65 ± 10%	13 ± 8%

Values on energy consumption during air-drying in the fishmeal and fish oil production. Calculations are based on values obtained from a Mollier diagram (Table A.4).

Table A.4
Calculations of the energy estimations for air-drying fishmeal and fish oil from a Mollier diagram.

	T [°C]	RH [%]	x [kg water/kg air]	i [kJ/kg air]
1	5	80	0.004	15
2	450	1	0.004	430
3	65	100	0.145	430

Midpoint analysis of the fishmeal and fish oil processing

Table A.5 demonstrates calculated values during a full capacity of the fishmeal and fish oil production plant, with a different cooking temperature in the cookers (85 °C and 90 °C). Values were calculated from heating the raw material from 50 °C to 85 °C or 90 °C with different raw materials. The mass balances during production can be seen in Fig. 3.

Table A.5
Power usage (kWh) during full operational capacity at each processing steps during fishmeal and fish oil production at 85 °C and 90 °C cooking temperature.

kWh during full capacity	Capelin		Mackerel/herring blend		Blue Whiting	
Processing steps	90 °C	85 °C	90 °C	85 °C	90 °C	85 °C
Cookers	241	211	301	263	267	234
Presses	254		254		254	
Decanters	144		144		144	
Centrifuges 1	38		38		38	
Evaporator	765		916		782	
Centrifuges 2	51		51		51	
Steam dryer	1277		645		1175	
Air dryer	1293		1331		1090	
Centrifuges 3	76		76		76	

References

Cashion, T., Tyedmers, P., Parker, R.W.R., 2017. Global reduction fisheries and their products in the context of sustainable limits. Fish Fish. 18 (6), 1026–1037. https://doi.org/10.1111/faf.12222.

Einarsson, M.I., Jokumsen, A., Bæk, A.M., Jacobsen, C., Pedersen, S.A., Samuelsen, T.A., Palsson, J., Eliasen, O., Flesland, O., 2019. Nordic Centre of Excellence Network in Fishmeal and Fish Oil, vol. 119. https://doi.org/10.5281/ZENODO.3243334.

EUfishmeal, 2019. EUfishmeal Input to the BREF SA TWG.

FAO, 1986. The Production of Fish Meal and Oil, ISBN 92-5-102464-2. FAO Fisheries Technical Paper 142.

FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Food and Agriculture Organization of the United Nations.

Fellows, P., 2000. Food Processing Technology: Principles and Practice, second ed. Woodhead Pub. ; CRC Press.

FIF, 2019. Energy Usage in the Factories from 2003.

Préon, P., Durand, H., Avadí, A., Huaranca, S., Orozco Moreyra, R., 2017. Life cycle assessment of three Peruvian fishmeal plants: toward a cleaner production. J. Clean. Prod. 145, 50–63. https://doi.org/10.1016/j.jclepro.2017.01.036.

Geankoplis, C.J., 1993. Transport Processes and Unit Operations, third ed. Prentice-Hall International, Inc.

Hall, G.M., 2010. Fishmeal production and sustainability. In: Hall, G.M. (Ed.), Fish Processing. Wiley-Blackwell, pp. 207–235. https://doi.org/10.1002/9781444328585.ch9.

Hauschild, M.Z., Bjørn, A., Owsianiak, M., Laurent, A., Olsen, S.I., Corona, A., 2017. Life cycle assessment. Theory and practise. In: Hauschild, M.Z., Rosenbaum, R.K., Olsen, S.I. (Eds.), Life Cycle Assessment: Theory and Practice. Springer, pp. 75–116. https://doi.org/10.1007/978-3-319-56475-3_6.

Hilmarsdottir, G.S., Ogmundarson, Ó., Arason, S., Gudjónsdóttir, M., 2020. The effects of varying heat treatments on lipid composition during pelagic fishmeal production. Processes 8 (9), 1142. https://doi.org/10.3390/pr8091142.

Hilmarsdottir, G.S., Ogmundarson, Ó., Arason, S., Gudjónsdóttir, M., 2021. Efficiency of fishmeal and fish oil processing of different pelagic fish species: identification of processing steps for potential optimization toward protein production for human consumption. J. Food Process. Preserv. 45 (4) https://doi.org/10.1111/jfpp.15294.

ISO, 2006. Environmental Management—Life Cycle Assessment—Principles and Framework. ISO, p. 14040.

Morgunbladid, 2021. Land connection a positive step in energy exchange. https://www.mbl.is/200milur/frettir/2021/09/07/landtenging_jakvaett_skref_i_orkuskiptum/.

Nygaard, H., 2010. Standard Norwegian fishmeal- and fishoil process (Nofima). Nofima 33 (November), 25.

Ögmundarson, Ó., Sukumara, S., Laurent, A., Fantke, P., 2020. Environmental hotspots of lactic acid production systems. GCB Bioenergy 12 (1), 19–38. https://doi.org/10.1111/gcbb.12652.

Papatryphon, E., Petit, J., Kaushik, S.J., Van Der Werf, H.M.G., 2004. Environmental impact assessment of salmonid feeds using Life Cycle Assessment (LCA). Ambio 33 (6), 316–323. https://doi.org/10.1579/0044-7447-33.6.316.

Pelletier, N., Tyedmers, P., 2010. Life cycle assessment of frozen tilapia fillets from Indonesian lake-based and pond-based intensive aquaculture systems. J. Ind. Ecol. 14 (3), 467–481. https://doi.org/10.1111/j.1530-9290.2010.00244.x.

Romotowska, P.E., Karlsdóttir, M.G., Gudjónsdóttir, M., Kristinsson, H.G., Arason, S., 2016. Seasonal and geographical variation in chemical composition and lipid stability of Atlantic mackerel (Scomber scombrus) caught in Icelandic waters. J. Food Compos. Anal. 49, 9–18. https://doi.org/10.1016/j.jfca.2016.03.005.

Samuel-Fitwi, B., Meyer, S., Reckmann, K., Schroeder, J.P., Schulz, C., 2013. Aspiring for environmentally conscious aquafeed: comparative LCA of aquafeed manufacturing using different protein sources. J. Clean. Prod. 52, 225–233. https://doi.org/10.1016/j.jclepro.2013.02.031.

Schau, E.M., Ellingsen, H., Endal, A., Aanondsen, S.A., 2009. Energy consumption in the Norwegian fisheries. J. Clean. Prod. 17 (3), 325–334. https://doi.org/10.1016/j.jclepro.2008.08.015.

Síldarvinnslan, 2018. Green Accounting for Síldarvinnslan Hf.

Smárason, B.O., Ogmundarson, Ó., Árnason, J., Bjornsdóttir, R., Davíðsdóttir, B., 2017. Life cycle assessment of Icelandic arctic char fed three different feed types. Turk. J. Fish. Aquat. Sci. 17, 1387–1395. https://doi.org/10.4194/1303-2712-v17.

Statistics Iceland, 2019. Profitability in Fishing and Fish Processing 2018.

The Directorate of Fisheries, 2020. Quotas and catches. http://www.fiskistofa.is/english.

The Marine, Freshwater Research Institute, 2019. Advice and TAC Overview.

Thorkelsson, G., Slizyte, R., Gildberg, A., Kristinsson, H.G., 2009. Fish proteins and peptides. Processing methods, quality and functionality. In: Luten, J.B. (Ed.), Marine Functional Foods. Wageningen Academic Publishers, pp. 115–135.

Thrane, M., Nielsen, E.H., Christensen, P., 2009. Cleaner production in Danish fish processing—experiences, status and possible future strategies. J. Clean. Prod. 17 (3), 350–360. https://doi.org/10.1016/j.jclepro.2008.08.006.

Paper V

Near infrared spectroscopy and chemometrics for effective online quality monitoring and control during pelagic fishmeal and oil production

María Gudjónsdóttir[ab*], Gudrun Svana Hilmarsdóttir[a], Ólafur Ogmundarson [a], Sigurjón Arason[ab]

[a]University of Iceland; Faculty of Food Science and Nutrition, Aragata 14, 102 Reykjavík, Iceland

[b]Matis ohf, Icelandic Food and Biotech R&D, Vínlandsleið 12, 113 Reykjavík, Iceland

Abstract

Near infrared spectroscopy has become a common quality assessment tool for fishmeal products during the last two decades. However, to date it has not been used for active online quality monitoring during fishmeal processing. The aim was to investigate whether NIR spectroscopy, in combination with multivariate chemometrics, could actively predict the changes in the main chemical quality parameters of pelagic fishmeal and oil ***during processing***, with an emphasis on lipid quality changes. Results indicated that partial-least square regression (PLSR) models from the NIR data effectively predicted proximate composition changes and were successful in distinguishing between fatty acids according to their level of saturation (SFA, MUFA, PUFA). The technique also allowed prediction of phospholipids (PL), and EPA and DHA content throughout the processing. NIR spectroscopy in combination with chemometrics is thus a powerful quality assessment tool that can be applied for active online quality monitoring during fishmeal and oil processing.

Keywords: Near infrared (NIR) spectroscopy, chemometrics, process monitoring, quality assessment, process optimization; product optimization

* Corresponding author
E-mail address: mariagu@hi.is (María Gudjónsdóttir)

1 Introduction

Recently pelagic fishmeal for the use in fish and animal feed has been lowering in value due to the high competition with alternative protein meals. However, pelagic fishes, such as Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*), and blue whiting (*Micromesistius poutassou*) are nutritious raw materials, containing several valuable fatty acids, essential proteins, and minerals (Einarsson et al. (2019). This calls for increased utilization of pelagic fish raw materials and optimization of production processes of higher value products, including products for human consumption. Traditional fishmeal and oil processes from pelagic fish species have remained unchanged for decades (Hall, 2010; Oterhals and Vogt, 2013; Einarsson et al., 2019). Moreover, the variation in water, protein, and lipid content in the processing streams during fishmeal processing of pelagic fishes is extreme due to the high variation in the raw material that enters the process (Cozzolino et al., 2002; Hilmarsdóttir et al., 2020; 2021). The raw material often includes several species, cut-offs, heads, bones and guts, and the availability of species is highly seasonal (Romotowska et al., 2016; Hilmarsdóttir et al., 2021). The share of side-streams from pelagic fish processing to human consumption has increased in recent years and will increase significantly in the future. This expected demand of products from pelagic side-stream processing calls for effective raw material and process monitoring of the chemical changes occurring at each processing step. Such monitoring is critical for process optimization and fast decision making towards the production of higher value products. However, traditional quality assessment methods performed in the laboratory are commonly sample destructive, time consuming and expensive, which does not fit well into the need for fast decision making within the processing environment (Cozzolini et al., 2002).

Near infrared spectroscopy (NIR) is a fast, non-destructive, analytical method that has been used for some time for quality assessment of seafood quality, including the quality of the final fishmeal products (Cozzolino et al., 2002; 2009). The main chemical constituents of fish and fishmeal are rich in C-

H, O-H, N-H and S-H chemical bonds, which overtones and combination bands are absorbing strongly in the NIR spectral range (McClure, 2003). NIR analysis has therefore shown to be useful in predicting several chemical (Cozzolini et al., 2002; 2009; Gudjónsdóttir et al. 2011) and derived physical (Stevik et al., 2010) and sensory quality parameters (Bøknæs et al., 2002; Nilsen and Esaiassen, 2005; Ellekjær et al., 2006) in diverse agricultural and marine food applications (Blanco & Villarroya, 2002). However, due to the complex calibration work needed, and the earlier mentioned variations in the chemical composition of the streams during processing, little effort has been put into collecting live online processing of data to allow active, live quality monitoring during fishmeal and fish oil processing. This kind of quality monitoring is, however, necessary if products of higher quality are to be produced. The use of chemometric data processing simplifies this necessary calibration work and takes multiple variables into consideration during prediction model building (Gomes et al., 2022). The combination of spectroscopy and multivariate chemometrics is, therefore, highly effective in the development of fast online prediction tools (Blanco & Villarroya, 2002; Rinnan et al., 2009; Cozzolino et al, 2009).

The aim of the current study was, therefore, to investigate whether NIR spectroscopy, in combination with multivariate chemometrics, could actively predict the changes in the main chemical quality parameters of pelagic fishmeal and fish oil ***online during processing*** and to assess if the technique can be actively used for process control and optimization, potentially towards the production of high-quality products for human consumption and customized high-quality feed for aquaculture and pets.

2 Materials and methods

2.1 Raw materials and process description

A mixture of several pelagic fish species was caught of the east and southeast coast of Iceland with a purse seiner during September 3rd to 6th 2017. The total landings arriving to the fishmeal factory contained a mixture of Atlantic mackerel (*Scomber scombrus*, 58%), Atlantic herring (*Clupea harengus*, 37%), blue

whiting (*Micromesistius poutassou*, 4.5%), and <0.5% of by-catch from other species. The fishmeal process commenced three days post landing. The fishmeal and oil production process, which can be seen in Figure 1, was described in detail by Hilmarsdóttir et al. (2020). Three processing runs were analysed, each with a different temperature in the cooker, i.e. 85°C, 90°C and 95°C, respectively to give more variation to the sampling set. Samples were taken in triplicate from each processing step (as marked with red dots on Figure 1) from raw material to final product during each process run to follow the quality characteristics of the processing streams throughout processing. The samples were analysed for water, lipids, fat free dry matter, fatty acid composition (FAC), free fatty acids (FFA) and phospholipids (PL), as well as their NIR spectra.

2.2 Near infrared spectroscopy

Near infrared (NIR) reflection of the samples were analysed with a Bruker Multi Purpose Analyzer (MPA) spectrometer (Bruker Optics GmbH, Ettlingen Germany), using a handheld optic fibre probe. NIR measurements were performed and all NIR data was collected in the Opus software (Bruker Optics GmbH, Ettlingen Germany). Five scans were measured from each sample to increase the signal-to-noise ratio (SNR) and their average spectrum used to represent each sample. Each sample from each sampling location (m=30) was measured in triplicate (n=3), giving a total of 90 NIR spectra throughout the fishmeal and oil processing.

2.3 Chemical reference measurements

The physicochemical quality parameters of water, lipids, fat free dry matter, fatty acid composition, free fatty acids and phospholipids were analysed in triplicate (n=3) throughout the processing to give a detailed view of the quality changes occurring to the pelagic raw material during processing. The water, lipid and fat free dry matter were measured at all sampling points (m=30), while FAC, FFA and PL were measured at key locations throughout processing (m=22 for FFA and PL, m=17 for FAC).

89 The water content of all processing samples was measured by drying of 5 g samples in an oven at
90 104±4°C for 4 hours according to ISO 6496 (1999). The water content in the final oil samples was, however,
91 measured by calorimetric titration in an 851 Titrando titrator (Metrohm, Herisau, Switzerland). The water
92 content was measured in triplicate and expressed as g water/100 g sample.

93 The total lipid (TL) content of the samples was obtained by Bligh and Dyer extraction (Bligh and
94 Dyer, 1959). The extracts were then used for analysis of free fatty acids (FFA) with the Lowry and Tinsley
95 (1976) method with modifications described by Bernárdez et al. (2005), as well as colorimetric analysis of
96 phospholipids in the form of phosphatidylcholine according to Stewart (1980). The total lipids results were
97 expressed as g lipids/100 g sample, while FFA and PL concentrations were expressed as g/100 g TL.

98 Fat free dry matter (FFDM) was calculated as the percentage of remaining weight once the water
99 and lipid contents had been subtracted from the total sample weight as seen in equation 1:

100 $$FFDM\ (\%) = 100 - water\% - TL\% \qquad (1)$$

101 The FFDM results were expressed as g FFDM/100 g sample.

102 The fatty acid composition (FAC) of the samples was determined by gas chromatography in a Varian
103 3900 gas chromatography instrument (Varian Inc., Walnut Creek, CA, USA) as described in the AOAC
104 Official method (1998). The fatty acids were classified according to their level of saturation, i.e. into
105 saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA),
106 respectively. The fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were also
107 analysed specifically due to their potential health benefits (Fournier et al., 2006; Larsen et al., 2011; Zheng
108 et al., 2013). All FAC results were expressed as g FA/100 g TL.

109 The obtained range of each reference chemical variable can be seen in Table S.1 in the
110 Supplementary materials. However, a detailed analysis of the chemical changes occurring during

processing and interpretation of the reference measurements was performed and discussed by Hilmarsdóttir et al. (2020).

2.4 NIR PLSR prediction models

Data from the NIR spectral analysis and the reference measurements were imported to Unscrambler X®(Camo, Oslo, Norway) for further data analysis. Mean Centred Partial Least Square Regression (PLSR) models were built to test the ability of the NIR spectra to predict the various analysed reference parameters.

Two spectra from each sampling location during processing were used for building the training set for the PLSR calibration models compared to the reference measurements. The third sample spectrum from each sampling location was used as an independent validation test set of the models. The effectiveness of using different spectral treatments was also tested, i.e. applying i) no spectral treatments, ii) using a baseline correction, iii) a first derivative model using the Savitzky-Golay method with 11 smoothing points at each end of the spectra, iv) a Savitzky-Golay second derivative model of the second polynomial order with 11 smoothing points, and finally v) a full multiplicative scatter correction (MSC) of the spectral data. The score plots of the first two principal components (PCs) and predicted versus reference results from each obtained PLSR model, were assessed both graphically and numerically to easily identify trends, outliers, stability, and precision of each prediction model. The efficiency of the models was evaluated according to the model coefficients of determination (R^2_{CV}) and coefficient of determination (R^2_P), and the representative root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) as described by Gomes et al. (2022).

3 Results and discussion

3.1 Chemical changes during fishmeal and oil processing

The main chemical changes occurring during fishmeal and oil processing are involving the separation of water and lipids from the fat free dry matter, to form a low-fat fishmeal and a pure fish oil. The chemical changes observed in the present processes were described in detail by Hilmarsdóttir et al. (2020), but the range of each of the chemical reference variables are given in Table S2 in the supplementary materials. The process reflects great variation in chemical composition between processing steps, varying from high liquid streams rich in water and oil (separated press liquid, first concentrate, final oil), to more solid streams containing high dry matter content (press cake, sludge, second concentrate and final fishmeal). The chemical analysis indicated that several processing steps in the current, traditional processes were inefficient both with regards to energy and material efficiency (Hilmarsdóttir et al., 2020). Lowering the temperature to 85°C resulted in more effective lipid separation during the process, and thus also a lower lipid content in the final fishmeal, making it both more valuable and more stable. Life cycle assessment (LCA) of the process furthermore indicated that lowering the cooking temperature to 85°C had a positive impact on evaluated environmental categories (Hilmarsdóttir et al. 2022), indicating that small process changes can also benefit the sustainability of the process. The two studies, furthermore, identified that the drying and evaporation stages required optimization to allow the production of food-grade products for human consumption, instead of the current lower value fishmeal and fish oil.

The traditional chemical reference assessment methods applied in the studies were very time consuming, required the use of environmentally harmful solvents and chemicals, and the reliability (precision, accuracy, repeatability) of the method may in some cases also depend on the individual performing the analysis. The disadvantages of applying these methods, as well as the need for process

optimization, highlights the need for improved process monitoring on-site. Spectroscopic methods, such as NIR spectroscopy might be a solution to this problem, as investigated in the following sections.

3.2 NIR spectra and Principal Component Analysis

The obtained NIR spectra showed clear differences between the samples according to where they were sampled during the fishmeal process (Figure 2). Dominating absorption peaks of overtones and combination bands due the vibrations of chemical bonds such as -CH, -NH, OH, C=O, etc., which can be found in the main constituents of fishmeal, were assigned (Figure 2; Table S1 in Supplementary materials). The peak assignment indicated that the main differences between samples could be found in the water and lipid content and characteristics of the samples. To get a better overview of the connection between these main variables a Principal Component Analysis (PCA) was performed on the NIR spectral data (Figure 3). The PCA, which described 99% of the variation between samples, effectively distinguished between the raw materials, products (fishmeal and oil), and processing streams. Clear groupings could also be seen within the processing streams distinguishing streams of higher water and lipid content (liquid streams), such as after cooking and draining, the separated press liquid, and first concentrate, compared to streams with higher protein content (solid streams), such as from the press cake, sludge and second concentrate. These results indicate that the NIR spectral information obtained during processing had potential to be used for process optimization for the main chemical components. This led to the construction and assessment of the prediction potential of linear partial least square models to all chemical components investigated by Hilmarsdóttir et al. (2020) as discussed in the following section.

3.3 PLS quality prediction models

Results of valid concentration ranges, correlation factors (R^2), root-mean-square errors (RMSE), standard error of calibration and validation (RMSEC and RMSEP, respectively), the optimal number of model factors, and the number of measurements used both for the calibration (CAL) models, and the

176 independent validations (VAL) of the built PLS models were listed and compared to assess which data
177 treatments resulted in the best prediction models (Table S2 in supplementary materials). An overview of
178 the best performing PLS models for each chemical variable is shown in Figures 4-6.

179 Several combinations of variable selections, including either the whole spectrum or chosen
180 spectral regions were tested. Selecting the whole spectrum for the modelling, may lead to the inclusion
181 of noise in the modelling data (Gomes et al., 2022). Appropriate data pre-processing, such as smoothing
182 of the spectra can be applied to decrease such detrimental effects and improve the signal-to-noise ratio
183 (Rinnan et al., 2009). Several spectral data pre-processing treatments were tested to find the strongest
184 prediction models, including using the raw data directly, applying data normalization and baseline
185 corrections, first and second derivatives, and multiplicative scatter signal correction (MSC). During the
186 evaluation of the first and second derivative spectra, the data was smoothed by removing 11 spectral
187 points from each end of the spectra to improve to signal-to-noise ratio and make the prediction models
188 stronger as described by Rinnan et al. (2009).

189 Nevertheless, the comparison indicated that using the whole spectrum seemed to result in the
190 strongest prediction models, potentially due to the high overlap of absorption bands within the NIR
191 spectra, especially C-H and O-H bands relating to moisture, proteins, and lipids content of the samples
192 (Díaz et al. 2019). Selecting the whole spectrum did thus not seem to interfere with the prediction
193 performance of the PLS models in the current study. Furthermore, applying no spectral pre-treatment
194 (raw data), only baseline correction in the case of lipid content prediction, or MSC data pre-treatment
195 provided the overall strongest prediction models. Potentially can even more precise models be obtained
196 by applying ordered predictors selection (OPS) algorithms as described by Roque et al. (2019), but that is
197 left for further studies. The best data treatment obtained for each variable in the current study are
198 discussed in the following section.

3.3.1 Water content prediction

The water content of the samples obtained throughout processing ranged from 0.3 to 93 g water/100 g sample. The strongest PLRS models predicting the water content of the samples were obtained by using the no spectral data treatment, resulting in calibration and validation correlation coefficients of R^2_{CV} of 0.9995, and R^2_P of 0.9938, respectively. The obtained model had a RMSEC of 0.67 g/100 g sample and RMSEP of 2.41 g/100 g sample, indicating that the model was effective for process assessment and precise enough for process decision making, even though the RMSEP could be improved. The RMSEP is still higher than values obtained in other studies, such as Cozzolino et al. (2002), who obtained standard errors of calibration and cross-validation (SEC and SECV, respectively) of 3.9 g/kg dry weight in the final fishmeal alone (equivalent to a RMSEC of approximately 0.4 g/100 g sample). However, since the range in the chemical composition in the samples throughout the processing is much wider than in fishmeal alone, the achieved model parameters are well within acceptable levels for water content prediction.

A robust way of predicting water content simultaneous to optimize or redesign any food production process with water content ranging from 0 to 93% is a valuable tool. With active monitoring can any changes in the process be assessed, and the production can be directed towards processing of products that better fit the processing stream characteristics immediately. As water and lipids account for 85-90% of the raw materials entering the fishmeal and fish oil production process (FAO, 1986), controlling, and ensuring effective water- and lipid removal from the solid streams remains a priority during the production of high-quality protein products (Hilmarsdóttir et al. 2020;2021). Furthermore, the abundance of water can stimulate hydrolyzation of the raw material along with heat and oxygen (Jacobsen, 2015) which can have serious degrading effects on the quality and storage stability of the products. This further emphasizes the need of immediate actions if negative characteristics of the processing material are monitored during production. The developed NIR water prediction model is fully capable of predicting such quality changes during the process.

3.3.2 Total lipid content prediction

The total lipid content ranged from 1.2 to 99.7 g/100 g sample in the samples during the fishmeal processing, although the raw material entering the process had an average lipid content of 19.5±2.0 g/100 g sample. This lipid content agrees with the lipid content obtained in Atlantic herring and mackerel of earlier studies (Romotowska et al., 2016; Huong et al., 2018). Similar prediction model results were obtained for the lipid prediction models as for the water, with regards to that the MSC gave the overall strongest prediction model, when taking both the calibration and validation parameters into account. Interestingly, adequate models were achieved with all spectral data treatments each having calibration and validation coefficients R^2 in the range from 0.9265 to 0.9773, except the second derivative, in which the model validation was far below expectation with a R_p^2 of 0.5314. The lower performance of the second derivative models may be caused by the variable selection for the modelling (full spectra with smoothing), in which the smoothing may have been insufficient, or the full second derivative spectra may contain too much noise for the modelling. However, since acceptable prediction models were achieved with other spectral treatments the optimization of variable selection of the second derivative spectra was not investigated further. It is also possible that the choice of validation samples might have influenced the validation of the models. A few different validation setups were therefore tested as well, including randomized validation and full validations, but these did not perform any better than the external, independent test set validation.

Measuring lipid quality, including lipid content with traditional laboratory methods can take several months of labour work when sampling numbers are high and frequent (Hilmarsdóttir et al., 2021), and includes high material costs involved with glassware, chemicals etc. Furthermore, the traditional analysis commonly uses toxic solvents, such as chloroform, which may have a negative impact on the health of the staff performing the analysis as well as having negative environmental impacts during disposal (Watts et al., 2004). Hence, predicting the lipid content by NIR spectroscopy may save a lot of

247 time and money, is safer to the analytical staff, and has a lower environmental impact. Furthermore,
248 human error can be minimized by applying the NIR analysis compared to traditional lipid extraction
249 protocols, such as the Bligh and Dyer (1959) method, and the staff training periods could be reduced when
250 using NIR spectroscopy for the task. The fishmeal produced under the current traditional processing
251 methods resulted in a high lipid content (11-14 g/100 g sample), which classifies it as a Type C fish protein
252 concentrate (FPC) according to FAO (1986). Online monitoring of the lipid content during processing could
253 allow the improved process monitoring and thus lowering of the lipid content in the final product. If the
254 lipid content is lowered below 0.75 g/100 g the product would be classified as a Type A FPC, allowed for
255 human consumption (Windsor, 2001). Direct monitoring and appropriate process adjustments could
256 therefore increase the value of the produced products substantially, as well as increase the possibility of
257 producing more standardized products.

258 ### 3.3.3 Fat free dry matter prediction

259 The fat free dry matter mainly consisted of proteins, but also smaller amounts (<2 g/100 g sample) of
260 salts, minerals, and other trace elements. The obtained FFDM content during the processing ranged from
261 0 to 84.4%. Again, the MSC model performed best, resulting in a R^2_{CV} and R^2_P of 0.9183 and 0.9356,
262 respectively. The RMSE were though relatively high, or 6.23 and 5.58 g/100 g sample for the calibration
263 and prediction, respectively, of the MSC prediction model. Visual evaluation of the sample distribution
264 during modelling showed that the oil samples appeared as extremes during the modelling. However, the
265 model strength became weaker if the oil samples were removed, indicating the importance of including a
266 wide range of FFDM content samples to obtain strong prediction models.

267 The main FFDM component in fishmeal and its production streams is crude protein which
268 traditionally is measured by determining the nitrogen content, e.g., with the Kjeldahl method (ISO, 2009)
269 or the Dumas method (ISO, 2008). Neither the Kjeldahl nor the Dumas method distinguishes between

V

protein and non-protein nitrogen (ISO, 2008). However, since the NIR absorption is highly dependent on both the molecular composition, the micro-structure of the molecule, and the environment that the molecule is in (Rinnan et al., 2009), NIR spectroscopy has a potential to assess the protein content with higher precision than the traditional methods. This also highlights the importance of choosing the reference method wisely when building the NIR prediction models, as this could affect the prediction ability and precision of the models.

The FFDM was in this study expressed as what is left when the water and lipid content has been removed from the total weight of the sample. Even more precise prediction of the protein content and FFDM might have been achieved if direct measurements of the protein content through the Kjeldahl or Dumas method had been applied as reference data during the FFDM NIR prediction model building, along with more precise analyses of the vitamins and minerals potentially present in the samples. The precision of the Kjeldahl and Dumas methods in assessing protein content have, however, also been criticized lately and there is not a uniform consensus on which multiplication factors should be used when converting the analyzed nitrogen content to crude protein content. Most studies refer to the 6.25 multiplication factor, as derived from the standard Kjeldahl method, while others have shown that the factor requires modifications dependent on the characteristics of the sample as well as the choice of analytical method (Kjeldahl or Dumas) (ISO, 2008). However, this comparison between the precision of different protein assessment methods is outside the scope of the current study but is worthy of further comparisons in the future. Nevertheless, the current study clearly shows that both time and cost can be saved by applying NIR spectroscopy to analyse FFDM during fishmeal processing. As the value of fishmeal depends on a high protein content, an online system monitoring the protein content throughout each operational step could help keep the final product quality consistent which is of great importance to both the producers and consumers (Kristinsson, 2007).

3.3.4 Fatty acid composition (FAC) prediction

The fatty acid composition was analysed and NIR prediction models built for the fatty acid classes SFA, MUFA and PUFA, along with DHA and EPA. Excellent prediction models were achieved for all parameters (Figure 5), especially SFA, DHA and EPA, which had high R^2 parameters (>0.98), and low prediction errors (<1 g/100 g lipid) for all lipid class parameters. Slightly higher prediction errors were obtained in the MUFA (RMSEP=1.5 g/100 g lipid) and PUFA (RMSEP=2.1 g/100 g lipid) compared to the SFA (RMSEP=0.23 g/100 g lipid), potentially due to the spectral similarities and high absorption peak overlap between the unsaturated bonds in the two fatty acid classes, making it difficult to distinguish between the level of unsaturation. However, the performance of the MUFA and PUFA prediction models is still within satisfactory limits for process monitoring and optimization purposes.

Prediction models for DHA and EPA using the MSC spectral data treatment, furthermore, turned out to be highly efficient during processing, showing R_P^2 parameters of 0.8785 and 0.8689 and RMSEP values of 0.89 and 0.62 g/100 g sample for the DHA and EPA, respectively. DHA and EPA are desirable omega-3 fatty acids which can be destroyed during drying, as high temperatures drastically affect the long-chain PUFAs (Fournier et al., 2006). Hence, heating, and drying techniques need to be monitored closely for high yield. As DHA and EPA are among desirable PUFAs due to various health effects (Larsen et al., 2011; Zheng et al., 2013), a higher yield would be beneficial. The prediction of these fatty acids during processing is therefore especially important due to their potential health effects and their influence on product value. Furthermore, no other studies were found in literature predicting these parameters in fishmeal or during fishmeal production, supporting the novelty of the current study results.

3.3.5 Free fatty acids (FFA) and phospholipids (PL) prediction

No significant correlations were obtained for the free fatty acids in the samples, possibly due to the narrow range of obtained FFA values (ranging between 0 to 5 g FFA/100 g sample). However, a nice PLS

316 model was achieved for the phospholipid content (PL) in the range between 0 to 1.4 g PL/100 g sample
317 with the MSC spectral data treatment, resulting in a prediction model with a R^2_{CV} and R^2_P of 0.9617 and
318 0.8617, respectively. The low RMSEC and RMSEP of 0.06 and 0.11 g/100 g sample furthermore indicated
319 excellent prediction precision of the model compared to the reference measurements.

320 Free fatty acids and phospholipid content are strong indications of lipid and protein degradation, and
321 analysis thereof can emphasize and identify problematic areas or processing steps which involve
322 hydrolyzation or lipid oxidation induced degradation (Jacobsen, 2015; Hilmarsdóttir et al., 2020;2021).
323 However, further investigations are proposed with a wider range of FFAs in samples for potential FFA
324 prediction model building using the NIR technology.

325 4 Conclusions

326 NIR spectroscopy based PLS modelling were shown to be a precise and accurate tool for simultaneous
327 assessment of water-, lipid- and FFDM content, EPA, DHA, and PLs during fishmeal processing. Overall,
328 two spectral treatments resulted in stronger prediction models than the others, i.e. the spectral models
329 using no spectral treatments (raw spectra) and the MSC treated spectra, respectively. The models can
330 potentially be improved even further by adding an even wider range of raw materials (i.e. include data on
331 more species during processing) or applying more detailed variable selections as inputs for the
332 multivariate PLS model building. Investigations of this is left for future studies. However, the developed
333 composition models can substantially decrease labour work and material costs. Integrating NIR for
334 process monitoring can be promoted as a more sustainable method to monitor processing induced
335 changes with lower environmental impacts compared to the traditional quality measurements and
336 processing standards. More effective process control leads to further increase of production yield and
337 better utilization of the available biomass, which also has substantial environmental benefits. How large

these potentially positive environmental impacts are, and which categories are most affected, is though left for further studies.

Overall, NIR online monitoring allows better process control and directing the process towards the production of products of higher value, potentially even for human consumption, and facilitate that production of more specialized feed for aquaculture, agriculture, and pets.

5 Acknowledgements

The authors would like to acknowledge the Added Value of Seafood (AVS) fund of the Ministry of Fisheries and Agriculture in Iceland (grant number R18 031-18), the Rannís Technology Development Fund (Production development from flexible fish processing, grant number 198883-0611 and BIOZOOSTAIN grant number 2021267-0611), the ERA-NET BlueBio Cofund (BIOZOOSTAIN) and the University of Iceland Research fund. The authors are also grateful to Síldarvinnslan (SVN) pelagic fish processing company, which provided raw materials and industrial facilities to perform the study in, and valuable knowledge and insight into the processing.

6 References

AOCS (1998). Official Method and Recommended Practices of the AOC, (7th ed.). American Oil Chemists' Society. AOCS Official Method Ce 1b-89.

Bernárdez, M., Pastoriza, L., Sampedro, G., Herrera, J.J.R., & Cabo, M.L. (2005). Modified method for the analysis of free fatty acids in fish. *Journal of Agricultural and Food Chemistry*, 53 (6), 1903–1906. https://doi.org/10.1021/jf040282c

Blanco, M. & Villaroya, I. (2002). NIR spectroscopy: a rapid-response analytical tool. *Trends in Analytical Chemistry*, 21, 240–250. https://doi.org/10.1016/S0165-9936(02)00404-1

359 Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal*
360 *of Biochemistry and Physiology*, *37* (8), 911–917. https://doi.org/10.1139/y59-099

361 Bøknæs, N., Jensen, K.N., Andersen, C.M., & Martens, H. (2002). Fresh assessment of thawed and chilled
362 cod fillets packed in Modified atmosphere using near infrared spectroscopy. *LWT–Food Science and*
363 *Technology,* 35, 628-634. https://doi.org/10.1006/fstl.2002.0923

364 Cozzolino, D., Chree, A., Murray, I. & Scaife, J.R. (2002). The assessment of the composition of fishmeal by
365 near infrared reflectance spectroscopy. Aquaculture Nutrition, 8 (2), 147–
366 155. https://doi.org/10.1046/j.1365-2095.2002.00206.x

367 Cozzolino D., Chree, A., Murray, I., & Scaife JR. (2009). Usefulness of near infrared spectroscopy to monitor
368 the extent of heat treatment in fish meal. International *Journal of Food Science and Technology*, 44,
369 1579-1584. https://doi.org/10.1111/j.1365-2621.2008.01845.x

370 Dang, T.T.H., Gudjónsdóttir M., Ren, D., Karlsdóttir M.G., Nguyen, M.V., Tómasson, T., & Arason, A. 2018.
371 Effects of pre and postrigor freezing and temperature stability during frozen storage on
372 physicochemical stability of Atlantic herring (*Clupea harengus*) muscle). *Journal of Food Processing*
373 *and Preservation*, 42(9), Article e13754. https://doi.org/10.1111/jfpp.13754

374 Díaz, E.O., Kawamura, S., Matsuo, M., Kato, M., & Koseki, K. (2019). Combined analysis of near-infrared
375 spectra, colour, and physicochemical information of brown rice to develop accurate calibration
376 models for determining amylose content. *Food Chemistry*, 286, 297-306.
377 https://doi.org/10.1016/j.foodchem.2019.02.005

378 Einarsson, M.I., Bæk, A.M., Eliasen, O., Flesland, O., Jacobsen, C., Jokumsen, A., Pálsson, J., Pedersen, S.A.,
379 & Samuelsen, T.A. (2019). Nordic center of excellence network in fishmeal and fish oil. Matís ohf.
380 Pp. 124. https://doi.org/10.5281/zenodo.3243334

Ellekjær, M.R., Isaksson, T., & Solheim, R. (2006). Assessment of sensory quality of meat sausages using near infrared spectroscopy. *Journal of Food Science* 59 (3), 456-464. https://doi.org/10.1111/j.1365-2621.1994.tb05538.x

FAO. (1986). *The production of fish meal and oil* (FAO Fisheries Technical Paper - 142 ISBN 92-5-102464-2; Aquaculture Feed and Fertilizer Resources Information System). http://www.fao.org/3/X6899e/X6899E00.HTM

Fournier, V., Destaillats, F., Juanéda, P., Dionisi, F., Lambelet, P., Sébédio, J.-L., & Berdeaux, O. (2006). Thermal degradation of long-chain polyunsaturated fatty acids during deodorization of fish oil. *European Journal of Lipid Science and Technology*, *108* (1), 33–42. https://doi.org/10.1002/ejlt.200500290

Gomes, A.A., Azcarate, S.M., Goncalves, Dias Diniz, P.H., de Sousa Fernandes, D.D., & Veras, G. (2022). Variable selection in the chemometric treatment of food data: A tutorial review. *Food Chemistry* 370, Article 131072. https://doi.org/10.1016/j.foodchem.2021.131072

Gudjónsdóttir, M., Jónsson, Á., Bergsson, A.B., Arason, S., Margeirsson, S., & Rustad, T. (2011). Shrimp processing assessed by low field Nuclear Magnetic Resonance, Near Infrared spectroscopy and physicochemical measurements The effect of polyphosphate content and length of pre-brining on shrimp muscle. *Journal of Food Science* 76 (4), E357-367. https://doi.org/10.1111/j.1750-3841.2011.02112.x

Hall, G.M. (2010). Fishmeal production and sustainability. In: *Fish Processing: Sustainability and new opportunities*. Hall, G.M., (ed.); Wiley-Blackwell. pp. 207–235.

Hilmarsdóttir, G.S., Ogmundarson, Ó., Arason, S., & Gudjónsdóttir, M. (2020). The effects of varying heat treatments on lipid composition during pelagic fishmeal production. *Processes*, 8 (9), 1142 https://doi.org/10.3390/pr8091142

Hilmarsdóttir, G.S., Ogmundarson, Ó., Arason, S., & Gudjónsdóttir, M. (2021). Efficiency of fishmeal and fish oil processing of different pelagic fish species: Identification of processing steps for potential optimization toward protein production for human consumption. *Journal of Food Processing and Preservation*, 45 (4), e15294. https://doi.org/10.1111/jfpp.15294

Hilmarsdóttir, G.S., Ogmundarson, Ó., Arason, S., & Gudjónsdóttir, M. (2022). Identification of environmental hotspots in fishmeal and fish oil production for process optimization purposes. *Journal of Cleaner Production (In Press)*.

ISO (1999). ISO 6496:1999. Animal Feeding Stuffs – Determination of moisture and other volatile matter content. International Organization for Standardization: Geneva, Switzerland, 1999; Available online: https://www.iso.org/obp/ui/#iso:std:iso:6496:ed-2:v1:en (accessed on March 10th, 2020).

ISO (2008) ISO 16634-1:2008. Food products – Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content – Part 1: Oilseeds and animal feeding stuffs. International Organization for Standardization: Geneva, Switzerland, 1999; Available online: https://www.iso.org/obp/ui/#iso:std:iso:16634:-1:ed-1:v1:en (accessed on January 5th, 2022).

ISO (2009) ISO 5983-2:2009. Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content–Part 2: Block digestion and steam distillation method. International Organization for Standardization: Geneva, Switzerland, 1999; Available online: https://www.iso.org/standard/52199.html (accessed on January 5th, 2022).

Jacobsen, C. (2015). Some strategies for the stabilization of long chain n-3 PUFA-enriched foods: A review. *European Journal of Lipid Science and Technology*, *117* (11), 1853–1866. https://doi.org/10.1002/ejlt.201500137

Kristinsson, H. G. (2007). Aquatic food protein hydrolysates. In: *Maximising the Value of Marine By-Products* (pp. 229–248). Elsevier. https://doi.org/10.1533/9781845692087.2.229

Larsen, R., Eilertsen, K.-E., & Elvevoll, E. O. (2011). Health benefits of marine foods and ingredients. *Biotechnology Advances*, *29*(5), 508–518. https://doi.org/10.1016/j.biotechadv.2011.05.017

Lowry, R.R.; & Tinsley, I.J. (1976). Rapid colorimetric determination of free fatty acids. *Journal of American Oil Chemists Society*, 53, 470–472. https://doi.org/10.1007/BF02636814

McClure, F.W. (2003). 204 years of near infrared technology: 1800–2003. *Journal of Near Infrared Spectroscopy*, 11, 487–518. https://doi.org/10.1255/jnirs.399

Nilsen, H., & Esaiassen, M. (2005). Predicting sensory score of cod (*Gadus morhua*) from visible spectroscopy. *LWT–Food Science and Technology* 38 1), 95-99. https://doi.org/10.1016/j.lwt.2004.05.001

Oliveira, U. F., Costa, A. M., Roque, J. V., Cardoso, W., Motoike, S. Y., Barbosa, M. H. P., & Teofilo, R. F. (2021). Predicting oil content in ripe Macaw fruits (Acrocomia aculeata) from unripe ones by near infrared spectroscopy and PLS regression. *Food Chemistry*, 351, 129314. https://doi.org/10.1016/j.foodchem.2021.129314.

Oterhals, Å., & Vogt, G. (2013). Impact of extraction, refining and concentration stages on the stability of fish oil. In: Jacobsen, C., Nielsen, N.S., Horn, A.F., & Sørensen, A.-D.M. (Eds.), *Food enrichment with omega-3 fatty acids (*pp. 111–129*)*, Woodhead Publishing.

Rinnan, Å., van den Berg, F. & Engelsen, S.B. (2009). Review of the most common pre-processing techniques for near-infrared spectra. *Trends in Analytical Chemistry*, 28 (10), 1201-1222. https://doi.org/10.1016/j.trac.2009.07.007

Romotowska, P.E., Karlsdóttir, M.G., Gudjónsdóttir, M., Kristinsson, H.G., & Arason, S. (2016). Seasonal and geographical variation in chemical composition and lipid stability of Atlantic mackerel (Scomber scombrus) caught in Icelandic waters. *Journal of Food Composition and Analysis,* 49, 9-18. http://dx.doi.org/10.1016/j.fjca.2016.03.005

Roque, J.V., Cardoso, W., Peternelli, L.A., & Teófilo, R.F. (2019). Comprehensive new approaches for variable selection using ordered predictors selection. *Analytica Chimica Acta*, 1075, 57-70. https://doi.org/10.1016/j.aca.2019.05.039

Shiroma, C., & Rodriguez-Saona, L. (2009). Application of NIR and MIR spectroscopy in quality control of potato chips. *Journal of Food Composition and Analysis*, 22 (6), 596-605. https://doi.org/10.1016/j.jfca.2008.09.003

Stevik, A.M., Duun, A.S., Rustad, T., O'Farrel, M., Schulerud, H., & Ottestad, S. (2010). Ice fraction assessment by near infrared spectroscopy enhancing automated superchilling process lines. *Journal of Food Engineering*, 100, 169-177. https://doi.org/10.1016/j.jfoodeng.2010.03.042

Stewart, J.C.M. (1980). Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Analytical Biochemistry*, 104 (1), 10–14. https://doi.org/10.1016/0003-2697(80)90269-9

Watts, P., Long, G., & Meek, M. E. (2004). Concise International Chemical Assessment Document 58. *Chloroform*. World Health Organization, Geneva. Switzerland. https://inchem.org/documents/cicads/cicads/cicad58.htm

Windsor, M.L. (2001). Fish Protein Concentrate. FAO Fisheries and Aquaculture Department 2001. Available online: http://www.fao.org/3/x5917e00.htm#Contents (accessed on February 7th, 2020).

Zheng, J.-S., Hu, X.-J., Zhao, Y.-M., Yang, J., & Li, D. (2013). Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: Meta-analysis of data from 21 independent prospective cohort studies. *BMJ*, *346*, f3706–f3706. https://doi.org/10.1136/bmj.f3706

CRediT author statement

María Gudjónsdóttir: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft; Writing - Reviewing and editing. **Gudrún Svana Hilmarsdóttir**: Conceptualization, Formal analysis; Methodology; Validation: Writing – Review and editing. **Ólafur Ögmundarson**: Supervision, Writing- Review and editing. **Sigurjón Arason:** Funding acquisition, Methodology; Resources; Supervision, Writing – Review and editing.

Supplementary material

Table S1: Assignment of NIR overtone and combination bands obtained during pelagic fishmeal and oil processing. Adapted and modified from Shiroma and Rodriguez-Saona (2009) and Cozzolini et al. (2009).

Approximate peak maximum wave numbers (cm^{-1})	Functional group assignment	Dominant chemical component
10600	O-H stretch 2nd overtone	Water
8600-8150	C-H 2nd overtone	Lipids
7400-7000	2nd overtone of C-H combinations	Lipids
6800,	O-H 1st overtone	Water
5750	C-H 1st overtone of CH_3	Lipids
5500	C-H 1st overtone of -HC=CH-	Lipids
5200-5000	O-H stretch 1st overtone, O-H combinations	Water
5100-4500	N-H combination peptide absorption bands of amide groups	Proteins
4370	C-H+C-H combination bands $-CH_2$, $-CH_3$	Lipids
4350-4150	C-H combination bands, C=C stretch, -CH, $-CH_2$	Lipids

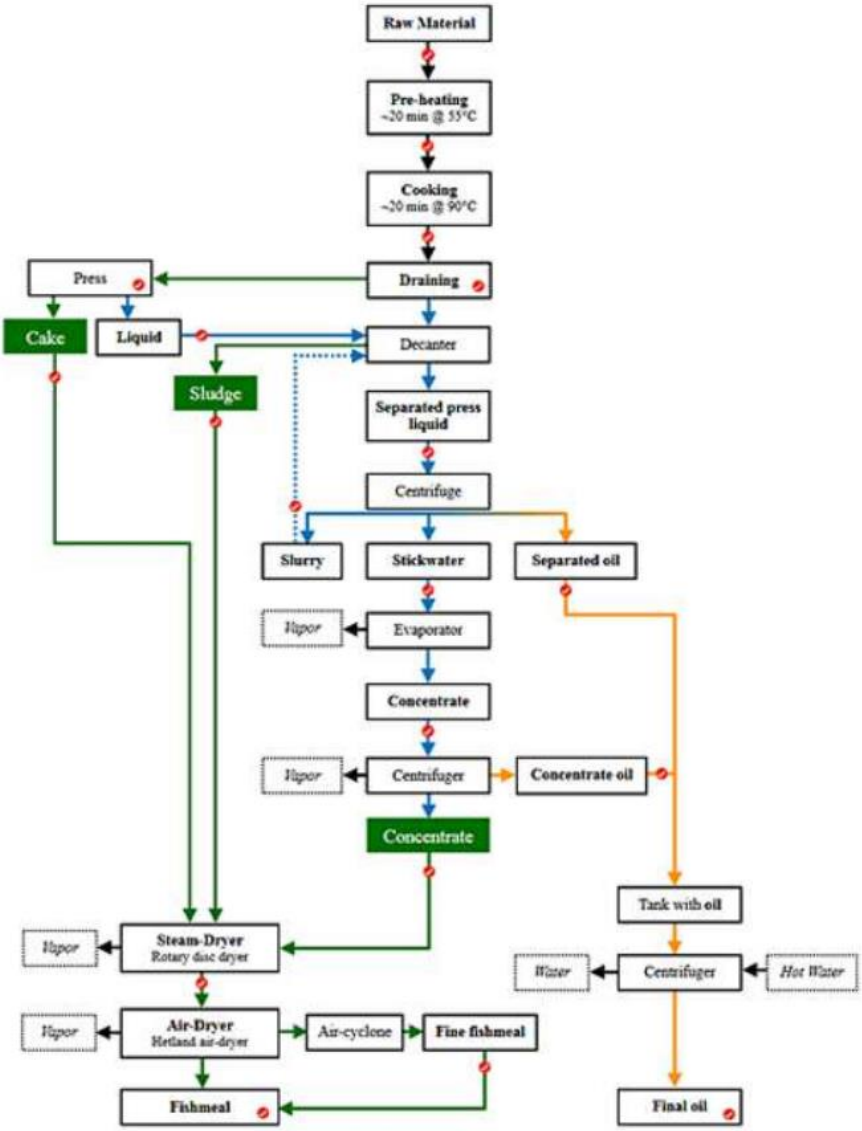
19

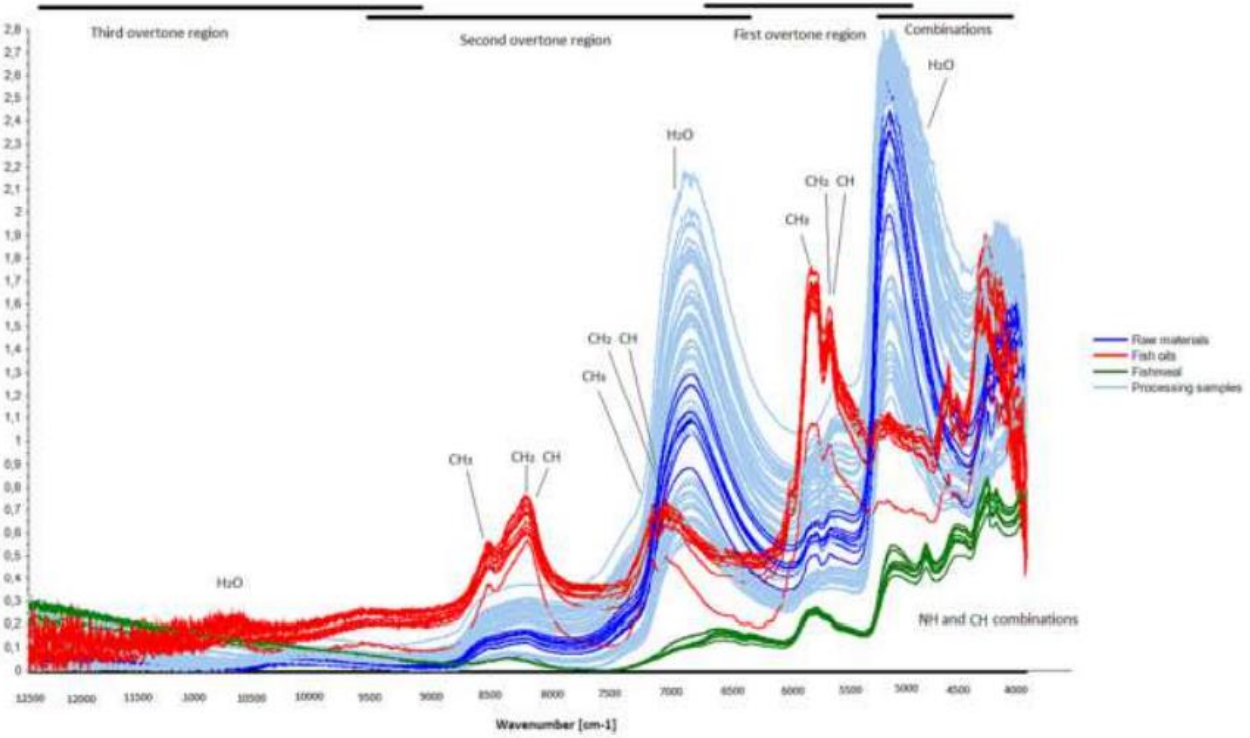
Table S2. NIR prediction model summary for the prediction of the chemical composition of fishmeal and oil during processing.

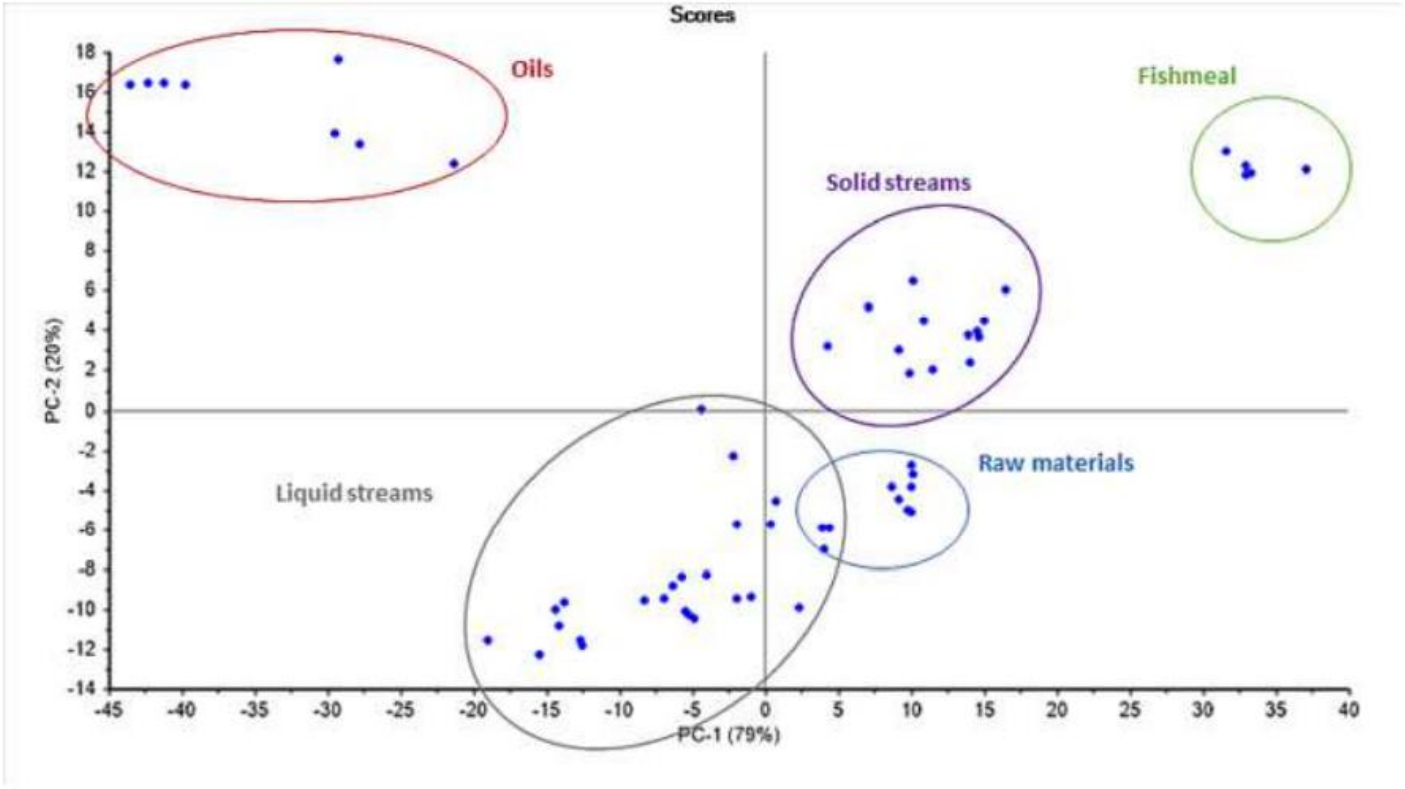
		Calibration				Independent validation		
Spectral data pre-treatment	**Valid range of reference parameter**	n	p	R^2_{CV}	RMSEC	n	R^2_P	RMSEP
Water (g/100 g sample)	**0.3-93.0**							
None		**60**	**2**	**0.9995**	**0.67**	**30**	**0.9938**	**2.41**
Baseline correction		60	3	0.9666	5.56	30	0.9631	5.88
1st derivative		60	3	0.9404	7.42	30	0.9348	7.82
2nd derivative		60	5	0.9793	4.38	30	0.8142	13.20
MSC		60	3	0.9498	6.81	30	0.9477	7.00
Lipids (g/100 g sample)	**0-100**							
None		60	4	0.9640	4.11	30	0.9749	4.97
Baseline correction		**60**	**3**	**0.9669**	**3.94**	**30**	**0.9773**	**3.94**
1st derivative		60	2	0.9265	5.87	30	0.9404	7.66
2nd derivative		60	3	0.9828	2.84	30	0.5314	21.49
MSC		60	3	**0.9681**	**3.86**	30	**0.9592**	**6.34**
FFDM (g/100 g sample)	**0-84.4**							
None		60	4	0.8899	8.36	30	0.9063	7.01
Baseline correction		60	4	0.8473	8.52	30	0.8969	7.06
1st derivative		60	5	0.6743	12.44	30	0.8590	8.25
2nd derivative		60	4	0.8570	8.24	30	0.8003	9.82
MSC		60	7	**0.9183**	**6.23**	30	**0.9356**	**5.58**
FFA (g/100 g sample)	**0-5**							
None		52	1	0.0911	0.91	25	0.0242	0.99
MSC		52	2	0.0785	0.92	25	0.0583	0.98
PL (g/100 g sample)	**0-1.4**							
None		44	6	0.4476	0.24	22	0.4841	0.22
Baseline correction		44	8	0.6506	0.19	22	0.5453	0.20
1st derivative		44	9	0.8888	0.11	22	0.6033	0.19
2nd derivative		44	1	0.3427	0.26	22	0.3055	0.25
MSC		44	10	**0.9617**	**0.06**	22	**0.8617**	**0.11**
SFA (g/100 g lipids)	**20-32**							
None		**34**	**6**	**0.9953**	**0.20**	**17**	**0.9928**	**0.24**
Baseline correction		34	9	0.9464	0.68	17	0.6922	1.56
1st derivative		34	15	0.9989	0.10	17	0.6668	1.62
2nd derivative		34	1	0.4147	2.26	17	0.3149	2.32
MSC		**34**	**12**	**0.9889**	**0.31**	**17**	**0.8363**	**1.13**
MUFA (g/100 g lipids)	**38-54**							
None		**34**	**9**	**0.9062**	**1.15**	**17**	**0.7968**	**1.62**

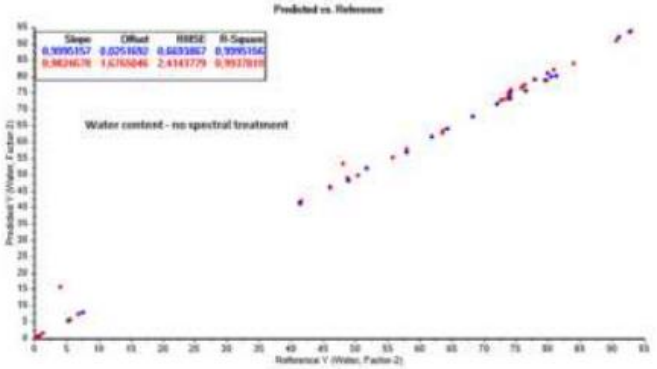
Baseline correction		34	5	0.6815	2.11	17	0.6200	2.22
1st derivative		34	4	0.3882	2.93	17	0.4230	2.74
2nd derivative		34	1	0.2863	3.16	17	0.2532	3.11
MSC		**34**	**9**	**0.9462**	**0.87**	**17**	**0.8291**	**1.49**
PUFA (g/100 g lipids)	**12-36**							
None		**34**	**9**	**0.9371**	**1.53**	**17**	**0.8461**	**2.20**
Baseline correction		34	6	0.7060	3.30	17	0.6051	3.53
1st derivative		34	4	0.4791	4.39	17	0.4924	4.00
2nd derivative		34	1	0.3196	5.02	17	0.3087	4.67
MSC		**34**	**11**	**0.9816**	**0.83**	**17**	**0.8588**	**2.11**
DHA (g/100 g lipids)	**4-17**							
None		**34**	**8**	**0.9073**	**0.84**	**17**	**0.8146**	**1.09**
Baseline correction		34	6	0.7320	1.43	17	0.6302	1.55
1st derivative		34	4	0.5864	1.78	17	0.6197	1.57
2nd derivative		34	14	0.9982	0.12	17	0.3380	2.07
MSC		**34**	**9**	**0.9623**	**0.54**	**17**	**0.8785**	**0.89**
EPA (g/100 g lipids)	**2-10**							
None		**34**	**10**	**0.9536**	**0.40**	**17**	**0.8278**	**0.71**
Baseline correction		34	4	0.5110	1.30	17	0.6366	1.03
1st derivative		34	4	0.5146	1.30	17	0.5077	1.20
2nd derivative		34	1	0.3437	1.51	17	0.3335	1.40
MSC		**34**	**11**	**0.9791**	**0.27**	**17**	**0.8689**	**0.62**

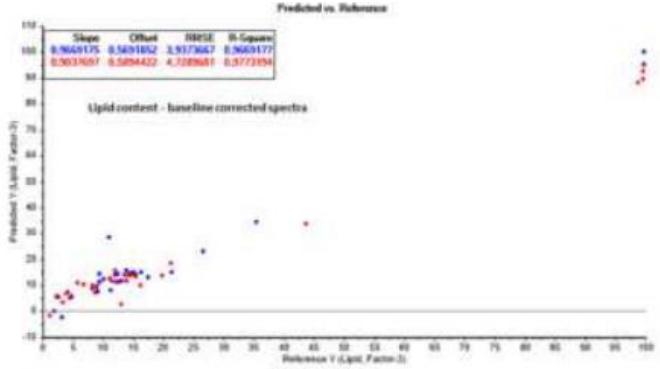
n=number of samples, p=model prediction factor, R^2_{CV}=calibration correlation factor, R^2_P=prediction correlation factor, RMSEC=root-mean-square error of calibration, RMSEP=root-mean-square error of prediction

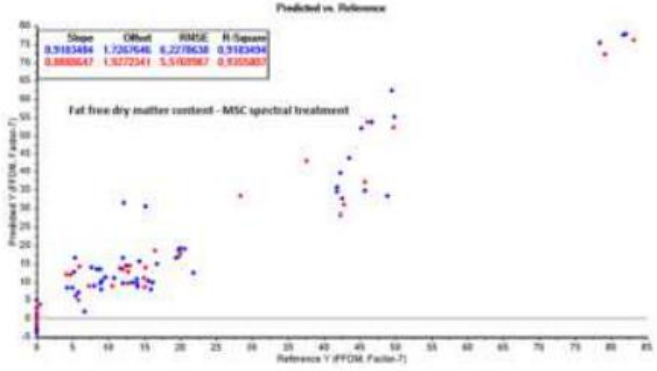

Raw Material
Pre-heating
~20 min @ 55°C
Cooking
~20 min @ 90°C
Draining
Press
Cake
Liquid
Sludge
Decanter
Separated press liquid
Centrifuge
Slurry
Stickwater
Separated oil
Vapor
Evaporator
Concentrate
Vapor
Centrifuger
Concentrate oil
Concentrate
Tank with oil
Vapor
Steam-Dryer
Rotary disc dryer
Water
Centrifuger
Hot Water
Vapor
Air-Dryer
Hetland air-dryer
Air-cyclone
Fine fishmeal
Fishmeal
Final oil

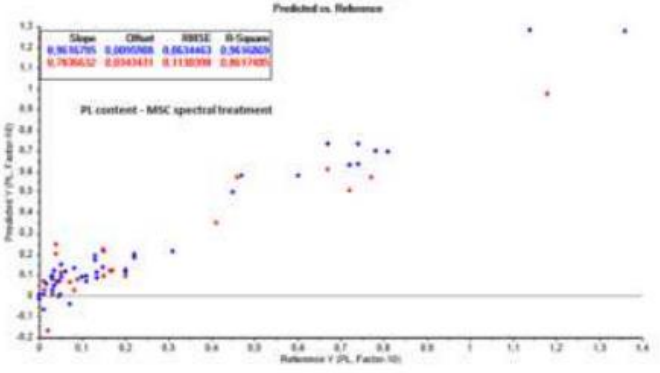

Third overtone region
Second overtone region
First overtone region
Combinations
H₂O
H₂O
CH₂ CH
CH₃
CH₂ CH
CH₃
CH₂ CH
CH₃
H₂O
NH and CH combinations
Raw materials
Fish oils
Fishmeal
Processing samples
Wavenumber [cm-1]

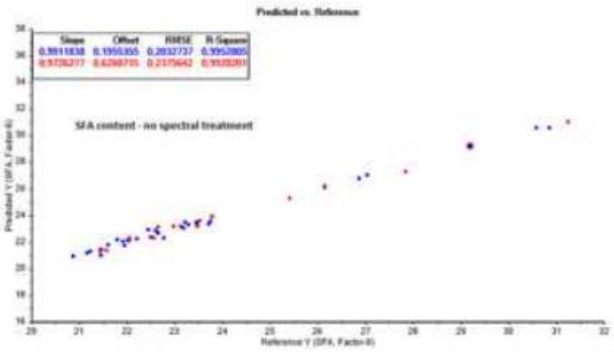

Scores
Oils
Fishmeal
Solid streams
Raw materials
Liquid streams
PC-2 (20%)
PC-1 (79%)

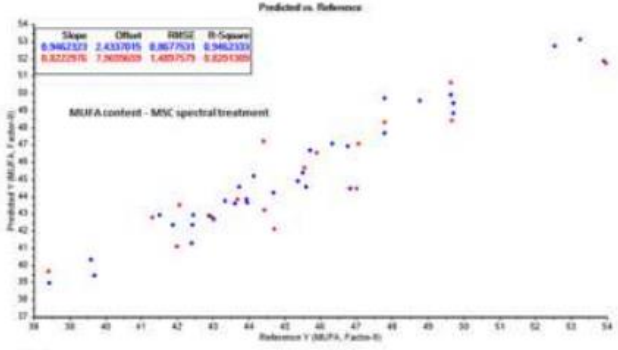

Predicted vs. Reference
Slope Offset RMSE R-Square
0,9995157 0,0251692 0,6693867 0,9995154
0,9824670 1,6765046 2,4141779 0,9937810
Water content - no spectral treatment
Predicted Y (Water, Factor-2)
Reference Y (Water, Factor-2)

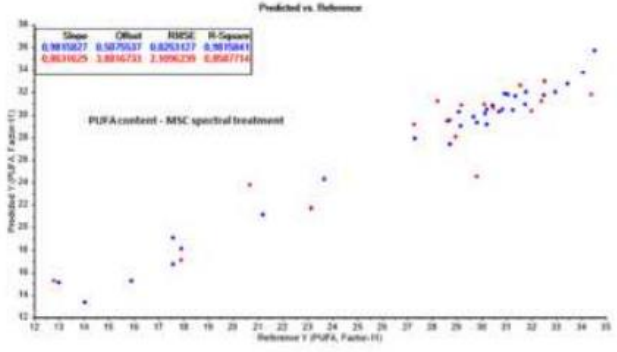

Predicted vs. Reference
Slope Offset RMSE R-Square
0,9669175 0,5691852 3,9373667 0,9669177
0,9037697 0,5894422 4,7189681 0,9773394
Lipid content - baseline corrected spectra
Predicted Y (Lipid, Factor-3)
Reference Y (Lipid, Factor-3)

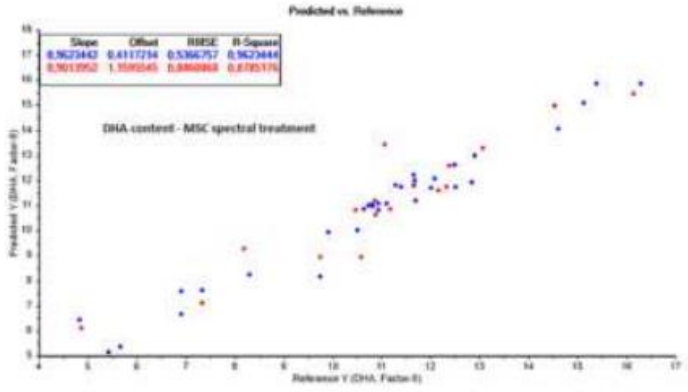

Predicted vs. Reference
Slope Offset RMSE R-Square
0,9183494 1,7267646 6,2278638 0,9183494
0,8880647 1,9272341 5,5703987 0,9305807
Fat free dry matter content - MSC spectral treatment
Predicted Y (FFDM, Factor-7)
Reference Y (FFDM, Factor-7)

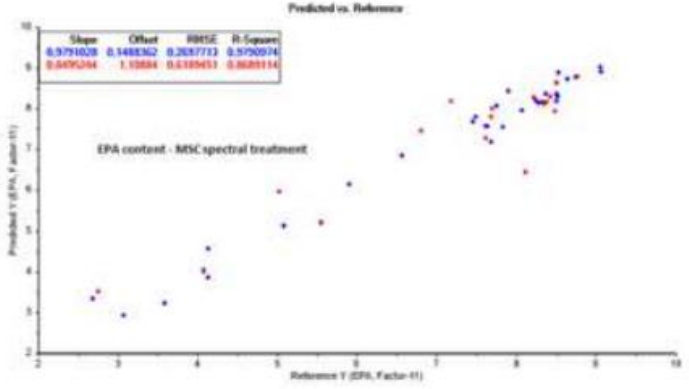

Predicted vs. Reference
Slope Offset RMSE R-Square
0,9616795 0,0095068 0,0634463 0,9616269
0,7936652 0,0343431 0,1138599 0,8617495
PL content - MSC spectral treatment
Predicted Y (PL, Factor-10)
Reference Y (PL, Factor-10)


Predicted vs. Reference
Slope Offset RMSE R-Square
0.9911838 0.1955355 0.2032737 0.9952805
0.9726277 0.6268715 0.2375642 0.9935093
SFA content - no spectral treatment
Predicted Y (SFA, Factor-I)
Reference Y (SFA, Factor-I)


Predicted vs. Reference
Slope Offset RMSE R-Square
0.9462323 2.4337015 0.8677531 0.9462333
0.8222576 7.9059659 1.4857579 0.8251385
MUFA content - MSC spectral treatment
Predicted Y (MUFA, Factor-II)
Reference Y (MUFA, Factor-II)


Predicted vs. Reference
Slope Offset RMSE R-Square
0.9815627 0.5875537 0.8253127 0.9815841
0.8631625 3.8816733 2.1096239 0.8587714
PUFA content - MSC spectral treatment
Predicted Y (PUFA, Factor-II)
Reference Y (PUFA, Factor-II)


Predicted vs. Reference
Slope Offset RMSE R-Square
0.9623442 0.4117234 0.5366757 0.9623444
0.9613952 1.1595245 0.8860863 0.8785176
DHA content - MSC spectral treatment
Predicted Y (DHA, Factor-5)
Reference Y (DHA, Factor-5)


Predicted vs. Reference
Slope Offset RMSE R-Square
0.9791028 0.1488362 0.2687713 0.9790974
0.9499284 1.10884 0.5389453 0.8685114
EPA content - MSC spectral treatment
Predicted Y (EPA, Factor-11)
Reference Y (EPA, Factor-11)

NIR spectroscopy for effective online process monitoring and quality prediction