The Fatty Acid Profile of Eulachon (*Thaleichthys pacificus*) Grease: an Invaluable Traditional Food of the Coastal First Nations of British Columbia

by
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Abstract

A lesser-known and under-studied, but prized oil-rich fish species for coastal First Nations of British Columbia is the eulachon (*Thaleichthys pacificus*). Each family has individual techniques to ferment, cook, and strain this cultural keystone species to render the fat, often called "grease" (*ɬi'na*) which is of great cultural, nutritional, social, and economic value. In this study, the nutritional profiles of eulachon grease are explored by chemical analysis and traditional knowledge obtained through interviews with Knowledge Holders. Lipidomic techniques were applied using two different chemical analysis methods (i.e., gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry) to identify and quantify the individual fatty acid levels in seven eulachon grease samples collected in Alert Bay, BC, in July 2023. Fish oil supplement samples were bought from the Canadian market in January 2023 and analyzed for comparison. The results show that there are significant variations in the lipid profiles of the eulachon grease samples regardless of preparation techniques (i.e., length of eulachon fermentation, cooking, etc.). Eulachon grease samples contain unique saturated, polyunsaturated, and monounsaturated fatty acids that are beneficial to health (i.e., promote cardiovascular health, reduce the risk of cardiovascular diseases, and provide anti-inflammatory, immunoregulatory, and neuroprotective effects). In comparison, fish oil supplements from the Canadian market were found to have relatively high levels of saturated fatty acids. Traditional Knowledge also supports the many benefits of eulachon grease. Eulachon grease fills a critical niche in the diet, health, and well-being of BC coastal First Nations People.

Résumé

Un poisson riche en huile et peu étudié, mais valorisé par les Premières Nations côtières de la Colombie-Britannique, est l'eulachon (*Thaleichthys pacificus*). Chaque famille possède des techniques spécifiques pour fermenter, cuire et filtrer cette espèce clé de voûte culturelle afin d'en extraire la graisse (*ɬi'na*), qui a une grande importance culturelle, nutritionnelle, sociale et économique. Dans cette étude, les profils nutritionnels de la graisse d'eulachon sont explorés à travers une analyse chimique et les savoirs traditionnels obtenus lors d'entretiens avec des Gardiens du savoir. Des techniques lipidomiques ont été appliquées en utilisant deux méthodes d'analyse chimique différentes (c'est-à-dire la CL-MS et la CG-MS) afin d'identifier et de quantifier les niveaux individuels d'acides gras dans sept échantillons de graisse d'eulachon collectés à Alert Bay, C-B, en juillet 2023. Des échantillons de suppléments d'huile de poisson ont été achetés sur le marché canadien en janvier 2023 et analysés pour comparaison. Les résultats montrent qu'il existe des variations significatives dans les profils lipidiques des échantillons de graisse d'eulachon, indépendamment des techniques de préparation (c'est-à-dire la durée de fermentation de l'eulachon, la cuisson, etc.). Les échantillons de graisse d'eulachon contiennent des acides gras saturés, polyinsaturés et monoinsaturés uniques, bénéfiques pour la santé (c'est-à-dire qu'ils favorisent la santé cardiovasculaire, réduisent le risque de maladies cardiovasculaires, et offrent des effets anti-inflammatoires, immuno-régulateurs et neuroprotecteurs). En comparaison, les suppléments d'huile de poisson du marché canadien présentaient des niveaux relativement élevés d'acides gras saturés. Les savoirs traditionnels confirment également les nombreux bienfaits de la graisse d'eulachon. La graisse d'eulachon remplit une niche essentielle dans l'alimentation, la santé et le bien-être des Premières Nations côtières de la Colombie-Britannique.

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Abbreviations

ALA	Alpha-Linolenic Acid
ANOVA	Analysis of Variance
AVG	Average
BC	British Colombia
BCFA	Branched-Chain Fatty Acid
%C	Percent Carryover
CMSC	Carleton Mass Spectrometry Centre
CVD	Cardiovascular Diseases
CVH	Cardiovascular Health
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
%FA	Percent Fatty Acid
GC-MS	Gas Chromatography-Mass Spectrometry
GLA	Gamma-Linoleic Acid
HPLC	High-Performance Liquid Chromatography
ICP-MS	Inductively Coupled Mass Spectrometry
IHD	Ischemic Heart Disease
IK	Indigenous Knowledge
JLHMS	John L. Holmes Mass Spectrometry Facility
LC-MS	Liquid Chromatography-Mass Spectrometry
LDL-C	Low-Density Lipoprotein Cholesterol
LMSD	LIPID MAPS® Structure Database
PCA	Principal Component Analysis
POP	Persistent Organic Pollutants
SFA	Saturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
NRCD	Nutrition-Related Chronic Disease
PUFA	Polyunsaturated Fatty Acid
TEK	Traditional Ecological Knowledge
TK	Traditional Knowledge

Kwak’wala Glossary

Dzawadi	Knight Inlet
Gilakas’la	Welcome and/or thank you
Ł̓axwe’gila	Strength gaining or building
Samgaẗsi	Container to boil eulachons over a fire (like a vat)
Taga’ł	Eulachon net
Ṫł̓i’na	Eulachon grease
Ṫł̓i’nagila	Making eulachon grease
Ṫsapa	Dip food in eulachon grease
‘Yalis	Alert Bay

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1. Introduction

1.1 Project Background

This master's thesis is part of a larger global project titled *Climate Change Adaptation and Food Security for First Nations (CCFS4N)*. It is funded by the Canadian Institutes of Health Research (CIHR). The partners on the global project include First Nation Peoples from Tla'amin Nation, 'Namgis First Nation, Skidegate Band Council, and Nuxalk Nation as well as academic researchers from the University of Ottawa, Université Laval, Université de Montréal, University of British Columbia, Simon Fraser University, and the First Nations Health Authority. The objectives of the global project are based on concerns raised by the Steering Committee about the effects of climate change on First Nation fisheries and consequently, the increased risk of food insecurity and non-communicable nutrition-related diseases in coastal seafood-dependent communities. The intentions and principles of this thesis align with that of the global project in using Western science and Traditional Knowledge to promote management authority, food security, and food independence for coastal First Nations in British Columbia. The global project and this thesis follow the First Nations principles of ownership, control, access, and possession (OCAP®). Ethics approval was obtained through the University of Ottawa's Behavioural Research Ethics Board (certificate number H-04-23-9108).

1.2 Literature Review

In North America, "Indigenous peoples" is a collective name for the original inhabitants of the land and their descendants (CIRNAC, 2022). Canada recognizes three groups of Indigenous peoples: First Nations, Inuit, and Métis (Canadian Constitution Act, 1982). These are three distinct peoples with unique histories, languages, cultural practices, and spiritual beliefs (CIRNAC, 2022). Unlike Inuit and Métis, most First Nations have tracts of land held by the

Crown that fall under the reserve system, First Nation members may live on or off these reserves (Parrott et al., 2023). "First Nations people" include Status and non-Status Indians and represent approximately 3% of the Canadian population (Statistics Canada, 2023; CIRNAC, 2021). There are 634 First Nation communities in Canada, which represent more than 50 Nations and 50 Indigenous languages (AFN, 2023; CIRNAC, 2021). The area now known as British Columbia (BC) has supported First Nations people for more than 10 000 years (BCAFN, 2023). There are 203 First Nations in BC, the larger ethnic groupings include the Coast Salish, Dakelh, Dene, Haida, Ktunaxa, Kwakwaka'wakw, Nisga'a, Okanagan, Secwepemc, Sekani, Stl'atl'imx, Sto:lo, Tlingit, Tsilhqot'in, Tsimshian and Wet'suwet'en (BCAFN, 2023; James-Abra, 2023).

Since time immemorial, First Nations people have relied on food harvesting strategies (e.g., hunting, foraging, and fishing) and food production practices (e.g., clam gardens, berry patches, and species domestication) to procure their traditional foods (Kuhnlein et al., 2013; Power, 2008, Deur & Turner, 2005). "Traditional food" (TF) is a collective term for all foods available from local natural resources that are culturally accepted within a particular Nation (Kuhnlein & Receveur, 1996). "Traditional food systems" also include the sociocultural meanings, acquisition/processing techniques, use, composition, and nutritional consequences for the people using and consuming the food (Kuhnlein & Receveur, 1996). The traditional food systems of First Nations people in Canada are diverse and include a large variety of game, fish, birds, berries, and other plant and tree foods (Batal et al., 2021a). Traditional foods are a significant source of energy, essential vitamins, minerals, and polyunsaturated fatty acids (PUFAs). They are low in saturated fat and carbohydrates and have important social, health, and cultural benefits (Batal et al., 2021b**;** Gagné et al., 2012; Kuhnlein & Receveur, 2007). When comparing days on which TF was consumed against days where it was not, more vitamins A, D,

E, and B-6, riboflavin, iron, zinc, copper, magnesium, manganese, phosphorus, potassium, and selenium were part of the diet and there were lower amounts of undesirable sodium, sugar, carbohydrates, and fats (Kuhnlein & Receveur, 2007; Kuhnlein et al., 2004).

First Nations rely strongly on the anticipated seasonal abundance of resources and predictable environmental conditions to harvest the TFs they need for sustenance and economic stability and to carry out cultural activities (Turner & Cilfton, 2009). However, climate change is causing anomalies in weather, animal behaviour, and overall environmental health making it more difficult for First Nations people to predict, prepare, and adapt to environmental changes (McLean et al., 2009; Turner & Cilfton 2009). Consequently, these effects have led to a dietary transition, resulting in a decline in the availability, quality, safety, and access to TF (Batal et al., 2021a; McLean et al., 2009). The reduction of TFs in the diet of First Nations people is worsening the ongoing health disparities between First Nations and non-Indigenous Canadians (NCCIH, 2022; Kuhnlein et al., 2013; Adelson, 2005). First Nations in BC experience poorer health status and a more significant burden from chronic conditions compared with the general population of BC (FNHA, 2012). For example, First Nations people experience shorter life expectancy and higher rates of mortality and chronic conditions including obesity, type 2 diabetes, cancer, respiratory and cardiovascular diseases (CVD) (NCCIH, 2022; Batal et al., 2021c). The health discrepancies that exist between First Nations and non-Indigenous Canadians amplify the importance of maintaining sustained access to TFs and promoting the use of TFs in the diet of First Nations people (Batal et al., 2021b).

Among First Nations in BC, particularly in coastal communities, there is a greater reliance on marine foods in the traditional diet (Batal et al., 2021a; Marushka et al., 2018). A lesser-known and under-studied but prized oil-rich fish species for BC coastal First Nations

people is the eulachon (*Thaleichthys pacificus*) (Patton et al., 2019; Payne et al., 1999). This cultural keystone species is commonly spelt as ooligan, eulachon, eulachen, olachen, olachon, oulachan, or oolachan. The origin of its name is derived from the Chinook trade language, but each First Nation group possesses a different word for the fish specific to their language. Eulachon has also been coined the "candlefish" due to its high-fat content that allows it to be burnt like a candle when dried, and the "salvation fish" as it historically arrived as First Nations people were starving or low on winter food stores. In this study, "eulachon" will be used as this is the most common spelling in today's literature.

Eulachon is a small smelt species (Family *Osmeridae*) that grows to a maximum length of 30 cm. Mature eulachons are dark blue/grey with black speckling and a silvery white underbelly. Eulachons are found only along the North American Pacific Coast from northern California to southwestern Alaska. Eulachons are an anadromous fish that return in the spring to freshwater river systems for spawning after spending 2-3 years at sea. In coastal BC, glacial-fed rivers, such as the Stikine, the Nass, the Skeena, the Kitimat, the Bella Coola, the Kingcome, the Klinaklini (Knight Inlet), and the Fraser are important for harvesting eulachons (MacNair, 1971). Eulachons also play a crucial role in ecological function by providing a large amount of energy-rich food for marine and freshwater species such as seagulls, crabs, seals, eagles, sea lions, porpoises, fin back whales, and even marine fish such as salmon, hake, halibut, and cod (Marston et al., 2002).

The cultural significance of the eulachon to First Nations people cannot be underestimated (Patton et al., 2019). The fish can be eaten fresh, baked, grilled, or boiled or preserved by smoking, drying, salting, or freezing (Kuhnlein & Chan, 1998; Kuhnlein et al., 1982). The fat rendered from eulachons, often called "grease" (or *ɫi'na*), is also of great

cultural, nutritional, social, and economic value to BC coastal First Nations people. The people of ‘Namgis First Nation in Alert Bay (*‘Yalis*), BC, prepare eulachon grease from the eulachons entering Knight Inlet (*Dzawadi*) around the 3rd week of April. The fishermen use a traditional conical net (*taga’ł*) to catch the eulachons (MacNair, 1971).

Each family has individual techniques to ferment (or “ripen”), cook, and strain the eulachons to produce eulachon grease (Kuhnlein et al., 1982). For example, after the eulachons have been harvested and before they are cooked, the eulachons are left to ripen in pits on the riverbank for 4-14 days, depending on weather conditions (Kuhnlein et al., 1982). Each family has their own way of telling when the ripening is complete-either by smell, or by feeling the texture of the decomposing fish (Kuhnlein et al., 1982). Fermenting fish increases the levels of some fatty acids (FAs) (e.g., capric acid, stearic acid, EPA, and DHA) and decreases the levels of others (e.g., myristic acid, palmitic acid, oleic acid, and linolenic acid) (Anggo et al., 2015). Studies regarding the effects of fermentation time on fish FAs are limited. However, potential explanations include the degree of eulachon carcass decomposition, which promotes the release of select fats during cooking and/or the microbial conversion that occurs during the ripening process (Kuhnlein et al., 1996; Kuhnlein et al., 1982). After the eulachons have sufficiently ripened, they are moved to vats (*samgaẋsi*) that have been filled 1/3 with water and warmed to the perfect temperature. As the eulachons cook, the oil from the fish melts off the bones and rises to the surface of the water layer. The oil is skimmed off the top, placed in jugs, and then strained several times through various cloths, sheets, and mesh to remove any remaining eulachon or other debris. After preparation, eulachon grease is stored in a cool, dry place, and can last several years. It can also be frozen; some believe this stops the flavour from becoming “strong”.

Eulachon grease is used as a condiment for potatoes, fish, and berries (*ɨsa̱pa*) or as an ingredient in the preparation of bread, soup, and salads (Kuhnlein & Chan, 1998; Kuhnlein et al., 1996). Eulachon grease contains less PUFAs (e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) than whole eulachon fish, allowing reduced peroxidability and improving its capability to be stored and transported which would have been of high importance to First Nations Peoples who travelled long distances to trade eulachon grease (Phinney et al., 2009). However, when consumed as a dietary staple, eulachon grease still satisfies the nutritional requirements for omega(ω)-3 FAs of First Nations Peoples (Phinney et al., 2009; Iverson et al., 2002; Kuhnlein & Chan, 1998; Kuhnlein et al., 1996). The benefits of diets high in PUFAs are well documented, especially regarding cardio-protection and reduced CVD risk (i.e., the favourable effects on lipid and lipoprotein metabolism, blood pressure, platelet function, etc.) (Kapoor et al., 2021; Shibabaw, 2021; Shahidi & Ambigaipalan, 2018; Fleming & Kris-Etherton, 2014; Siriwardhana et al., 2012).

Moreover, eulachon grease is used as a medicinal ointment to treat colds, eczema, and wounds (Kuhnlein & Chan, 1998; Kuhnlein et al., 1982). The high monounsaturated fatty acid (MUFA) (e.g., palmitoleic acid, oleic acid, and cetoleic acid) levels in eulachon grease provide anti-inflammatory effects to the treated area (Farag & Gad, 2022; Yang et al., 2020; García, 2019). Additionally, the grease is superior at providing vitamins A, E, and K when compared to other common fat sources (Kuhnlein et al. 1996; Kuhnlein et al. 1982). Eulachon grease's distinct nutritional profile makes it the preferred fat source in the traditional diet (Phinney et al., 2009; Iverson et al., 2002; Kuhnlein & Chan, 1998).

In addition to providing nutrition and healing, eulachon grease is key to the social, mental, and cultural wellbeing of coastal BC First Nations (Patton et al., 2019). Eulachon grease

is highly prized and historically distributed at potlatches, given as a gift, and traded with neighbouring Nations. The importance of eulachon grease is best signified by the ancient trade routes used to link the coastal First Nations with the interior First Nations. These routes are famously referred to as "grease trails" as the heaviest traffic occurred during the eulachon season to trade for grease (Collision, 1941). The oral history and actions pertaining to eulachon grease-making foster harmonious intergenerational relationships and an intimate connection to the land (Patton et al., 2019).

However, climate change is altering the availability of the eulachons, making it difficult to pass these traditions on to younger generations (Personal Communications, 'Namgis First Nation member). It is proposed that warming ocean waters will cause the eulachons to migrate northward approximately 37km per decade (Lemmen et al., 2016; Weatherdon et al., 2016). Furthermore, it is predicted that changes in relative catch potential for eulachons in the BC central coast region will decrease by 30-40% between now and 2050 (Weatherdon et al., 2016). In the absence of eulachon fisheries, there will be a loss of invaluable traditional knowledge and an increased risk of food insecurity and non-communicable nutrition-related chronic diseases (NRCDs) in coastal-seafood-dependent First Nation communities (Batal et al., 2021b; Marushka et al., 2019; Gagné et al., 2012).

1.3 Problem Statement

Researchers in many disciplines have become increasingly aware of the values of a large body of information held by Indigenous Peoples, known as Traditional Knowledge (TK), Indigenous Knowledge (IK), or Traditional Ecological Knowledge (TEK), amongst other terms (Bruchac, 2014). These knowledge systems, developed over countless generations, are based on individual and collectively learned experiences and explanations of the world, verified by elders,

and conveyed and guided by experiential learning and by oral traditions and other means of record keeping (Pearce et al., 2015; Bruchac, 2014; Nakashima et al., 2012). Western and IK share several important and fundamental attributes as ways of knowing. Both are constantly verified through repetition and verification, inference and prediction, empirical observations, and recognition of pattern events (NCCIH, 2022).

However, many scientists often assume that Western Sciences are more "advanced" or "robust", and TK needs to be verified by Western Science (Bruchac, 2014). The reality is that there are many cases where Western science is behind or is just catching up with what Indigenous peoples have long known (Bruchac, 2014). For example, the medicinal properties of plants or insights into caribou migration patterns are better understood by local Knowledge Holders (Uprety et al., 2012; Reyes-García, 2010; Kendrick & Manseau, 2008; Parlee et al., 2005). It is important to embrace the knowledge gained by the two systems. Employing TK-based observations and explanations within multiple working hypotheses ensures consideration of a variety of predictive, interpretive, or explanatory possibilities not constrained by Western expectation or logic. Hypotheses that compliment TK-based information can lead the way toward unanticipated insights. Therefore, rather than treating TK as an adjunct or element to be incorporated or integrated into Western scientific studies (too often used as euphemisms for assimilating), TK should instead ground our understanding of the environmental, social, and biomedical determinants of health and improve our understanding of health and disease.

Two-Eyed Seeing (*Etuaptmumk* in Mi'kmaw) is an approach of inquiry and solutions, envisaged by Elder Dr. Albert Marshall, in which people come together to view the world from one eye with the strengths of IK and ways of knowing, and from the other eye with the strengths of Western knowledge and ways of knowing, and to use both these eyes together, for the benefit

of all (Reid et al., 2021; Forbes et al., 2020; Bartlett et al., 2012). Relationship building, community control, collaborative data analysis, and results that fostered change were recognized as common principles for successfully applying Two-Eyed Seeing (Bartlett et al., 2012). The research team has developed an excellent partnership with 'Namgis First Nation. The proposed study will allow cutting-edge studies to be conducted to optimize the use of TFs and to promote the nutritional health of Indigenous Peoples using the Two-Eyed Seeing approach.

In this study, Western science techniques (e.g., interviews, chemical analyses, and statistics) will be used to determine the optimal preparation methods of eulachon grease to promote nutritional and health benefits. This study will substantiate the TK that eulachon grease is critical to the diet, health, and well-being of coastal BC First Nations people. Through these efforts, the value of the First Nations' eulachon fishery will be highlighted. Thus, strengthening First Nations' management authority in protecting First Nations fisheries and advancing First Nations' food independence and well-being in coastal BC.

1.4 Research Objectives

In this study, the first objective is to employ lipidomic techniques to identify and quantify individual FA levels in eulachon grease samples and compare them to those of ω-3/fish oil supplements from the Canadian market. The second objective of this study is to compare the findings of Western science (i.e., the FA profile of eulachon grease and ω-3/fish oil supplements from the Canadian market) to TK (i.e., the preparation techniques and use of eulachon grease). The ultimate purpose of this study is to support First Nations TK with chemical analyses to determine which preparation methods of eulachon grease provide optimal nutritional and/or medicinal effects.

The first hypothesis of this research project is that there will be characteristics in the FA profile of eulachon grease that will be unique from that of the ω-3/fish oil supplements. The prediction is that eulachon grease will have lower levels of PUFAs (e.g., linoleic acid, EPA, and DHA) and higher levels of MUFAs (e.g., oleic acid, palmitoleic acid, and cetoleic acid) than the supplements. This prediction assumes that fish oil supplements available on the Canadian market have been chemically altered to produce high quantities of PUFAs to enhance the consumer's diet with ω-3/6/9s (Karsli, 2021; Brotas et al., 2020). Whereas previous studies show that eulachon grease processed by First Nations People has elevated levels of MUFAs (Phinney et al., 2009; Iverson et al., 2002).

The second hypothesis of this research project is that different preparation techniques will significantly change the FA profile of eulachon grease. One variable in preparation techniques is the length of eulachon fermentation (or "ripening") periods. Thus, the prediction is that longer eulachon fermentation periods prior to grease processing will produce grease with enhanced FA concentrations (Kuhnlein & Chan, 1998; Edwards, 1978). In essence, eulachon carcass decomposition and/or the microbial conversion during the fermentation period could promote the release of select fats which can increase the level of some FAs (e.g., capric acid, stearic acid, EPA, and DHA) and decrease the level of others (e.g., myristic acid, palmitic acid, oleic acid, and linolenic acid) (Anggo et al., 2015; Kuhnlein et al., 1996; Kuhnlein et al., 1982).

The third hypothesis of this research project is that the unique FA composition of eulachon grease and the differences in the preparation methods will substantiate the traditional uses of eulachon grease by First Nations people. The prediction that eulachon grease will contain beneficial PUFAs (e.g., linoleic acid, EPA, and DHA) and MUFAs (e.g., palmitoleic acid, oleic acid, and cetoleic acid), supporting both its use as a condiment/ingredient (i.e.,

promoting heart health) and as a medicine (i.e., reducing inflammation) for First Nations people (Phinney et al., 2009; Iverson et al., 2002; Kuhnlein & Chan, 1998; Kuhnlein et al., 1996).

1.5 Significance

Previous studies documenting the use and FA composition of eulachon fish and grease are more than 20 years old (e.g., Iverson et al., 2002; Kuhnlein & Chan, 1998; Kuhnlein et al., 1996; Kuhnlein et al., 1982). A more recent study (Phinney et al., 2009) discovered discrepancies in the reported FA concentrations of eulachon grease in these prior studies. Phinney et al., (2009) agreed with the results of Iverson et al., (2002) and refuted some of the FA concentrations observed by Kuhnlein et al., (1996). The present study will employ lipidomic techniques to provide the most complete and up-to-date FA profile of eulachon grease. The results from the Western science used in this study will support the TK of First Nation Peoples regarding the benefits of using eulachon grease as a food source and for medicinal purposes.

This study intends to discover unique FAs in eulachon grease and significant differences between the FA profile of eulachon grease and fish oil supplements available on the Canadian market. Therefore, it will demonstrate that eulachon grease fills an important niche in the diet of BC coastal First Nations Peoples while potentially linking unconventional FAs to health benefits (e.g., anti-inflammatory effects, reducing CVD risk, lowering cholesterol levels, etc.). Secondly, this study could identify differences in the FA profile between preparation techniques of eulachon grease. Thus, determining the optimal preparation methods of eulachon grease to be employed based on its intended use (e.g., food vs. medicine) and to promote health effects. Moreover, this study will highlight the cultural and social importance of the traditional eulachon harvest and grease-making process while possibly emphasizing concerns about conservation and climate change regarding the eulachon harvest or other TFs.

1.6 Thesis Outline

This thesis is organized into five chapters. The first chapter gives background information on the larger research program (*CCFS4N*) that this project contributes to, provides information on eulachon/eulachon grease and highlights its importance to the coastal First Nations of BC. Chapter one also describes the problem statement, the research objectives, the significance of the work, and outlines the structure of this monograph. The second chapter of this thesis summarizes the findings from the expert interviews with local Knowledge Holders conducted in Alert Bay, BC, in July 2023. Chapter two gives important context to the time, money, and resources required for the eulachon harvest and eulachon grease processing while explaining the taste, colour, and uses of eulachon grease. In chapter two, there are also important details regarding the preparation techniques of eulachon grease, the perceived nutritional composition of eulachon grease, and concerns regarding the eulachon fishery and climate change. Chapter three investigates the identified and quantified FAs in eulachon grease samples from ‘Namgis First Nation and ω-3/fish oil supplement samples from the Canadian market using gas chromatography-mass spectrometry (GC-MS) methodologies. In chapter three, the FA profiles are presented, the amount of PUFAs, MUFAs, and SFAs are stated, and the results of the statistical analyses (e.g., principal component analyses (PCAs), heatmaps, ANOVAs, t-tests, etc.) for eulachon grease and supplement samples are shown. Later in chapter three, the potential health benefits of eulachon grease and supplement samples are explored using the P:S ratio, and the results of the GC-MS analyses are compared to those of previous studies. Chapter four highlights the identified and quantified FAs in eulachon grease samples from ‘Namgis First Nation and ω-3/fish oil supplement samples from the Canadian market using liquid chromatography-mass spectrometry (LC-MS) methodologies. The faults of this method are

presented, and potential explanations are given, the results of the FA profile and amounts of PUFAs, MUFAs, and SFAs are stated and compared for eulachon grease and supplement samples. In chapter three, statistical results are shown (e.g., PCAs, heatmaps, etc.), and the P:S ratios for eulachon grease and supplement samples are stated. The fifth and final chapter of this thesis presents a summary of the results and the conclusion. In chapter five, the results from the GC-MS and LC-MS analyses are compared, the research objectives, hypotheses, and predictions are reviewed and discussed, and lastly, future directions are explored.

1.7 References

Adelson, N. (2005). The Embodiment of Inequity: Health Disparities in Aboriginal Canada. Canadian Journal of Public Health, 96(S2), S45–S61.

Assembly of First Nations (AFN). (2023). Retrieved from https://afn.ca

Anggo, A. D., Ma'ruf, W. F., Swastawati, F., & Rianingsih, L. (2015). Changes of Amino and Fatty Acids in Anchovy (Stolephorus Sp) Fermented Fish Paste with Different Fermentation Periods. Procedia Environmental Sciences, 23, 58–63.

Batal, M., Chan, H. M., Fediuk, K., Ing, A., Berti, P., Sadik, T., & Johnson-Down, L. (2021a). Importance of the traditional food systems for First Nations adults living on reserves in Canada. Canadian Journal of Public Health, 112(S1), 20–28.

Batal, M., Chan, H. M., Ing, A., Fediuk, K., Berti, P., Sadik, T., & Johnson-Down, L. (2021b). Nutrient adequacy and nutrient sources of adults among ninety-two First Nations communities across Canada. Canadian Journal of Public Health, 112(S1), 29–40.

Batal, M., Chan, H. M., Fediuk, K., Ing, A., Berti, P., Sadik, T., & Johnson-Down, L. (2021c). Associations of health status and diabetes among First Nations Peoples living on-reserve in Canada. Canadian Journal of Public Health, 112(S1), 154–167.

Bartlett, C., Marshall, M., & Marshall, A. (2012). Two-Eyed Seeing and other lessons learned within a co-learning journey of bringing together indigenous and mainstream knowledges and ways of knowing. Journal of Environmental Studies and Sciences, 2(4), 331–340.

British Columbia Assembly of First Nations (BCAFN). (2023). Retrieved from http://bcafn.ca/

Brotas, M., Carvalho, G., & Pereira, P. (2020). Determination, through Derivatization and Gas Chromatography-Mass Spectrometry Analysis, of Omega-3 and Omega-6 Fatty Acids in Fish Oil Capsules Sold in Salvador, Bahia. Journal of the Brazilian Chemical Society.

Bruchac, M. M. (2014). Indigenous Knowledge and Traditional Knowledge. Encyclopedia of Global Archaeology, Chapter 10, 3814-3824.

Canadian Constitution Act, Canada. (1982). Part II Rights of the Aboriginal Peoples of Canada Sections 35.1–37.1, p. 56.

Collison, H. (1941). The Oolachon Fishery. British Columbia Historical Quarterly, 5(1), 25-31.

Crown-Indigenous Relations and Northern Affairs Canada (CIRNAC). (2022, August 30). Indigenous peoples and Communities. Government of Canada. Retrieved from https://www.rcaanc-cirnac.gc.ca/eng/1100100013785/1529102490303#

Crown-Indigenous Relations and Northern Affairs Canada (CIRNAC). (2021, October 15). Indigenous peoples and Communities. Government of Canada. Retrieved from https://www.rcaanc-cirnac.gc.ca/eng/1100100013791/1535470872302

Deur, D. & Turner, N. (2005). Keeping It Living: Traditions of Plant Use and Cultivation on the Northwest Coast of North America. Vancouver: UBC Press.

Edwards, G. T. (1978). Oolachen Time in Bella Coola. The Beaver, 32-37.

Farag, M. A., & Gad, M. Z. (2022). Omega-9 fatty acids: Potential roles in inflammation and cancer management. Journal of Genetic Engineering and Biotechnology, 20(1), 48.

First Nations Health Authority (FNHA). (2012). Healthy children, healthy families, healthy communities: BC provincial results 2008-10 First Nations Regional Health Survey.

Fleming, J. A., & Kris-Etherton, P. M. (2014). The Evidence for α-Linolenic Acid and Cardiovascular Disease Benefits: Comparisons with Eicosapentaenoic Acid and Docosahexaenoic Acid. Advances in Nutrition, 5(6), 863S-876S.

Forbes, A., Ritchie, S., Walker, J., & Young, N. (2020). Applications of Two-Eyed Seeing in Primary Research Focused on Indigenous Health: A Scoping Review. International Journal of Qualitative Methods, 19, 160940692092911.

Gagné, D., Blanchet, R., Lauzière, J., Vaissière, É., Vézina, C., Ayotte, P., Déry, S., & O'Brien, H. T. (2012). Traditional food consumption is associated with higher nutrient intakes in Inuit children attending childcare centres in Nunavik. International Journal of Circumpolar Health, 71, 1-10.

García, V. L. (2019). The Omega 7 as a Health Strategy for the Skin and Mucous Membranes. EC Nutrition, 14.6, 484-489.

Iverson, S., Frost, K., & Lang, S. (2002). Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: Factors contributing to among and within species variability. Marine Ecology Progress Series, 241, 161–181.

James-Abra, E. (2023, February 6). First Nations in British Columbia. The Canadian Encyclopedia. Retrieved from https://www.thecanadianencyclopedia.ca/en/article/first-nations-in-british-columbia

Kapoor, B., Kapoor, D., Gautam, S., Singh, R., & Bhardwaj, S. (2021). Dietary Polyunsaturated Fatty Acids (PUFAs): Uses and Potential Health Benefits. Current Nutrition Reports, 10(3), 232–242.

Karsli, B. (2021). Comparative analysis of the fatty acid composition of commercially available fish oil supplements in Turkey: Public health risks and benefits. Journal of Food Composition and Analysis, 103, 104105.

Kendrick, A., & Manseau, M. (2008). Representing Traditional Knowledge: Resource Management and Inuit Knowledge of Barren-Ground Caribou. Society & Natural Resources, 21(5), 404–418.

Kuhnlein, H. V., Erasmus, B., Spigelski, D., & Burlingame, B. (Eds.). (2013). Indigenous peoples' food systems & wellbeing: Interventions & policies for healthy communities. Food and Agriculture Organization of the United Nations; Centre for Indigenous Peoples' Nutrition and Environment.

Kuhnlein, H. V., & Receveur, O. (2007). Local Cultural Animal Food Contributes High Levels of Nutrients for Arctic Canadian Indigenous Adults and Children1,. The Journal of Nutrition, 137(4), 1110–1114.

Kuhnlein, H. V., Receveur, O., Soueida, R., & Egeland, G. M. (2004). Arctic Indigenous Peoples Experience the Nutrition Transition with Changing Dietary Patterns and Obesity. The Journal of Nutrition, 134(6), 1447–1453.

Kuhnlein, H. V., & Chan, H. M. (1998). Ooligan grease: A traditional food fat of western Canada and Alaska. International Journal of Circumpolar Health, 57, 211–214.

Kuhnlein, H. V., & Receveur, O. (1996). Dietary Change and Traditional Food Systems of Indigenous Peoples. Annual Review of Nutrition, 16, 417–442.

Kuhnlein, H. V., Yeboah, F., Sedgemore, M., Sedgemore, S., & Chan, H. M. (1996). Nutritional qualities of ooligan grease: A traditional food fat of British Columbia First Nations. Journal of Food Composition and Analysis, 9, 18–31.

Kuhnlein, H. V., Chan, A. C., Thompson, J. N., & Nakai, S. (1982). Ooligan Grease: A Nutritious Fat Used by Native People of Coastal British Columbia. Journal of Ethnobiology, 2(2), 154–161.

Lemmen, D.S., Warren, F.J., James, T.S. and Mercer Clarke, C.S.L. editors (2016): Canada's Marine Coasts in a Changing Climate; Government of Canada, Ottawa, ON, 274p.

MacNair, P. L. (1971). Descriptive Notes on the Kwakiutl Manufacture of Eulachon Oil. Syesis, 4, 169-177.

Marston, B. H., Willson, M. F. & Gende, S. M. (2002). Predator aggregations during eulachon *Thaleichthys pacificus* spawning runs. Marine Ecology Progress Series, 231, 220-239.

Marushka, L., Kenny, T.-A., Batal, M., Cheung, W. W. L., Fediuk, K., Golden, C. D., Salomon, A. K., Sadik, T., Weatherdon, L. V., & Chan, H. M. (2019). Potential impacts of climate-related decline of seafood harvest on nutritional status of coastal First Nations in British Columbia, Canada. PLOS ONE, 14(2), e0211473.

Marushka, L., Batal, M., Sadik, T., Schwartz, H., Ing, A., Fediuk, K., Tikhonov, C., & Chan, H. M. (2018). Seafood consumption patterns, their nutritional benefits and associated sociodemographic and lifestyle factors among First Nations in British Columbia, Canada. Public Health Nutrition, 21(17), 3223–3236.

McLean, K. G., Castillo, A. R., Johnston, S., Gross, T., Vierros, M., & Rahera Noa. (2009). Report of the Indigenous Peoples' Global Summit on Climate Change. United Nations University - Traditional Knowledge Initiative.

Nakashima, D. J., Galloway McLean, K., Thulstrup, H. D., Ramos Castillo, A., & Rubis, J. T. (2012). Weathering uncertainty: Traditional knowledge for climate change assessment and adaptation. Paris, UNESCO, and Darwin, USU, 120 Pp.

National Collaborating Centre for Indigenous Health (NCCIH). (2022). Climate Change and Indigenous People's health in Canada. (Reprinted with permission from P. Berry & R. Schnitter [eds.], Health of Canadians in a changing climate: Advancing our knowledge for action [Chapter 2]. Government of Canada).

Patton, A. K., Martindale, A., Orchard, T. J., Vanier, S., & Coupland, G. (2019). Finding eulachon: The use and cultural importance of Thaleichthys pacificus on the northern Northwest Coast of North America. Journal of Archaeological Science: Reports, 23, 687–699.

Parlee, B., Manseau, M., & Łutsël K'é Dene First Nation. (2005). Using Traditional Knowledge to Adapt to Ecological Change: Denésǫłiné Monitoring of Caribou Movements. Arctic Institute of North America, 58(1), 26–37.

Parrott, Z., Gallant, D., Filice, M., & Dipple, J. (2023, April 5). First Nations in Canada. The Canadian Encyclopedia. Retrieved from https://www.thecanadianencyclopedia.ca/en/article/first-nations#:~:text=First%20Nations%20is%20a%20term,)%2C%20Inuit%20and%20Métis%20peoples

Payne, S. A., Johnson, B. A., & Otto, R. S. (1999). Proximate composition of some north-eastern Pacific forage fish species. Fisheries Oceanography, 8(3), 159–177.

Pearce, T., Ford, J., Willox, A. C., & Smit, B. (2015). Inuit Traditional Ecological Knowledge (TEK) Subsistence Hunting and Adaptation to Climate Change in the Canadian Arctic. ARCTIC, 68(2), 233.

Phinney, S. D., Wortman, J. A., & Bibus, D. (2009). Oolichan Grease: A Unique Marine Lipid and Dietary Staple of the North Pacific Coast. Lipids, 44(1), 47–51.

Power, E. M. (2008). Conceptualizing Food Security for Aboriginal People in Canada. Canadian Journal of Public Health, 99(2), 95–97.

Reid, A. J., Eckert, L. E., Lane, J., Young, N., Hinch, S. G., Darimont, C. T., Cooke, S. J., Ban, N. C., & Marshall, A. (2021). “Two-Eyed Seeing”: An Indigenous framework to transform fisheries research and management. Fish and Fisheries, 22(2), 243–261.

Reyes-García, V. (2010). The relevance of traditional knowledge systems for ethnopharmacological research: Theoretical and methodological contributions. Journal of Ethnobiology and Ethnomedicine, 6(32).

Shahidi, F., & Ambigaipalan, P. (2018). Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits. Annual Review of Food Science and Technology, 9(1), 345–381.

Shibabaw, T. (2021). Omega-3 polyunsaturated fatty acids: Anti-inflammatory and anti-hypertriglyceridemia mechanisms in cardiovascular disease. Molecular and Cellular Biochemistry, 476(2), 993–1003.

Siriwardhana, N., Kalupahana, N. S., & Moustaid-Moussa, N. (2012). Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. Advances in food and nutrition research, 65, 211-222.

Statistics Canada. (2023, July 19). Statistics on Indigenous Peoples. Government of Canada. Retrieved from https://www.statcan.gc.ca/en/subjects-start/indigenous_peoples

Turner, N. J., & Clifton, H. (2009). “It’s so different today”: Climate change and indigenous lifeways in British Columbia, Canada. Global Environmental Change, 19(2), 180–190.

Uprety, Y., Asselin, H., Dhakal, A., & Julien, N. (2012). Traditional use of medicinal plants in the boreal forest of Canada: Review and perspectives. Journal of Ethnobiology and Ethnomedicine, 8(1), 7.

Yang, Z.-H., Amar, M., Sorokin, A. V., Troendle, J., Courville, A. B., Sampson, M., Playford, M. P., Yang, S., Stagliano, M., Ling, C., Donkor, K., Shamburek, R. D., Mehta, N. N., & Remaley, A. T. (2020). Supplementation with saury oil, a fish oil high in omega-11 monounsaturated fatty acids, improves plasma lipids in healthy subjects. Journal of Clinical Lipidology, 14(1), 53-65.

Weatherdon, L. V., Ota, Y., Jones, M. C., Close, D. A., & Cheung, W. W. L. (2016). Projected Scenarios for Coastal First Nations' Fisheries Catch Potential under Climate Change: Management Challenges and Opportunities. PLOS ONE, 11(1), e0145285.

2. Traditional Knowledge and the Use of Eulachon Grease

2.1 Introduction

In this time of reconciliation, integrating "Two-Eyed Seeing" frameworks in scientific inquiries has gained recognition as an essential practice (e.g., Marsh et al., 2016; Clark, 2014; Moller et al., 2004). In the context of eulachon grease, an invaluable socio-cultural resource to the coastal First Nations of BC, employing a two-eyed seeing approach is critical. By engaging directly with Knowledge Holders, this research honours the expertise of 'Namgis First Nation members and enriches the scientific inquiry with perspectives that are often overlooked in conventional research. This collaboration exemplifies the principles of Two-Eyed Seeing, where the strengths of both TK and Western science are harnessed to create a more holistic and informed approach to understanding the nutritional value of eulachon grease.

In this project, the knowledge held by First Nation Peoples offers firsthand perspectives into the ecological patterns of the eulachon run, the eulachon harvest, the processing of eulachon grease, and the cultural significance of eulachon that cannot be obtained from any other source. Western science brings technology and methodologies for analyzing eulachon grease to identify and quantify the FAs within it. By combining these perspectives, we can develop a more comprehensive understanding of the critical role eulachon grease plays in nutritional, medicinal, and cultural systems.

This chapter conveys the TK related to the eulachon harvest and grease-making process. It highlights the environmental concerns of local Knowledge Holders obtained from five interviews conducted in Alert Bay, BC, in July 2023. Integrating this information through a collaborative approach ensures that the research is grounded in local knowledge and cultural practices, making it more relevant and impactful for the members of 'Namgis First Nation.

Ultimately, the Two-Eyed Seeing approach relies on this collaborative, transdisciplinary research that respects and integrates diverse ways of knowing.

2.2 Methods

In July 2023, in Alert Bay, BC, individual interviews of approximately 30 minutes in length were conducted with local Knowledge Holders who regularly participate or have previously participated in the eulachon harvest and eulachon grease making (*ɫli'nagila*). The first person approached for an interview was a steering committee member for the CCFS4N project from 'Namgis First Nation. Subsequently, the snowball sampling technique (Naderifar et al., 2027) was used to identify further key informants. Overall, five face-to-face semi-structured interviews were organized with local Knowledge Holders. The interviews were conducted in English.

The interviewees were provided with a questionnaire (template provided in Appendix 3) that guided the discussion. The questionnaire had 13 questions total and covered topics such as their view on the status of the eulachon harvest, their method of eulachon grease preparation, their use of eulachon grease, their perceived nutritional value of eulachon grease, and their experience with the impacts of climate change on the eulachon harvest. After each interview was completed, one eulachon grease sample of approximately five grams was collected from each Knowledge Holder who chose to contribute to this part of the study. Each participant received an honorarium to compensate them for their time during the interview as well as for the eulachon grease sample provided (if applicable). In sum, three interviewees provided two eulachon grease samples each, two interviewees did not provide eulachon grease samples, and one eulachon grease sample was gifted to the project anonymously (i.e., no interview was conducted).

Each interview was audio-recorded on iPhone with proper consent given by the Knowledge Holders in advance (example information sheet and consent form in Appendix 2). Upon completion of all interviews, the audio recording(s) from each interview were manually transcribed into individual Microsoft Word documents. Subsequently, the interview transcripts were used for thematic analysis (Braun & Clarke, 2006) to analyse the qualitative data and identify patterns and themes within and between interviews. Since only five interviews were conducted, common patterns and themes were extracted manually and reported on in section 2.3 of this thesis.

2.3 Results and Discussion

The interviews with Knowledge Holders conducted in Alert Bay in July 2023 focused on obtaining information about the eulachon harvest and grease processing. The questions pertaining to eulachon fish present in the questionnaire form (appendix 3) were not answered throughout the interviews. Eulachon fish samples could not be collected from the Knowledge Holders as some did not have eulachon fish or didn't want to part with the fish they did have stored.

The first and second questions in the questionnaire were for identification purposes (i.e., the first question asked for the Knowledge Holder's email or preferred method of contact and the second asked what they would like their sample of eulachon grease to be called (if applicable)). Hence, these answers varied based on the individual and will not be discussed further in this thesis.

Furthermore, the third and fourth questions asked the participant where and when the eulachon harvest occurred, respectively. Plus, the sixth question asked when the eulachon grease was made. These results are summarized in Table 2.1; all but one eulachon grease sample was

prepared in Knight Inlet (*Dzawadi*), and the other (sample 6) was prepared in Kingcome Inlet (Figure 2.1). Three of the seven eulachon grease samples were prepared in the spring of 2023, immediately following the eulachon harvest that same year. One sample was prepared in the spring of 2022 and another in spring 2019, following the eulachon harvest that year, and the other three samples' preparation dates were not specified or not known.

Table 2.1 – Summary of the characteristics of each eulachon grease sample collected from community members of 'Namgis First Nation in July 2023. Namely, the sample ID, the location of preparation, the year of preparation, whether the sample was kept frozen or not, and any notable differences/similarities (notes).

Sample Number	Location of Preparation	Year of Preparation	Kept Frozen?	Notes
1	Knight Inlet (*Dzawadi*)	Spring 2023	No	Made by same family as sample 2. Made at same time as samples 4 and 5.
2	Knight Inlet (*Dzawadi*)	Spring 2022	No	Made by the same family as sample 1.
3	Knight Inlet (*Dzawadi*)	Spring 2019	Yes	Made by the same person as sample 6.
4	Knight Inlet (*Dzawadi*)	Spring 2023	No	Unique straining technique. Made at same time as samples 1 and 5.
5	Knight Inlet (*Dzawadi*)	Spring 2023	No	Made at same time as samples 1 and 4.
6	Kingcome Inlet	Unknown	Yes	Made by the same person as sample 3.
7	Knight Inlet (*Dzawadi*)	Unknown	Yes	Anonymous sample

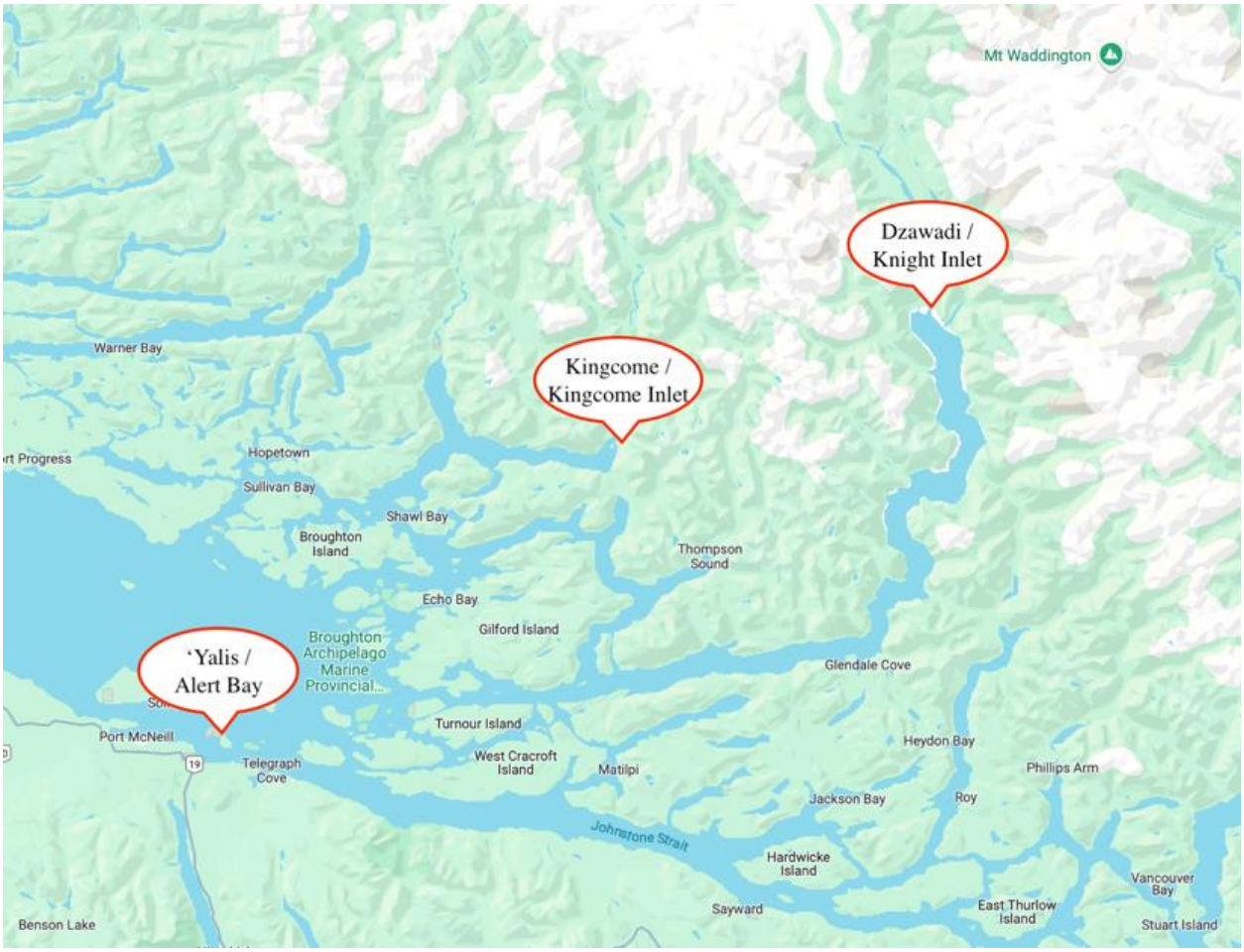

Figure 2.1 – Screenshot from Google Maps depicting part of the central coast of British Columbia with three locations of interest specified: Alert Bay ('Yalis) ('Namgis First Nation) (leftmost), Kingcome Inlet (Kingcome First Nation and where sample 6 was prepared) (middle), and Knight Inlet (Dzawadi) (where samples 1-5, and 7 were prepared) (rightmost).

The fifth question asked Knowledge Holders if the eulachon harvest had been scarce or plentiful in their experience. The consensus among the individual interviews was that the eulachon harvest has been consistently plentiful and that they have had success making eulachon grease year after year. However, topics such as climate change, fish farms, and logging were also mentioned as concerns about the health of the eulachon fishery.

"There were lots of eulachons; there have been lots of eulachons, the last 10 years, it's been pretty consistent with being able to harvest and have a pit, with being able to smoke or make eulachon grease […] The only underlying condition is the level of the water which determines whether we can harvest enough eulachons in a short period of time."

– Knowledge Holder #5

"Very plentiful; I made 200, and I processed the eulachon into approximately 200 gallons. And my friend worked with us on the same pit, and he processed 40 gallons."

– Knowledge Holder #4

"It was a good year; the net was like packed, and it just kept filling up as we were emptying it. So, we just, eventually once we had enough, we just like you know let it go […] But, yeah, it was a good year. There was a lot."

– Knowledge Holder #1

The seventh question asked Knowledge Holders to describe the process of making eulachon grease. Participants often started by describing the long journey from Alert Bay to Knight Inlet and the time, money, and resources required to make the trip possible. In early April each year, many will travel to Knight Inlet and stay there for 3-4 weeks to complete the entire process – waiting for the eulachon run to occur, catching the eulachon, processing the eulachon into grease, then straining and bottling the eulachon grease. Due to the remote location

of Knight Inlet, individuals and families going to make eulachon grease need to pack enough food and water to sustain themselves over the course of the process. They must also bring all the equipment that may be required for repairs or eulachon grease processing (e.g., wood, saws, drills, portable water pumps, tin, clay, tubs, shovels, rakes, jars, etc.). As people do not live in Knight Inlet year-round, when individuals and families arrive, they must assess the village site, assuring that everyone has a place to sleep, that the pits are raked and cleaned, that the vats are in good working condition and any necessary repairs are made.

"Equipment is very important in the whole process, you maintain your equipment, and you don't want any leaks in your corks or the sides of the vat. […] just maintain your equipment and it'll do a good job for you."

– Knowledge Holder #4

Once the area is prepared and everyone is given a task, the first step is to catch the eulachon. From the 1950s until the early 2000s, a boat was used with a drag seine net to catch the eulachon (MacNair, 1971). However, this technique was damaging the bottom of the river, affecting eulachon and other fish spawning. Thus, the fishermen switched back to the traditional technique of using a conical net (*taga'ɬ*), attaching either side of the net to posts/trees on the shore and allowing eulachons that have already gone upriver to spawn to swim back into the net. Often, fishing is done at night to allow cooking and other tasks to occur during the day.

"It's a herring web, it is used to catch herring, it has small, ¾ inch webs. It's like a vacuum cleaner, it's a 6-8-foot-wide net with the mouth about two feet deep that gets put out in the river and then we're catching them after they spawn. So, we call it a taga'ɬ, so as the fish go up and spawn and they come back, and they drift into this taga'ɬ,"

– Knowledge Holder #5

Once the net is full, the eulachons are emptied into buckets and brought to the "pits" on the riverbank. These pits vary in size but are typically square; the sides are made of cedar, and the bottom is clean sand. Some groups add drainage to the bottom of their pits, drawing channels into the sand to draw the blood that escapes the eulachon away from the fermentation process. Others don't and let it all pool together. Some believe this affects the taste.

"I don't like that blood so; I'll leave little channels all over the place for the blood to flow out so that's part of the quality control too. I don't want that blood in there so I try to drain as much as I can. And then what we do is we sweep the ground, we dig it all up, right down to the sand, rake it, rake it… nice and clean."

– Knowledge Holder #4

When the pit is full, the eulachons are left covered to ferment (or "ripen"). This process allows for the development of the flavour and permits the decomposition of the carcass for easy release of the fat during cooking (Kuhnlein et al., 1996). The eulachons are left in the pits for 4-14 days depending on weather conditions (MacNair, 1971). If it is a warm year, the eulachons may only be left for 4-8 days before the oil is visible on the surface of the eulachons and cooking needs to start. If it is a cool year, the eulachons may be left in the pits for 10-14 days. Although with climate change and increasing temperatures in Knight Inlet, the fermentation time has been on the lower end of this range over the last few years (Personal Communications, 'Namgis First Nation member).

"After you catch the fish, it ferments for six to 10 days, depending on the weather conditions it can ferment faster or slower. So, when you get warm weathers like we did, it fermented in six days."

– Knowledge Holder #5

"And that's the idea of leaving it, for 5-10 days, 10 days if the temperature is really cold, leave it for 10-12 days. But on a hot day like this, maybe only 5-6 days. Sometimes we'll get a hot climate in early… late April, early May, it gets too hot, eh? So, I gotta cook it right away."

– Knowledge Holder #4

While the eulachons ripen, the vats (*sa̲mgaẗsi*) are repaired (if necessary) and prepared. The vats are approximately 8ft long, 4ft tall, and 4ft wide with a chimney on one end. The sides are usually made of cedar and the bottom is made of tin (to distribute the heat evenly). Underneath the vats, wood is burned to cook the contents of the vat. To keep a more constant heat, around the underside of the vat, clay, rocks, mud, and/or cement are placed to insulate the fire. The vats are filled 2/3 full of water and warmed to the perfect temperature, ~125-140°F. The water should not froth or boil.

"We got our sa̲mgaẗsi ready which is the big cooker. It has a pit underneath; we lined it with bricks and clay and then put the sa̲mgaẗsi, the cooker on top of it and had a vent coming out. Then we light the fire, we make sure it's not too hot."

– Knowledge Holder #1

"I don't want it boiling hot, I want it at just the right temperature so I'm not turning the eulachon oil red."

– Knowledge Holder #4

Once the oil is visible on the surface of the eulachon, it is time to cook. The eulachons are transported to the vats in tubs and placed in warm water to cook. The fish will melt away from the bones, and the oil will rise to the surface of the water layer. Large wooden two-pronged forks are used to shake the remaining meat from the eulachon bones. Properly ripened fish will

give the most grease. Female eulachon are also oilier than males (Kuhnlein et al., 1982). The oil is skimmed off the water surface using wooden shell-shaped tools and is then placed into metal pots or bowls to be further purified. Once all the grease has been skimmed from that batch of fish, the remaining fish residue is released back into the river. The vats are rinsed with water and the process is repeated. Typically, families complete two renderings a day with people working from 5 am to 8 pm.

"I watch, I watch the sun, I watch the eulachons, and then sometimes, there's a little… might be a droplet of oil on top of the eulachons, and we gotta start cooking cause it's starting to come out of the fish."

– Knowledge Holder #4

"We'll fill the cooker up maybe about a third of the way with water and then fill the rest of it up with the fish, pretty much, scooping them out of big buckets. We have sticks and we break apart all the meat and the bones and because of that the oil will rise to the top."

– Knowledge Holder #1

"The extracted oil from the eulachon floats to the top and then we use a strainer and take it out. Then we repeat that process twice a day."

– Knowledge Holder #5

The eulachon grease is kept warm near the fire before being transferred to other pots/bowls. With each transfer, the oil is strained through different cloths, sheets, and towels to eliminate any impurities (e.g., remaining fish or bones, sticks, leaves, etc.). Once the straining process is complete, the oil is placed in green glass jugs. The oil can last several years when stored in a cool, dry place. It can also be frozen; some believe this stops the flavour from becoming "strong".

"I do the cheesecloth, maybe the next one like a towel, and the next one is a hotel quality sheet, right? Next one, sheet again. So, four filter systems, I do with the eulachon oil to get all the debris, scales, whatever. I want it pure."

– Knowledge Holder #4

"Then, it would go through a filtering system where they use cheesecloth or sheets to filter out the, you know, the bigger stuff. It's pretty much raw tli'na there and then after the purification, it goes into a jar, a bottle and is capped and it sits for a while."

– Knowledge Holder #2

In sum, all but one eulachon grease sample collected for this project was made in Knight Inlet, sample 6 being the exception, having been made in Kingcome Inlet. However, the same person who made sample 6 also made sample 3 in Knight Inlet. Thus, the same "recipe" was used to make samples 3 and 6; the only differences were location and year of manufacture. Additionally, samples 1, 4, and 5 were made at the same time (spring 2023), at the same location (Knight Inlet), and using the same "recipe", except the straining technique for sample 4 was unique. Samples 1 and 2 were made by the same person, using the same techniques, and in Knight Inlet. The only difference between samples 1 and 2 is that sample 2 was made the previous year (i.e., 2022). Furthermore, many could not remember the exact number of days the eulachons were left to ferment in each case. However, in recent years it has been quite warm, so generally, fermentation time was on the lower end of the spectrum (i.e., 4-8 days). This information will be important to consider in chapters 2 and 3 when the FA profiles of the different eulachon grease preparations are compared in accordance with the second research objective and hypothesis to determine which preparation methods may lead to optimal nutritional and medicinal effects.

Moreover, question eight asked if the grease had ever been kept frozen; the results are summarized in Table 2.1. Briefly, most eulachon grease samples had not been frozen as they had been recently made (i.e., samples 1, 2, 4, and 5), whereas the other samples that trended older had been kept frozen (i.e., 3, 6 and 7).

"It keeps the quality control for me, and it stays good."
– Knowledge Holder #4

"I don't have any right now, but I'll usually only freeze them after we've eaten some of it and you don't want it to go bad."
– Knowledge Holder #1

Question nine asked Knowledge Holders about the ways in which they use their eulachon grease. Mainly, people claim to eat their eulachon grease with seafood (*tsa̱pa*) such as halibut or BBQ salmon; others dip their berries in eulachon grease or to add it to soups. Additionally, some use their eulachon grease as a gift or payment to other families or other communities that can't make their own eulachon grease. Lastly, many Knowledge Holders spoke about the medicinal benefits of consuming eulachon grease when they feel they need an immunity boost. They also mentioned rubbing eulachon oil on their chests or under their noses when they have a cold. Most don't or haven't heard of using it as a moisturizing ointment in the case of dry skin or eczema. Again, this information will be important to consider in chapters 2 and 3 when we explore the third hypothesis: that the unique FA composition of eulachon grease substantiates the traditional uses (i.e., food and medicine) by First Nation Peoples.

"I absolutely know when I haven't had enough, I'll be feeling low, or I'll be feeling lethargic or feeling just kind of… or my tummy's giving me issue or somethings wrong. […] I try to eat it once a week."

– Knowledge Holder #2

"You could also take teaspoons a day, if you're not feeling well, you can take it just like cough medicine. You can put it on your chest. You can put it under your nose, like Vicks. Does exactly the same, just a lot harder smell."

– Knowledge Holder #5

Questions 10 and 11 asked Knowledge Holders to describe the taste and colour, respectively, of that year's batch compared to previous years. In general, everyone agreed that taste and colour are linked. Although there are slight differences in taste year to year or between the grease made in Kingcome Inlet versus Knight Inlet, everyone is quite happy with the taste of their grease. You can tell good-tasting grease and bad-tasting grease apart based on the colour. Good grease will be clear and golden, whereas bad grease will be hazy and brown, orange, or red. There were a few explanations for what would lead to the grease becoming murky or dark in colour. Namely, allowing the blood to pool under the eulachon when they sit in the pits, cooking the eulachons in water that is too hot, and/or a non-refined straining process.

"You can tell, eulachon oil year-to-year, you know, most of the time it's gold, a real golden colour but not all the time, it could be a little bit more tainted or brown or red. […] The temperature of that fire is critical to the colour of every grease. You know if you've cooked it too hot, it'll show when you pour it."

– Knowledge Holder #5

"Kingcome gold, got that nice colour. And they do a nice job in Knights Inlet, […] you get that nice colour, it's beautiful. You know what that comes from? The filtering. […] It's just nice and not thick and cloudy, it's clear."

– Knowledge Holder #4

Question 12 asked Knowledge Holders about their perception of the nutritional composition of eulachon grease. Some Knowledge Holders mentioned the vitamin and protein content of eulachon grease. Others hadn't really considered the "nutritional composition" but just know that it helps them when they are feeling ill, especially in the wintertime when fruits and vegetables are less readily available. Additionally, Knowledge Holders described the spiritual/communal aspect of consuming eulachon grease and how it helps with mental health and feeling connected to their ancestors and the creator.

"If I'm feeling sick, it gives me a bit more like you know, nutrition and I don't feel as bad anymore […] it gives me a little boost, just like makes me feel that I have more vitamins."

– Knowledge Holder #1

"I believe that historically our people found a way to keep us healthy over the winter and the eulachon oil was that. They put it in everything that we ate."

– Knowledge Holder #4

"What I know is that it's incredibly high in protein, and so obviously that's really beneficial when you're not feeling well or you're low or you know, you feel it coming on, right? A cold or the flu. […] Some people have said that it is a staple which might be a little bit more accurate in terms of what that means in English, but I'm saying something like it, there's another level, there's a spiritual level, like it's very personal to me, you know?"

– Knowledge Holder #2

The final question (#13) of the questionnaire, asked Knowledge Holders how they feel climate change is impacting their ability to harvest eulachon and make eulachon grease. There were two main concerns raised. The first being that the eulachon run has been delayed by approximately one day each year, and the second being the sudden high-water levels during the eulachon run or grease processing in Kingcome and Knight Inlet.

"Then, another thing is they're coming later and later every year […] because we have logs, we write down like journals every year that are coming later and later. Like our old people say they used to come at the end of March, but now they're coming at the end of April. That's strange. […] And it's not that there's less of them. It's just like this year it was a one day later, they say it's been like one day later and later every year"

– Knowledge Holder #1

"Because the runs are coming later, the river level's higher, and we're dealing with warmer weather. The climate change… there's two things: the fish are later in the season and we're dealing with warmer weather. So, water level is our main… what we're battling is the water level. […] If the water level is too high, we can't harvest."

– Knowledge Holder #5

"Fluctuations in the river and flooding are changing. Like I haven't fished in 3-4 years because of the flooding that's happening at the time of the eulachon run. You can't fish; it's just too dangerous."

– Knowledge Holder #4

Both Kingcome and Knight Inlet are a glacier-fed rivers; earlier ice melts due to climate change could explain this rise in water level (Islam et al., 2017; Jost et al., 2012; Larsen et al., 2007). However, climate change is also altering precipitation patterns in coastal BC (i.e., more frequent heavy downpours and fewer bouts of mist-like rainfall) (Schnorbus et al., 2014; Westra

et al., 2014; Trenberth, 2011). Plus, deforested areas on Kwakwaka'wakw territory are more conducive to quickflow, which reduces the resistance time of water in the soil (St-Hilaire et al., 2016).

"The logging has the biggest impact on the river because the banks and the land don't hold the water like they used to. When we get a rain, the impact is the next day… Used to be 3-4 days, now it is the next day and that is just because there are no trees to hold the water."
– Knowledge Holder #5

In concert, additional glacial melt, heavy rainfall, and the bare landscapes remaining from logging are resulting in increased erosion, floods, and landslides (Sobie, 2020; Islam et al., 2017; Loukas & Quick, 1999). Consequently, BC river systems are experiencing surges of high-water pressure (Sobie, 2020). This sudden outpouring of water results in dangerous (i.e., capsized boats, risk of drowning, etc.) and unsuitable (i.e., seine nets don't hold, loss of property, etc.) fishing conditions.

"I think the other thing that has happened is the erosion. We don't have much island left where we harvest; that is another thing we're going to have to look at in the future."
– Knowledge Holder #5

Knowledge Holders also identified both fish farms and commercial fisheries as major threats to the eulachon fishery. Fish farms, particularly those located near wild fish habitats, can introduce diseases, parasites such as sea lice, and pollutants into surrounding waters, which can negatively affect eulachon populations (Bostock et al., 2010; Holmer, 2010). The discharge of waste and chemicals from aquaculture operations can degrade water quality, further stressing these delicate ecosystems (Holmer, 2010). Furthermore, commercial fisheries targeting other

species, such as shrimp and groundfish, may inadvertently deplete eulachon through bycatch, reducing their numbers and disrupting their reproductive cycles (Gustafson et al., 2023). Competition for food resources, habitat disruption, and the alteration of marine food webs by industrial fishing practices also pose significant threats to the survival of eulachon (Komoroske & Lewison, 2015), compounding the impacts already felt due to climate change.

"Not just the climate change, we have people that are dragging for cod and shrimp, but they're killing our eulachons."
– Knowledge Holder #4

2.4 Summary and Conclusion

This study employs a Two-Eyed Seeing framework to combine the firsthand perspectives of First Nation Peoples and Western science technologies. Five interviews were conducted with local Knowledge Holders and seven eulachon grease samples were collected in Alert Bay, BC in July 2023 (characteristics summarized in Table 2.1). During these interviews, Knowledge Holders stated that the eulachon harvest had been plentiful and that they have had success making eulachon grease year after year. When asked about the eulachon harvest and grease-making processes, Knowledge Holders mentioned the journey from Alert Bay to Knight Inlet as well as the time, money, and resources required to make the trip possible. The eulachon harvest and grease preparation techniques were also described during the expert interviews (see section 2.3). Most Knowledge Holders agreed on the uses of eulachon grease; eating it with seafood and/or berries, adding it to soups and/or rubbing it on their chests/under their noses when they are sick. Many Knowledge Holders spoke about the medicinal benefits of using eulachon grease; how it can give a sense of an "immunity boost." The vitamin and protein content of eulachon grease was also mentioned during the expert interviews and how it is especially

important in the wintertime when fruits and vegetables are in low supply. The spiritual/communal aspect of consuming eulachon grease was discussed and some stated that it helped with mental wellbeing and feeling connected to their ancestors and/or the creator. Concerns regarding climate change and industry were also heavily voiced. It was stated that the eulachon run has been delayed by approximately one day each year, and that there have been dangerous, sudden high-water levels during the eulachon run or grease processing in Kingcome and Knight Inlet. Since Kingcome and Knight Inlet are glacial fed rivers, with the warmer weather due to climate change, glacial melt is occurring at an increased rate. Climate change is also altering precipitation patterns causing more frequent heavy downpours and fewer bouts of mist-like rainfall (Schnorbus et al., 2014; Westra et al., 2014; Trenberth, 2011). Plus, deforested areas are more conducive to quickflow (St-Hilaire et al., 2016). These natural and human-induced environmental changes are leading to increased erosion, floods, and landslides which result in dangerous and unsuitable fishing conditions during the traditional eulachon run and grease processing period (Sobie, 2020; Islam et al., 2017; Loukas & Quick, 1999). Knowledge Holders identified both fish farms and commercial fisheries as major threats to the eulachon fishery. Ultimately, the eulachon run has been plentiful, and grease has been produced but external factors like climate change and industry are limiting access to eulachon harvesting areas which may impact this cultural practice in the future.

This chapter summarizes the TK shared during interviews with local Knowledge Holders in Alert Bay, BC, in July 2023 about the eulachon harvest, eulachon grease processing, and environmental concerns regarding the eulachon fishery. This chapter affirms the importance of the eulachon fishery to the coastal First Nation Peoples of BC. Eulachon grease is known and recognized as a nutritious traditional food and medicine. The information in this chapter will be

referenced in subsequent chapters regarding the preparation techniques and uses of eulachon grease and how this might affect or be reflected in the FA profiles determined using Western science techniques.

2.5 References

Bartlett C., Marshall M., Marshall A. (2012). Two-Eyed Seeing and other lessons learned within a co-learning journey of bringing together Indigenous and mainstream knowledges and ways of knowing. Journal of Environmental Studies and Sciences, 2, 331–340.

Braun, V., & Clarke, V. (2006). Using thematic analysis in psychology. Qualitative Research in Psychology, 3(2), 77–101.

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., & Corner, R. (2010). Aquaculture: global status and trends. Philosophical Transactions of the Royal Society B, 365(1554), 2897–2912.

Clark, W. V. (2014). Diffusion of personal health information services: self-determining and empowering practices for Manitoba Inuit. Healthcare Quarterly, 17(1), 18–23.

Gustafson, R., Richerson, K. E., Somers, K. A., Tuttle, V. J., McVeigh, J. T. (2023). Observed and Estimated Bycatch of Eulachon in the 2002–21 U.S. West Coast Fisheries. NOAA technical memorandum, NMFS NWFSC, 188.

Holmer, M. (2010). Environmental issues of fish farming in offshore waters: perspectives, concerns and research needs. Aquaculture Environment Interactions, 1, 57–70.

Islam, S. U., Déry, S. J., & Werner, A. T. (2017). Future Climate Change Impacts on Snow and Water Resources of the Fraser River Basin, British Columbia. Journal of Hydrometeorology, 18(2), 473–496.

Jost, G., Moore, R. D., Menounos, B., & Wheate, R. (2012). Quantifying the contribution of glacier runoff to streamflow in the upper Columbia River Basin, Canada. Hydrology and Earth System Sciences, 16(3), 849–860.

Komoroske, L. M., & Lewison, R. L. (2015). Addressing fisheries bycatch in a changing world. Frontiers in Marine Science, 2, 2296-7745.

Kuhnlein, H. V., Yeboah, F., Sedgemore, M., Sedgemore, S., & Chan, H. M. (1996). Nutritional qualities of ooligan grease: A traditional food fat of British Columbia First Nations. Journal of Food Composition and Analysis, 9, 18–31.

Kuhnlein, H. V., Chan, A. C., Thompson, J. N., & Nakai, S. (1982). Ooligan Grease: A Nutritious Fat Used by Native People of Coastal British Columbia. Journal of Ethnobiology, 2(2), 154–161.

Larsen, C. F., Motyka, R. J., Arendt, A. A., Echelmeyer, K. A., & Geissler, P. E. (2007). Glacier changes in southeast Alaska and northwest British Columbia and contribution to sea level rise. Journal of Geophysical Research, 12, F01007.

Loukas, A., & Quick, M. C. (1999). The Effect of Climate Change on Floods in British Columbia. Hydrology Research, 30(3), 231–256.

MacNair, P. L. (1971). Descriptive Notes on the Kwakiutl Manufacture of Eulachon Oil. Syesis, 4, 169-177.

Marsh, T. N., Cote-Meek, S., Toulouse, P., Najavits, L. M., & Young, N. L. (2015). The Application of Two-Eyed Seeing Decolonizing Methodology in Qualitative and Quantitative Research for the Treatment of Intergenerational Trauma and Substance Use Disorders. International Journal of Qualitative Methods, 14(5).

Moller, H., Berkes, F., Lyver, P. O., & Kislalioglu, M. (2004). Combining Science and Traditional Ecological Knowledge: Monitoring Populations for Co-Management. Ecology and Society, 9(3).

Naderifar, M., Goli, H., & Ghaljaei, F. (2017). Snowball Sampling: A Purposeful Method of Sampling in Qualitative Research. Strides in Development of Medical Education, 14(3), e67670.

Schnorbus, M., Werner, A., & Bennett, K. (2014). Impacts of climate change in three hydrologic regimes in British Columbia, Canada. Hydrological Processes, 28, 1170–1189.

Sobie, S.R. (2020). Future changes in precipitation-caused landslide frequency in British Columbia. Climatic Change, 162, 465–484.

St-Hilaire, A., Duchesne, S., & Rousseau, A. N. (2016). Floods and water quality in Canada: A review of the interactions with urbanization, agriculture, and forestry. Canadian Water Resources Journal / Revue Canadienne Des Ressources Hydriques, 41(1–2), 273–287.

Trenberth, K. E. (2011). Changes in precipitation with climate change. Climate Research, 47, 123–138.

Westra, S., Fowler, H. J., Evans, J. P., Alexander, L. V., Berg, P., Johnson, F., Kendon, E. J., Lenderink, G., & Roberts, N. M. (2014). Future changes to the intensity and frequency of short-duration extreme rainfall, Reviews of Geophysics, 52, 522–555.

3. Fatty Acid Profile of Eulachon Grease Determined Using Gas Chromatography-Mass Spectrometry

3.1 Introduction

Eulachon grease is a traditional food derived from the eulachon fish and has been a vital dietary component for BC coastal First Nations communities for centuries (Patton et al., 2019). Known for its rich nutrient profile, eulachon grease is particularly valued for its high content of PUFAs and MUFAs, which contribute to its numerous uses and health benefits (Phinney et al., 2009; Iverson et al., 2002; Kuhnlein & Chan, 1998). Despite its cultural and nutritional significance, comprehensive studies on the FA profile of eulachon grease remain limited. Previous studies exploring the FA composition of eulachon grease using GC-MS methodologies are more than 15 years old (e.g., Phinney et al., 2009; Iverson et al., 2002; Kuhnlein et al., 1996). Moreover, discrepancies in the reported FA profiles have been highlighted between these studies. Thus, this chapter aims to fill this gap by using current GC-MS methodologies to provide the most complete and up-to-date FA profile of eulachon grease samples. The eulachon grease samples analyzed in this chapter were collected from Knowledge Holders in Alert Bay, BC in July 2023. Subsequently, the FA profile of ω-3/fish oil supplements available on the Canadian market were determined using the same GC-MS methodologies. To my knowledge, no studies have measured the FA profile of ω-3/fish oil supplements available on the Canadian market despite their promotion for health and frequent public use (Bailey et al., 2013; Barnes et al., 2008). The reported FA profile, the amounts of PUFAs, MUFAs, and saturated fatty acids (SFAs), as well as the individual FA concentrations in the eulachon grease samples will be compared to these same factors measured in the supplement samples to link potential health benefits or risks to their consumption/medicinal uses.

This chapter provides a comprehensive analysis of the FA profile of eulachon grease and commercially available ω-3/fish oil supplements using modern GC-MS methods. The identified FAs in eulachon grease and supplement samples will be quantified based on the percent fatty acid (%FA) and the percentage of the three FA groups (i.e., PUFA, MUFA, and SFA) levels will be stated. Statistical techniques such as PCAs, heatmaps, ANOVAs, t-tests, etc., have been completed based on the data from the eulachon grease samples and the supplement samples, and both have been combined. The results of this chapter not only update the scientific understanding of eulachon grease and ω-3/fish oil supplement samples but also reinforce the cultural, nutritional, and ecological importance of preserving eulachon grease as an invaluable traditional food resource.

3.2 Materials

3.2.1 Solvents and Reagents

Solvents and reagents were purchased from Sigma Aldrich (St. Louis, USA), glassware (i.e., 2mL amber HPLC vials and PTFE lined caps) were purchased from Agilent (Santa Clara, USA).

3.2.2 Supplement Samples

A single bottle of ω-3/fish oil supplements from 10 different brands readily available on the Canadian market was bought online or in superstores in January 2023. Nine ω-3/fish oil supplements were in the form of a softgel, and one was in liquid form. Seven of the supplements were manufactured in Canada and three were manufactured in the United States. All samples obtained were transferred to the laboratory and stored in a dark environment at room temperature. All samples were within their stated shelf lives.

Table 3.1 presents information about the supplements provided by their manufacturer (on the label), such as where they were manufactured, the type of product, the ingredient(s), the serving size, and the amount of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), total ω-3s, and fish oil (all measured in mg). It is important to note that sample 1 is a liquid and that sample 6 is considered a vegan ω-3 supplement, containing algal (*Schizochytrium spp*.) oil, not fish oil. Singular analysis was performed on all supplement samples (n=10).

Table 3.1 – Summary of the characteristics of each retail ω-3/fish oil supplement sample bought from the Canadian market. Namely, the sample ID, the country of manufacture, the product type, the ingredients, the suggested serving size, and the claimed content of EPA, DHA, and fish oil (all in mg) on the manufacturer's label.

Sample number	Country of Manufacture	Product Type	Ingredient(s)	Serving	EPA (mg*)	DHA (mg*)	Total omega-3s (mg*)	Fish Oil (mg*)
1	Canada	Liquid	Anchovies, Sardines, Mackerel, Herring	1 tsp. (5mL)	750	500	1250	4550
2	Canada	Softgel	Anchovies, Sardines, and/or Mackerel	1 Softgel	600	300	900	1425
3	Canada	Softgel	Anchovies, may also contain Sardines or Mackerel	2 Softgels	300	200	500	1000
4	Canada	Softgel	Anchovies, Sardines, and/or Mackerel	1 Softgel	600	300	900	1425
5	United States	Softgel	Wild Alaskan Pollock	1 Softgel	690	260	950 + 90 (other)	1250
6	Canada	Softgel	Algal oil (*Schizochytrium spp.*)	1 Softgel	nc	225	225 + 30 (DPA)	500**
7	United States	Softgel	Anchovies, Sardines, and/or Mackerel	1 Softgel	500	250	750	1200
8	United States	Softgel	Anchovies, Sardines, Mackerel, and/or Tuna	2 Softgels	360	240	600 + 40 (other)	2200
9	Canada	Softgel	Wild Alaskan Salmon	1 Softgel	70	80	150 + 50 (other)	1000
10	Canada	Softgel	Cod Liver (*Gadus morhua*)	2 Softgels	nc	nc	nc	1100

*mg per serving stated on the bottle (and in the table)
** Whole agal (*Schizochytrium spp.*) oil (vegan sample)
nc: not claimed

3.2.3 Eulachon Grease Samples

Eulachon grease samples were collected from local Knowledge Holders in Alert Bay on the traditional territory of ‘Namgis First Nation on the coast of BC, in July 2023. Eulachon grease samples were collected after the eulachon harvest and eulachon grease processing period in Spring 2023. Thus, there were samples from 2023 as well as previous years. Upon acquisition, the grease samples were sealed in individual amber glass jars and shipped frozen (-23 °C) to the laboratory in Ottawa, Ontario, Canada. Each grease sample represents an extract from approximately 600 lbs of fish (Kuhnlein & Chan, 1998; Kuhnlein et al., 1982). The characteristics of the eulachon grease samples are summarized in Table 2.1 (e.g., the sample number, the location of preparation, the year of preparation, whether the sample was kept frozen or not, and any notable differences (notes)). Triplicate analysis (n=3) was performed on each family’s eulachon grease preparation (n=7). Thus, 21 runs were completed in total (n=21).

3.3 Methods

3.3.1 Sample Preparation

To prepare the supplement samples for lipid extraction, a sub-sample of 10 softgels was randomly taken out of the bottle with the intention of representing the contents of each bottle of ω-3/fish oil supplements. From each of the 10 softgels, 0.1 mL of fish/algal oil was extracted using Hamilton syringes. The 10 0.1 mL sub-samples from each of the 10 supplement brands were combined in individual 1 mL amber glass vials. The samples were vortexed for 30 sec to ensure a proper mixture. For sample 1, which is in liquid form, two sub-samples of 0.5 mL were removed from the bottle, placed in a 1 mL amber glass vial, and vortexed for 30 sec. From these 10 1 mL sub-samples in 1 mL amber glass vials, 1 µL (0.085 mg) of each was then transferred to its own 1 mL amber glass vial.

To prepare the eulachon grease samples for lipid extraction, the grease was thawed and mixed well. Two sub-samples of 0.5 mL of each participant's preparation were removed from the container and placed into a 1 mL amber glass vial. The samples were vortexed for 30 sec. Then, from each of the seven eulachon grease 1 mL sub-samples in 1 mL amber glass vials, 1 µL (0.085 mg) of each was transferred to its own 1 mL amber glass vial.

600 µL of methanol with H2SO4 (19:1 v/v) and 16 µL of 2% (w/v) butylated hydroxytoluene in methanol were added to each vial containing 1 µL of eulachon grease or supplement sample. A matrix blank of all solvents and reagents was also prepared. Samples were heated at 90°C for 90 min. Samples were removed from heat and allowed to reach room temperature. Then, 900 µL of water with 0.9% (w/v) NaCl and 250 µL hexane were added. Samples were shaken vigorously and vortexed for 1min, and then centrifuged for 15 min. Subsequently, the hexane layer was removed and added to a glass vial, and then 250 µL of fresh hexane was added again to each sample. Again, the samples were shaken and vortexed for 1 min, centrifuged for 15 min, and the hexane layer was removed and combined with the first. Samples were then evaporated under N_2, and cyclohexane was added to each vial.

3.3.2 Data Acquisition

GC-MS analysis was completed at the Carleton Mass Spectrometry Centre (CMSC) at Carleton University in Ottawa, Ontario. Samples were analyzed on an Agilent 8890 GC with 5977B MSD with an Agilent HP-5MS UI column (30 m X 0.25 mm, 0.25 µm) and an Agilent 5190-2295; Lot 90444: 870 µL (Split, taper, wool, low-pressure drop) liner. A 1 µL injection with a 5:1 split ratio was used, and the inlet was set to 280 °C. The oven settings were 80 °C initial temperature, followed by a 30 °C /min ramp to 205 °C and then a 10 °C/min ramp to 320 °C and held for 8 min, the MS source and quad were set to 230 °C, and 150 °C, respectively.

3.3.3 Data Processing

GC-MS files were processed using Agilent MassHunter Qualitative version 10.0 using the "Find Compounds" feature and using "Find by Integration". The Agile 2 integrator was used, with a 10000-count peak filter and 0.0525 m/z + 7 ppm isotope grouping. For each compound, the spectra at the apex of the peak were used, and the first spectra of the peak were subtracted as background. Each compound was then searched against the NIST14 library using NIST MS Search software with height filters of 100 counts absolute or 0.005% relative to the largest peak and the following mass tolerances: -0.3 Da to +0.7 Da asymmetric ppm, 30 Da minimum m/z for matching, and 40% abundance ratio uncertainty. The results were then exported to a .csv and imported into a custom Microsoft Excel spreadsheet containing a macro to format and align all peak areas for each sample. Missing features in the aligned data are filled with a noise value that is 10% of the lowest peak area found in the dataset. Peak areas are then normalized to the sum of all peak areas for each sample.

Once all the results were imported into the custom Microsoft Excel spreadsheet, any FAs that were identified in supplement or eulachon grease samples but had no peak areas in the samples (i.e., peak areas were all zero), were removed from the dataset. Additionally, if the eulachon grease sample had two of three replicates that had peak areas of zero, the third replicate was changed to a zero for that FA identification. Whereas, if three of seven eulachon grease samples had zeros as their peak areas for a given FA, it was determined that FA did not exist in the sample and was removed from the dataset. Plus, a similar process was followed for the supplement samples, but since there was only one replicate, if the FA was not present in at least six of ten samples, then it was determined that FA was not actually in the samples, and it was

removed from the dataset. Statistics were then completed on this finalized version of the custom Microsoft Excel spreadsheet for both eulachon grease and supplement samples.

3.3.4 Statistical Analysis

The respective FA concentrations of each eulachon grease sample were presented as the mean of the peak areas from the triplicate runs. Subsequently, using the mean of the peak area from the triplicate runs for eulachon grease, the sum of all means for a given sample was calculated. Then, the %FA was calculated by dividing the mean peak area for each FA in each sample by the sum of all the peak areas for a given sample. The quotient was then multiplied by 100 to obtain the %FA for each FA in each sample. The same process was followed with the single runs from the supplement samples, without calculating the mean, to obtain the %FA for each FA of each supplement sample.

Once the %FA was determined for each FA and each sample for eulachon grease and supplements, the FAs identified were separated into one of the three FA groups: MUFA, PUFA, or SFA for eulachon grease and supplement samples, respectively. The sum of all the %FAs categorized as PUFA and SFA was calculated for each for eulachon grease and supplement samples, respectively. Then, the sum of the PUFA %FAs was divided by the sum of the SFA %FAs to determine the P:S ratio for eulachon grease and supplement samples.

The peak area data was subjected to two-way analysis of variance (ANOVA) and Tukey tests (when necessary) using RStudio 3 (RStudio Team, 2020) to test for the differences within and between the supplement and eulachon grease samples. PCA and cluster heatmap analyses were conducted using the peak area data and SRplot (Tang et al., 2023). Venn diagrams were made using SRplot (Tang et al., 2023) and any graphical visualizations (e.g., pie charts, scatter

plots, and bar charts) were made in Microsoft Excel (Microsoft Corporation, 2021) using the %FA data.

3.4 Results and Discussion

3.4.1 Identification and Quantification of the Fatty Acids in Eulachon Grease

There were 22 FAs identified in the eulachon grease samples using untargeted GC-MS methodologies, which are listed in Table 3.2, along with the %FA of each FA for each sample and the AVG %FA of all samples. Overall, the five most abundant FAs in eulachon grease samples were C18:0 (stearic acid) (30.67%), C20:4 (arachidonic acid) (16.64%), C18:3 (14.70%) (alpha-linolenic acid (ALA)), C22:1 (cetolic acid) (14.61%), and C20:5 (EPA) (9.89%) which don't necessarily corroborate with the most abundant FAs measured in previous studies (e.g., Phinney et al., 2009, Iverson et al., 2002, Kuhnlein et al., 1996) (see section 3.4.3).

Furthermore, there were four branched-chain fatty acids (BCFAs) (i.e., C17:0 (br), C17:1 (br), C19:0 (br), and C20:0 (br)) identified in the eulachon grease samples, which was unexpected as these had never been documented in previous literature on the FA profile of eulachon grease (e.g., Phinney et al., 2009; Iverson et al., 2002; Kuhnlein et al., 1996). BCFAs consist of a variety of non-straight-chain FAs, which are usually saturated (trace amount of monounsaturated), and the alkyl branch is a methyl group (Lu et al., 2024). Recently, an increasing number of researchers have become interested in BCFAs due to their unique biosynthesis pathway which makes them metabolically bioactive, and their potential health benefits (e.g., Yang et al., 2023; Wang et al., 2020; Dingess et al., 2017). BCFAs are present in various forms in living organisms, including both as free FAs and as part of more complex lipid structures (Gozdzik et al., 2023). BCFAs exist widely in animal fats and milk and are primarily derived from bacteria in the diet or from bacteria in animal digestive systems (Lu et al., 2024).

However, small amounts of BCFAs have been detected in fermented foods using GC-MS likely due to microbial exposure (Ran-Ressler et al., 2014). Hence, BCFAs were likely identified in eulachon grease samples due to bacteria in the eulachon themselves or because of microbial exposure during the fermentation process of the eulachon. Nonetheless, BCFAs demonstrate various potentially beneficial properties, including cancer prevention, anti-obesity agents, anti-inflammation, human intestinal function, immunoregulatory effects, and neuroprotective actions (Lu et al., 2024; Gozdzik et al., 2023).

Of the 22 FAs, nine were PUFAs, five were MUFAs, and eight were SFAs; the percentage of each group (i.e., PUFA, MUFA, and SFA) for each sample as well as the AVG of all samples is listed in Table 3.3. Based on the overall AVGs, eulachon grease is made up mostly of PUFAs (42.55%), followed by SFAs (33.03%), and lastly MUFAs (24.42%). The benefits of diets high in PUFAs are well documented, especially regarding cardio-protection and reduced CVD risk (i.e., the favourable effects on lipid and lipoprotein metabolism, blood pressure, platelet function, etc.) (Kapoor et al., 2021; Shibabaw, 2021; Shahidi & Ambigaipalan, 2018; Fleming & Kris-Etherton, 2014; Siriwardhana et al., 2012).

Through the analysis of the eulachon grease samples, it was determined that samples (2 and 5) are outliers because they contain very high concentrations of PUFAs (81.49% and 63.29%, respectively) and very low amounts of SFAs (2.42% and 3.08%, respectively). These two outliers (samples 2 and 5) are even more evident when observing the PCA plot (PC1-61.3% and PC2-18.7%) (Figure 3.1) and the heatmap (Figure 3.2). In the PCA, the replicates for samples 2 and 5 are grouped on the top left, sample 1's replicates are in the middle left, and the other samples and their replicates are grouped on the bottom left. The heatmap also follows this same pattern, but with samples 2 and 5 on the right side, the other samples are grouped in the

middle, and sample 1 is on the left side. It is possible to use the right-hand y-axis on the heatmap to explore which FAs are present in higher or lower concentrations between eulachon grease samples. Thus, what distinguishes samples 2 and 5 from the rest seems to be their high amounts of C17:0 (br), C18:2, C18:3, C20:5, C22:5, and C22:6 and their low amounts of C16:2, C17:1 (br), C18:0, C20:3, and C20:4.

It is difficult to assess what might have led to the high PUFA and low SFA levels in samples 2 and 5. At first, the age of the samples was considered, as sample 2 is an older sample (not from 2023), maybe FA concentrations changed or increased with age. However, this is not the case since sample 5 is from 2023 (a new sample) and shows the same high PUFA/low SFA pattern as sample 2, while sample 3 from 2019 does not show the same high PUFA/low SFA pattern as sample 2 and 5. Secondly, the location of eulachon grease preparation may be a factor, but both samples (2 and 5) were prepared in Knight Inlet. Thirdly, it was noted that neither samples 2 nor 5 had been previously frozen. It is possible that freezing the grease may change its FA composition. Nevertheless, sample 1 had also never been frozen and did not share the same high PUFA/low SFA pattern as samples 2 and 5. Lastly, potential differences in the preparation methods for samples 2 and 5 compared to the other eulachon grease samples were considered. However, samples 1 and 2, which do not share the same high PUFA/low SFA pattern were prepared by the same family (i.e., using the same methods) in the same location, simply in different years. Plus, samples 1, 4, and 5 were prepared by different families but using very similar methods (i.e., same fermentation length for the eulachon, same cooking temperature, etc.), in the same location, at the same time, and there is still such a drastic difference in the FA composition of sample 5 compared to samples 1 and 2. Thus, it is highly unlikely that the changes in the FA profile of eulachon grease are related to the preparation methods. A more

feasible factor could be the individual eulachons that make up each jar of eulachon grease. Namely, where the eulachon lived in the ocean (habitat), what the eulachon ate (diet), and potentially their sex or age at harvest (Kuhnlein et al., 1982). Additional eulachon grease samples and more detailed information about the eulachon harvest and eulachon grease preparation techniques would be required to make strong correlations between preparation methods and the FA profiles of eulachon grease.

Table 3.2 – Percent fatty acid (%FA) in eulachon grease samples. Each sample is the average of the triplicate runs. The %FA was calculated by dividing the peak area of each FA from each sample by the total of the peak areas for each sample. The quotient was then multiplied by 100. The first column (ID) contains FA identification, and the final column (AVG) contains the average %FA for each FA from all samples. The peak areas were obtained using GC-MS methodologies. Eulachon grease samples were collected in Alert Bay, BC from participating Knowledge Holders from 'Namgis First Nation in July 2023. A value of 0.00 means that no FA was recorded for that sample.

ID	1	2	3	4	5	6	7	AVG
C14:0	0.46	0.00	0.00	0.25	0.32	0.35	0.23	0.23
C15:0	0.31	0.15	0.28	0.29	0.25	0.37	0.15	0.26
C16:1	0.55	0.25	0.35	0.48	0.46	0.21	0.24	0.36
C16:2	0.15	0.00	0.09	0.15	0.00	0.09	0.09	0.08
C16:4	0.03	0.00	0.00	0.00	0.04	0.03	0.03	0.02
C17:0 (br)	0.49	0.49	0.39	0.00	0.52	0.27	0.34	0.36
C17:1 (br)	0.00	0.00	8.88	10.43	0.00	7.40	8.76	5.07
C18:0	41.46	0.31	41.18	44.69	0.26	37.43	49.34	30.67
C18:1	0.45	0.38	7.75	0.54	20.29	0.52	0.36	4.33
C18:2	0.00	0.09	0.06	0.07	0.11	0.00	0.00	0.05
C18:3	0.00	52.02	0.00	0.00	32.86	17.90	0.10	14.70
C19:0	1.23	0.94	0.94	0.94	1.07	0.90	0.93	0.99
C19:0 (br)	0.37	0.32	0.30	0.25	0.36	0.24	0.23	0.30
C20:0	0.07	0.04	0.00	0.00	0.07	0.06	0.05	0.04
C20:0 (br)	0.22	0.16	0.19	0.16	0.23	0.16	0.18	0.19
C20:3	0.13	0.00	0.10	0.11	0.00	0.10	0.12	0.08
C20:4	16.52	5.90	24.61	16.56	8.95	20.37	23.55	16.64
C20:5	17.18	22.01	0.17	9.41	20.01	0.22	0.24	9.89
C22:1	19.10	15.39	13.69	14.66	12.83	12.48	14.11	14.61
C22:5	0.91	0.94	0.75	0.70	0.95	0.63	0.63	0.79
C22:6	0.30	0.53	0.21	0.27	0.39	0.22	0.27	0.31
C24:1	0.08	0.06	0.05	0.07	0.06	0.05	0.04	0.06

br: branched

Table 3.3 – Percentage of each FA group (i.e., PUFA, MUFA, and SFA) in each eulachon grease sample as well as the total average of each group for all samples (final column - AVG). This was calculated by summing the individual %FAs based on the group they belong to for each sample. Eulachon grease samples were collected in Alert Bay, BC from participating Knowledge Holders from 'Namgis First Nation in July 2023.

FA Group	1	2	3	4	5	6	7	AVG
PUFA	35.22	81.49	43.28	27.26	63.29	39.56	25.02	42.55
MUFA	20.18	16.09	30.72	26.17	33.63	20.66	23.52	24.42
SFA	44.60	2.42	43.28	46.57	3.08	39.78	51.46	33.03

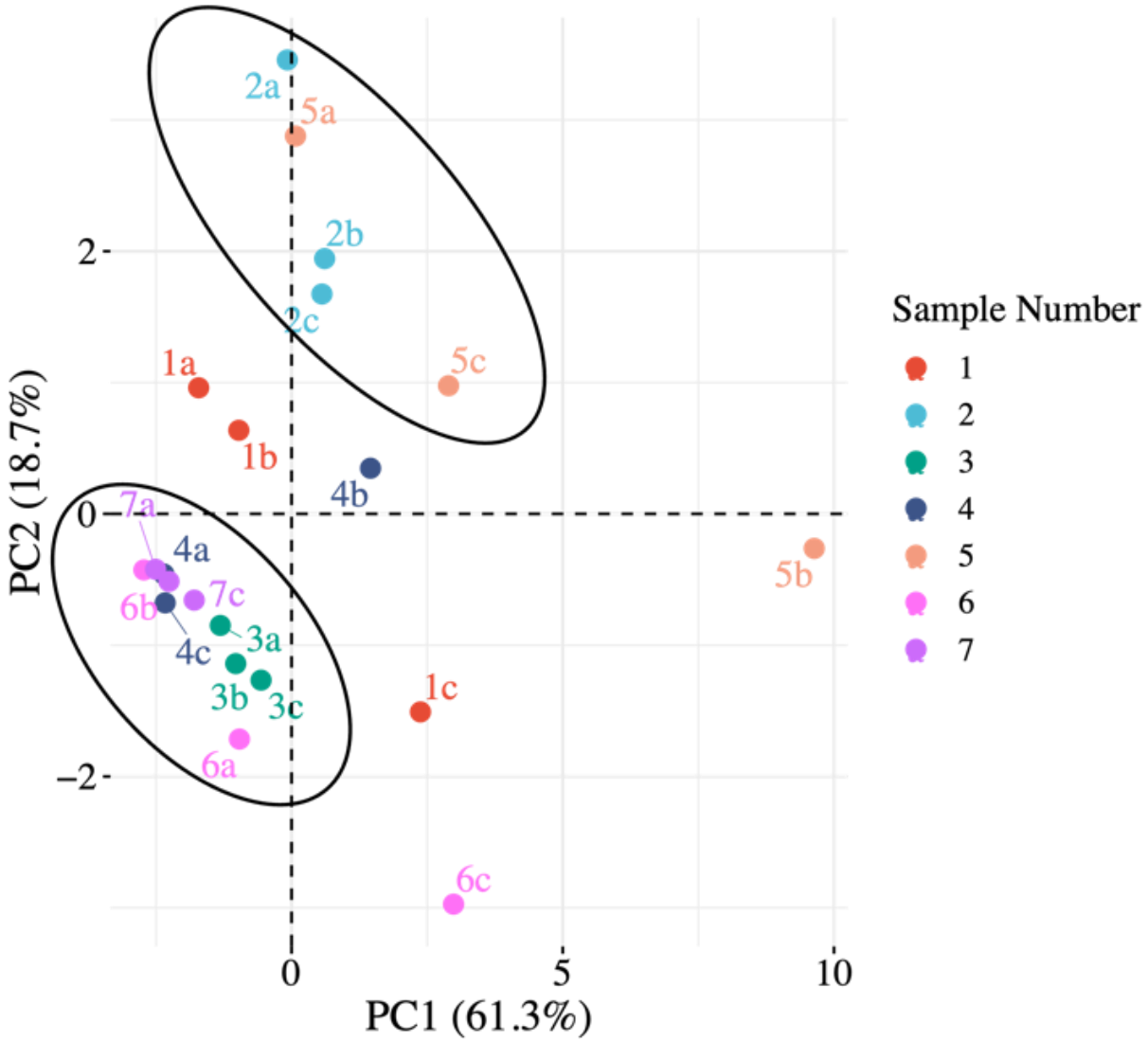

Figure 3.1 – PCA that shows the 3 replicates (a, b, c) for each of the seven eulachon grease samples (1, 2, 3…) (n=21) using the variable peak area and GC-MS methodologies.

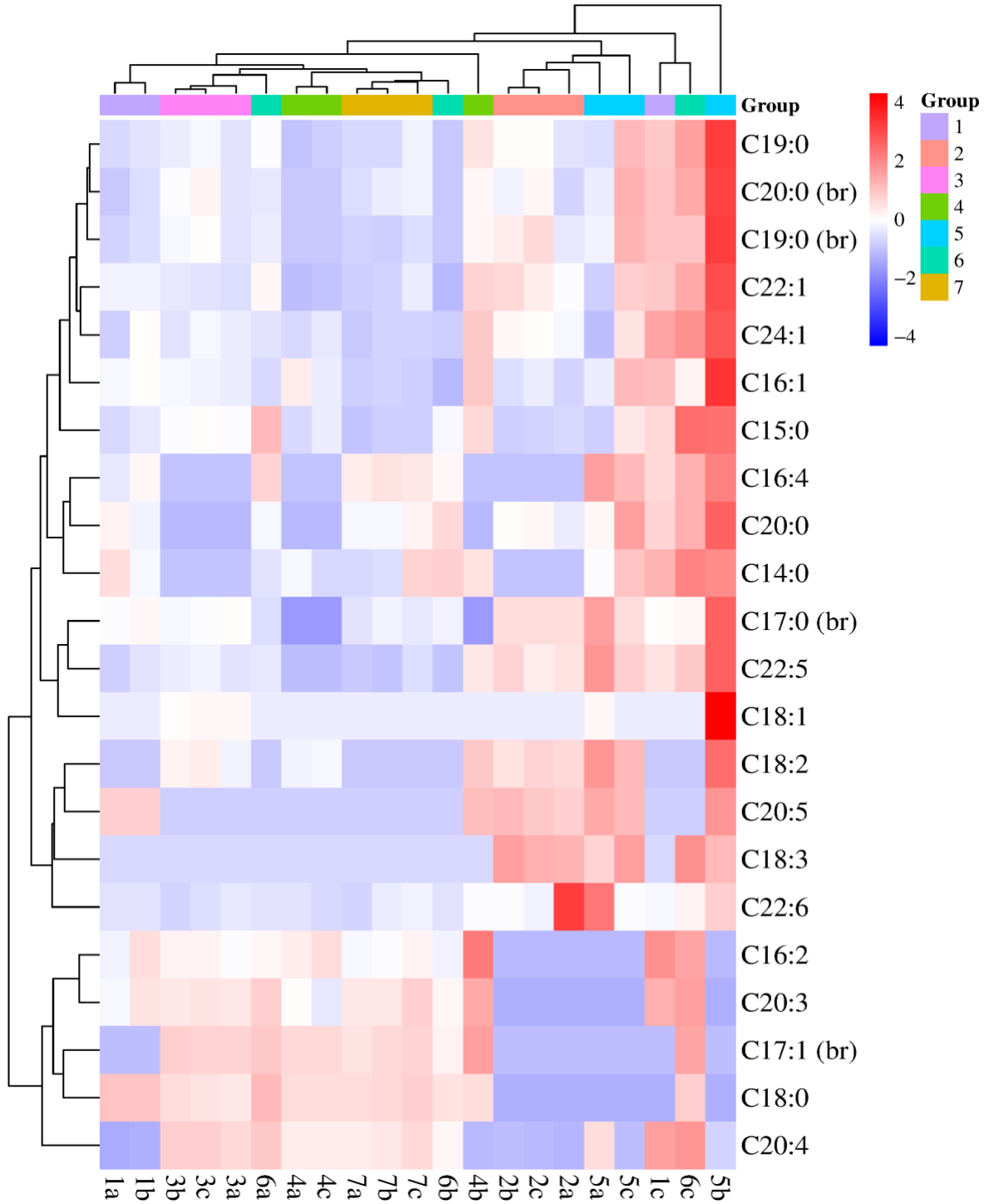

Figure 3.2 – Heatmap that compares the concentration of each FA (n=22) identified across the 3 replicates (a, b, c) for each of the seven eulachon grease sample (1, 2, 3…) (n=21) using the variable peak area and GC-MS methodologies, ((br): branched).

A two-way ANOVA was conducted on the eulachon grease samples' peak areas (Table 3.4). The two-way ANOVA tested 1) the effect of peak area on the amount of individual FAs (n=22) across eulachon grease samples and 2) the effect of peak area on the overall FA composition of eulachon grease samples (n=7). The first part of the two-way ANOVA had a statistically significant result ($\alpha<0.05$), meaning that there is a difference in the amount of individual FAs across eulachon grease samples. However, the second part of the two-way ANOVA had a statistically non-significant result, meaning that all the eulachon grease samples have relatively the same amount of total FA per sample.

Table 3.4 – Two-way ANOVA results which tests 1) The effect of peak area on the amount of individual FAs (n=22) across eulachon grease samples and 2) The effect of peak area on the overall FA composition of eulachon grease samples (n=7) collected from Alert Bay, BC, in July 2023. This was calculated using the peak area values determined using GC-MS methods. There is a significant difference in the amount of individual FAs present in eulachon grease samples (p<0.05). However, there is no significant difference in the total amount of FAs present between eulachon grease samples (p>0.05).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Fatty Acid	21	1.239e+16	5.899e+14	8.872	4.08e-15 ***
Sample	6	1.359e+14	2.265e+13	0.341	0.914
Residuals	105	6.982e+15	6.649e+13		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
21 observations deleted due to missingness.

To explore which FAs were contributing to the statistically significant result between eulachon grease samples, a Tukey test was conducted on the significant result from the ANOVA. The scatter plot in Figure 3.3 depicts the results of the Tukey test: there were 231 possible FA combinations, 49 of which contributed significantly ($\alpha<0.05$) to the positive ANOVA result. Of the 49 significant results, every combination contained at least one of three FAs, C18:0 (stearic acid), C20:4 (arachidonic acid), C18:3 (ALA), which are the top three most abundant FAs on AVG across the seven eulachon grease samples. Thus, these three FAs (C18:0, C20:4, and C18:3) are present at significantly different (i.e., higher) levels compared to the other FAs in eulachon grease samples.

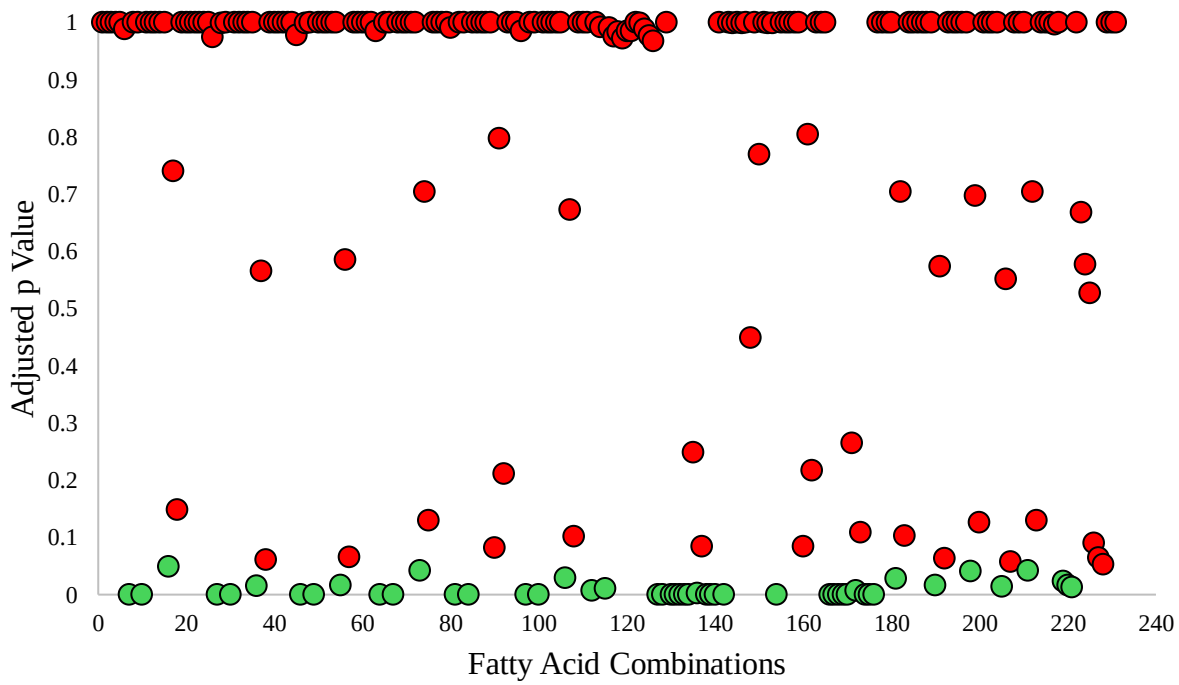

Figure 3.3 – Tukey test result following the significant two-way ANOVA ($p<0.05$) comparing the individual amounts of FAs ($n=22$) across eulachon grease samples ($n=7$). This scatter plot shows the adjusted p values for the FA combinations. There were 231 possible FA combinations, 49 of which were significant ($p<0.05$) (green points).

3.4.2 Identification and Quantification of the Fatty Acids in Supplement Samples

There were 22 FAs identified in the supplement samples using GC-MS methodologies, which are listed in Table 3.5, along with the %FA of each FA for each sample and the AVG %FA of all samples. It is interesting to note that these Canadian supplements only put EPA and DHA serving sizes on their labels (and no other FAs) while there are 20+ additional FAs present in the supplements. Overall, the five most abundant FAs in the supplement samples on AVG were C22:0 (behenic acid) (25.31%), C15:0 (br) (pentadecylic acid) (16.37%), C18:0 (8.93%) (stearic acid), C20:5 (EPA) (8.59%), and C20:1 (gondoic acid) (6.98%). Again, for brands that claim a particular serving size of EPA and DHA, it is interesting that these FAs do not rank within the top three most abundant FAs measured on AVG across the supplement samples. The results show that DHA is either entirely absent or present in very small (i.e., <2%) quantities in all 10 ω-3 supplements measured in this study. Plus, the relative levels of EPA reported per serving on the manufacturers' labels do not corroborate with the %FAs measured in this study. For example, supplement sample 1 claims to have the highest amount of EPA per serving (excluding supplement sample 6) with 750mg/serving. In this study, supplement sample 1 recorded a %FA of 15.91% for EPA. Meanwhile, supplement sample 8 actually recorded the highest amount of EPA across all supplement samples measured in this study, with 21.27%, while its label only claims to contain 360mg of EPA.

There were also four BCFAs identified in the supplement samples which was unexpected as they have, to my knowledge, never been recorded in fish oil supplements previously. Two of the BCFAs identified are the same as those found in eulachon grease samples (i.e., C19:0 (br) and C20:0 (br)), and two are unique to supplement samples (i.e., C14:0 (br) and C15:0 (br)). Since the supplement samples are made of various fish species (and not eulachon), it is likely

that these different fish species also carry some level of bacteria that contain BCFAs from their environments. It is also possible that these fish are exposed to microbes during processing which could introduce BCFAs to the fish oil supplements. Although little is known about these specific BCFAs (i.e., C14:0 (br), C15:0 (br), C19:0 (br), and C20:0 (br)), in general, BCFAs can provide many health benefits such as cancer prevention, anti-obesity agents, anti-inflammation, human intestinal function, immunoregulatory effects, and neuroprotective actions (Lu et al., 2024; Gozdzik et al., 2023).

Of the 22 FAs, five were PUFAs, five were MUFAs, and 12 were SFAs; the percentage of each group (i.e., PUFA, MUFA, and SFA) for each sample as well as the AVG of all samples is listed in Table 3.6. Based on the overall AVGs, the ω-3/fish oil supplement samples analyzed in this study are made up mostly of SFAs (65.67%), followed by MUFAs (21.37%), and lastly PUFAs (12.96%). A 2017 study by Mason & Sherratt that analyzed the FA profile of three fish oil supplements from the American market and a 2021 study by Nevigato et al., that analyzed the FA profile of three fish oil supplements from the Italian market both found considerable levels of SFAs among the ω-3/fish oil supplements studied, respectively. This high level of SFAs could indicate that the supplement samples underwent an incomplete purification process that did not eliminate the less valuable SFAs from the supplements (Nevigato et al., 2021). The purification process is equally important for the removal of toxic contaminants (Nevigato et al., 2021).

The findings from these studies, as well as the current study, are unexpected since, generally, the public believes that the consumption of fish oil supplements is a healthy dietary habit (Burger & Gochfeld, 2009). According to the National Health Statistics Report in 2008 and an analysis of National Health and Nutrition Examination Survey data from 2007 to 2010, fish oil supplements are the most used nonvitamin/nonmineral supplement among American

adults (Bailey et al., 2013; Barnes et al., 2008). In 2012, the National Health Interview Survey showed that 7.8% of adults (i.e., 19 million) had taken a fish oil supplement in the previous 30 days (Clarke, 2015). Ultimately, this misinformation is concerning given the variance in the amount of EPA, and DHA stated on the manufacturers' labels versus the levels measured in this study; patients cannot confidently know the amount of FAs they are consuming and may have to take an additional dose(s) which could lead to unintentional side effects. The presence of potential toxic contaminants in the supplement samples due to an incomplete purification process could cause a food safety problem, with adverse health effects on the consumer (Nevigato et al., 2021). Not to mention, a diet high in SFAs is associated with an increased risk for CVD (Stone et al., 2014).

Despite the high AVG amount of SFAs measured in the supplement samples analyzed in this study, sample 6 is an outlier as it is made up of 53.47% PUFAs, especially C20:5. The reason why sample 6 is an outlier is because it is a vegan sample made of algal oil. Since sample 6 is an outlier and does not contain fish oil like all other samples, it was omitted from all following statistical analyses. Moreover, samples 1 and 8 are outliers due to their high levels of MUFAs (i.e., ~40%) compared to the other samples (~16%). One potential explanation for their high MUFA levels is that samples 1 and 8 are the only two that definitely contain mackerel according to the ingredient label. These two samples (1 and 8) also contain the most different types of fish in their ingredient lists with potentially 4 species each. Thirdly, samples 9 and 10 are similar as they are composed of about 55% SFA, which is less than the rest of the samples (i.e., 2, 3, 4, 5, and 7), which are made up of approximately 80% SFA. Sample 9 may contain less SFAs than the other samples because it is the only sample that is made of wild salmon and

sample 10 may contain less SFAs than the other samples because it is the only sample that is made of cod liver.

These four outliers (samples 1, 8, 9, and 10) are even more evident when observing the PCA plot (PC1-50.6% and PC2-35.2%) (Figure 3.4) and the heatmap (Figure 3.5). In the PCA, sample 1 is by itself in the top left corner, sample 8 is in the middle, and samples 9 and 10 are close to one another in the middle bottom. Sample 1 is by itself in the top left corner of the PCA because it is the only liquid sample, and it is the sample with the highest dose of EPA and DHA per serving according to the manufacturer's label. Sample 8 also distinguishes itself from the other samples because it is the only sample that may contain tuna. The presence of a different fish species in sample 8 compared to the fish species present in the other samples could change the overall FA profile of sample 8. The other samples (i.e., 2, 3, 4, 5, and 7) are very closely grouped in the top right corner of the graph. These other samples all contain similarly high amounts of SFAs. This high level of SFAs could be due to the variation in fish species; many of these samples contain anchovies and/or sardines and/or mackerel. Also, sample 5 is made of Alaskan pollock, which is not known for its high levels of PUFAs (USDA, 2019). The heatmap also follows this same pattern, with samples 1, 8, 9, and 10 grouped on the right side and the other samples grouped on the left side. It is possible to use the right-hand y-axis on the heatmap to explore which FAs are present in higher or lower concentrations between supplement samples. Thus, what distinguishes samples 1 and 8 is their higher amounts of MUFAs such as C17:1, C18:1, and C22:1. What distinguishes samples 9 and 10 is their lower concentration of some SFAs like C15:0 (br), C20:0, C20:0 (br), and C22:0 whereas the rest of the samples (i.e., 2, 3, 4, 5, and 7) have higher amounts of those SFAs (i.e., C15:0 (br), C20:0, C20:0 (br), and C22:0) as well as C14:0 and C18:0.

Table 3.5 – Percent fatty acid (%FA) in supplement samples calculated by dividing the peak area of each FA from each sample by the total of the peak area for each sample, respectively. The quotient was then multiplied by 100. The first column (ID) contains FA identification, and the final column (AVG) contains the average %FA for each FA from all samples. The peak areas were obtained using GC-MS methodologies. Supplement samples were bought from common Canadian retailers, and all were within their stated shelf lives.

ID	1	2	3	4	5	6	7	8	9	10	AVG
C13:0	0.92	5.71	5.29	5.44	0.33	0.00	4.97	1.43	8.42	11.72	4.91
C14:0	0.41	0.61	0.90	0.62	0.00	0.00	0.60	0.29	0.36	0.00	0.42
C14:0 (br)	0.55	0.00	1.30	0.35	0.00	0.00	0.45	0.51	0.84	0.35	0.48
C15:0 (br)	0.00	24.58	23.75	23.55	26.26	0.00	23.23	14.57	11.37	0.00	16.37
C16:0	0.06	0.00	0.06	0.00	0.02	0.00	0.07	0.05	0.04	0.06	0.04
C16:3	2.17	2.71	2.38	3.21	1.27	0.00	2.81	1.77	0.51	0.37	1.91
C17:1	13.07	0.10	0.87	0.28	0.11	0.00	0.16	13.68	6.99	12.63	5.32
C18:0	11.59	0.40	3.58	0.68	0.79	5.97	1.34	13.65	25.37	22.92	8.93
C18:1	6.62	0.27	1.67	0.36	0.00	0.00	0.72	6.30	0.68	7.77	2.71
C19:0	0.00	0.86	1.33	0.93	0.00	0.00	1.11	2.26	9.94	0.00	1.83
C19:0 (br)	2.69	7.78	6.65	8.07	0.00	0.00	8.19	0.68	0.92	17.27	5.81
C19:1	1.31	0.23	0.00	0.86	0.00	0.88	0.85	0.00	1.45	0.83	0.62
C20:0	0.18	0.35	0.37	0.30	0.00	0.19	0.31	0.17	0.06	0.00	0.19
C20:0 (br)	0.00	1.37	1.48	1.42	0.00	0.31	0.00	0.44	0.12	0.12	0.55
C20:1	5.75	10.10	10.99	8.69	7.90	0.00	10.04	4.07	3.43	1.87	6.98
C20:4	0.82	0.00	0.93	0.35	0.05	0.30	0.12	0.49	0.83	13.42	1.89
C20:5	15.91	4.25	3.88	4.46	4.09	53.49	4.54	21.27	17.77	1.15	8.59
C21:0	3.84	0.00	0.07	0.02	0.00	0.00	0.00	2.78	0.34	0.45	0.84
C22:0	20.13	39.95	30.45	39.45	59.03	0.00	38.74	0.00	0.00	0.00	25.31
C22:1	12.65	0.57	3.57	0.78	0.07	38.68	1.54	14.44	9.49	8.63	5.75
C22:5	0.11	0.15	0.37	0.20	0.00	0.00	0.21	0.12	0.11	0.00	0.14
C22:6	1.21	0.00	0.12	0.00	0.08	0.18	0.00	1.02	0.97	0.44	0.43

br: branched

Table 3.6 – Percentage of each FA group (i.e., PUFA, MUFA, and SFA) in each supplement sample as well as the total average of each group for all samples (final column – AVG). This was calculated by summing the individual %FAs based on the group they belong to for each sample. Supplement samples were bought from common Canadian retailers, and all were within their stated shelf lives.

FA Group	1	2	3	4	5	6	7	8	9	10	AVG
PUFA	20.22	7.11	7.68	8.21	5.49	53.47	7.69	24.67	20.18	15.38	12.96
MUFA	39.41	11.27	17.09	10.97	8.08	38.56	13.30	38.49	22.03	31.72	21.37
SFA	40.37	81.62	75.23	80.82	86.43	6.47	79.01	36.84	57.79	52.90	65.67

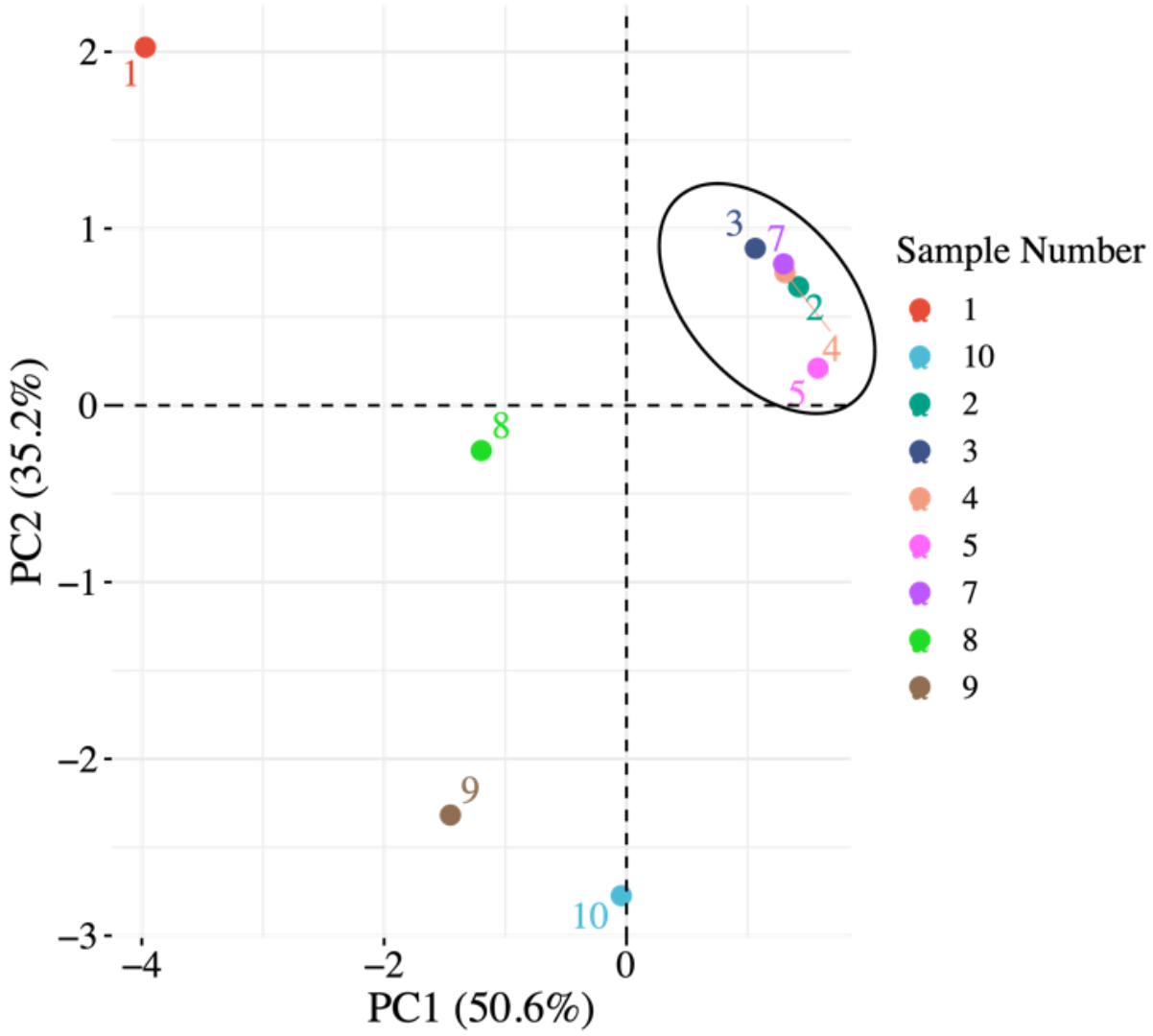

Figure 3.4 – PCA that shows the single replicate for each of the 10 supplement samples (1, 2, 3…) (n=9) using the variable peak area and GC-MS methodologies. (Supplement sample 6 omitted due to it not being fish oil.)

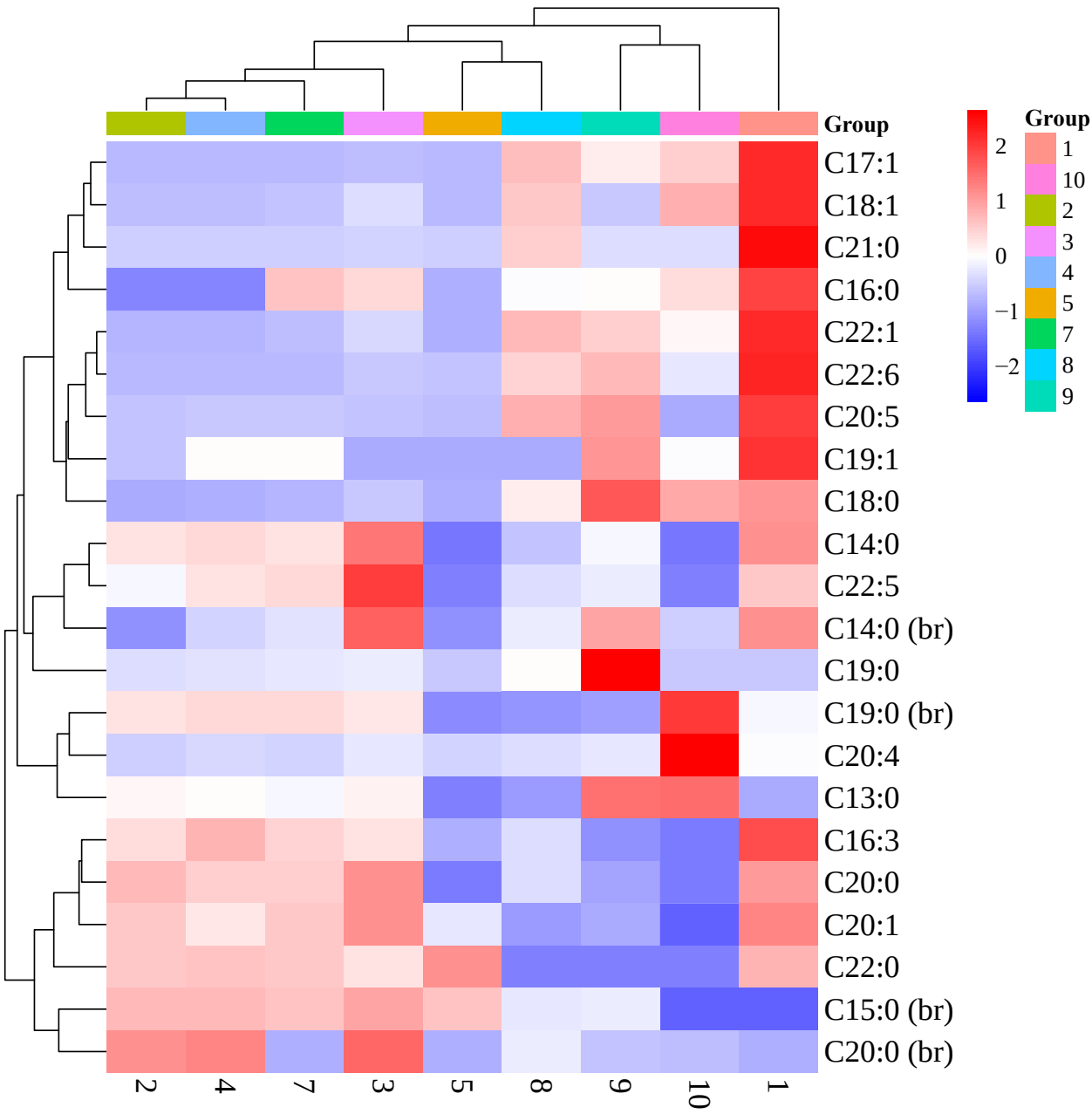

Figure 3.5 – Heatmap that compares the concentration of each FA (n=22) identified across the single replicate for each of the 10 supplement samples (1, 2, 3…) (n=9) using the variable peak area and GC-MS methodologies, ((br): branched). (Supplement sample 6 omitted due to it not being fish oil.)

A two-way ANOVA was conducted on the supplement samples' peak areas (Table 3.7). The two-way ANOVA tested 1) the effect of peak area on the amount of individual FAs (n=22) across supplement samples and 2) the effect of peak area on the overall FA composition of supplement samples (n=9). The first part of the two-way ANOVA had a statistically significant result ($\alpha<0.05$), meaning that there is a difference in the amount of individual FAs across supplement samples. The second part of the two-way ANOVA also had a statistically significant result ($\alpha<0.05$), meaning that there is a difference in the total amount of FAs per supplement sample.

Table 3.7 – Two-way ANOVA results which tests 1) The effect of peak area on the amount of individual FAs (n=22) across supplement samples and 2) The effect of peak area on the overall FA composition of supplement samples (n=9) bought from the Canadian market. This was calculated using the peak area values determined using GC-MS methods. There is a significant difference in the amount of individual FAs present in supplement samples (p<0.05). Plus, there is a significant difference in the total amount of FAs present between supplement samples (p<0.05).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Fatty Acid	21	2.571e+16	1.224e+15	18.45	< 2e-16 ***
Sample	8	2.198e+15	2.747e+14	4.14	0.00019 ***
Residuals	35	8.957e+15	6.635e+13		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
42 observations deleted due to missingness.

Two Tukey tests (Figures 3.6 and 3.7) were conducted to explore which FAs were contributing to the statistically significant results between supplement samples. The scatter plot in Figure 3.6 depicts the results of the first Tukey test. There were 231 possible FA combinations, 51 of which were contributing significantly ($\alpha<0.05$) to the first positive ANOVA result. Of the 51 significant results, every combination contained at least one of four FAs, C22:0 (behenic acid), C15:0 (br) (pentadecylic acid), C18:0 (stearic acid), and C20:5 (EPA), which are the top four most abundant FAs on AVG across the ten supplement samples. Moreover, the scatter plot in Figure 3.7 depicts the results of the second Tukey test: there were 36 possible sample combinations, 5 of which contributed significantly ($\alpha<0.05$) to the second positive ANOVA result. Of the 5 significant results, every combination contained sample 1. Sample 1 was paired against samples 2, 3, 4, 5, and 7 in the five significant combinations which means that samples 8, 9 and 10 were relatively comparable to sample 1 (i.e., not significantly different in total FA concentration). Sample 1 is likely significantly different from samples 2, 3, 4, 5, and 7 because of its high MUFA content, the fact that it is the only liquid sample, and because it is the only sample that contains herring in the ingredient list.

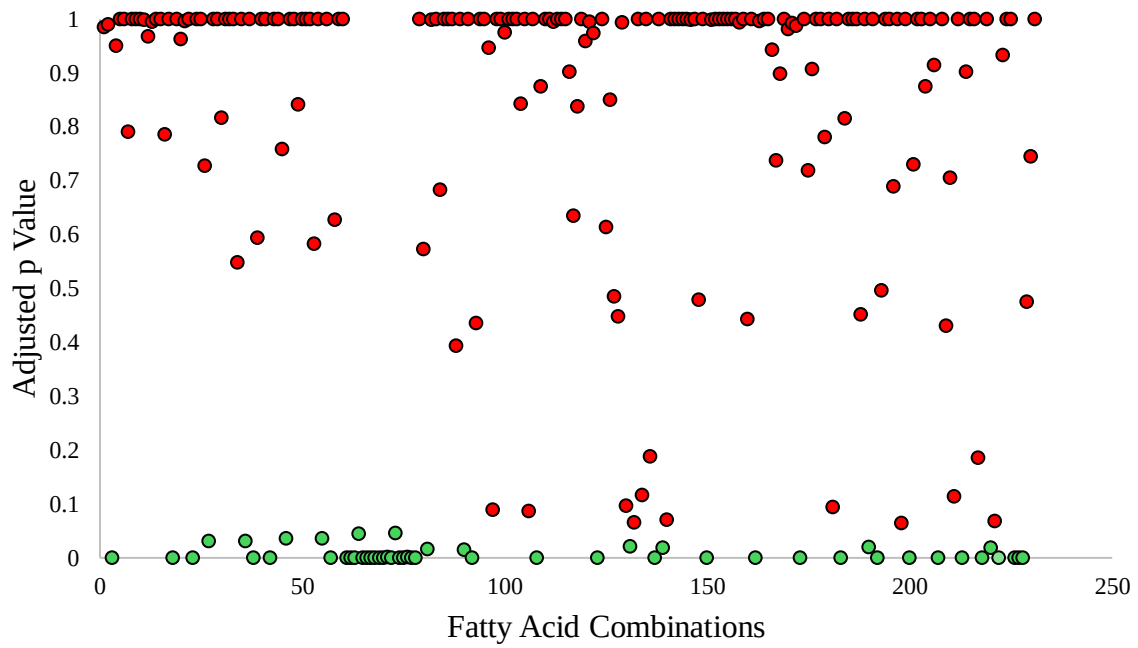

Figure 3.6 – Tukey test results following the significant two-way ANOVA ($p<0.05$) comparing the individual amounts of FAs ($n=22$) across supplement samples ($n=9$) (Supplement sample 6 omitted due to it not being fish oil), using GC-MS methodologies. This scatter plot shows the adjusted p values for the FA combinations. There were 231 possible FA combinations, 51 of which were significant ($p<0.05$) (green points).

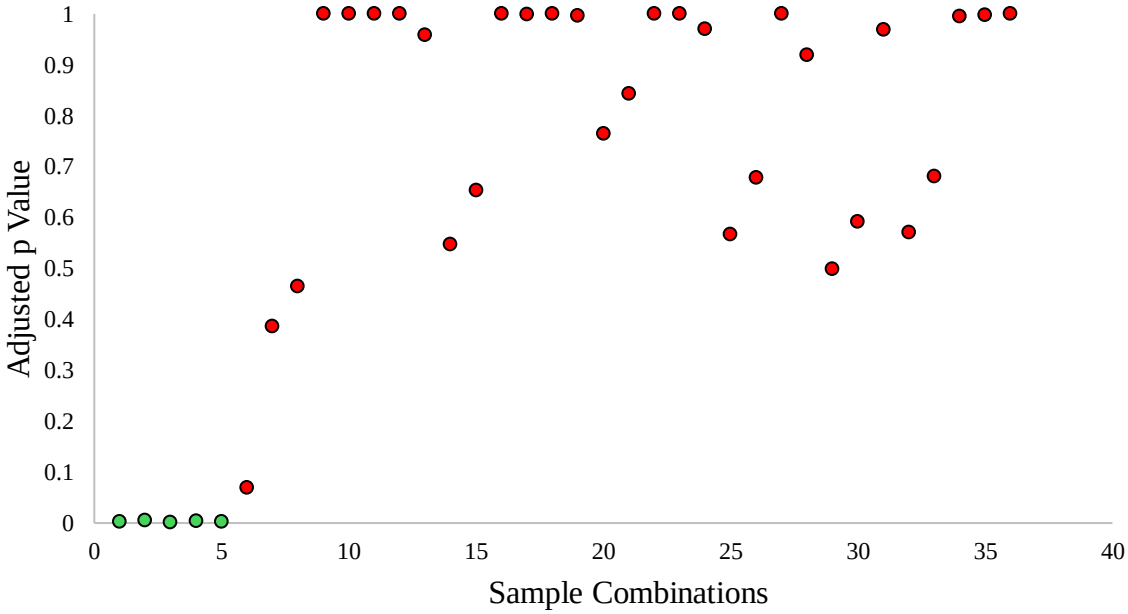

Figure 3.7 – Tukey test results following the significant two-way ANOVA ($p<0.05$) comparing the effect of peak area on the overall FA composition of supplement samples ($n=9$) (Supplement sample 6 omitted due to it not being fish oil), using GC-MS methods. This scatter plot shows the adjusted p values for the sample combinations. There were 36 possible sample combinations, 5 of which were significant ($p<0.05$) (green points).

3.4.3 Comparing the GC-MS Results of Eulachon Grease and Supplement Samples

To begin, in both eulachon grease and supplement samples, 22 FAs were identified but they were not the same 22 FAs in both cases. In fact, there were 12 FAs that were common between eulachon grease and supplement samples, 10 that were unique to eulachon grease samples, and 10 that were unique to supplement samples (Figure 3.8). The 12 FAs that both sample types had in common were: C14:0, C18:0, C18:1, C19:0, C19:0 (br), C20:0, C20:0 (br), C20:4, C20:5, C22:1, C22:5, C22:6. The FAs that were unique to eulachon grease samples were: C15:0, C16:1, C16:2, C16:4, C17:0 (br), C17:1 (br), C18:2, C18:3, C20:3, C24:1. The FAs that were unique to supplement samples were: C13:0, C14:0 (br), C15:0 (br), C16:0, C16:3, C17:1, C19:1, C20:1, C21:0, C22:0. Thus, there were 32 FAs total identified across all eulachon grease and supplement samples using GC-MS methods.

The 10 FAs identified in this study that are unique to eulachon grease samples provide exclusive health benefits. For example, C15:0 (pentadecanoic acid) is a FA that was only identified in eulachon grease samples in this study, not the supplement samples. C15:0 is an essential odd-chain SFA with broad activities relevant to protecting cardiometabolic, immune, and liver health (Venn-Watson & Schork, 2023). C15:0 is also known to enhance processes associated with human longevity and healthspan (Venn-Watson & Schork, 2023). Furthermore, C18:3 (ALA) is a FA that was only present in eulachon grease samples in this study, not the supplement samples. C18:3 is an essential PUFA that can reduce the risk of ischemic heart disease (IHD), mortality, and arrhythmia and may reduce the risk of CVD events (Abdelhamid et al., 2018). Thirdly, C24:1 (nervonic acid) is a MUFA that was only found in eulachon grease samples in this study, not the supplement samples. C24:1 is closely associated with the development and maintenance of the brain and the biosynthesis and improvement of nerve cells

(Li et al., 2019). C24:1 can also be effective in the treatment of neurological diseases (Li et al., 2019). Evidently, the FAs identified in this study that are unique to the eulachon grease samples provide exclusive health benefits. The FAs identified in this study that are unique to supplement samples may also provide exclusive health benefits. However, most of the FAs unique to the supplement samples are SFAs which typically result in negative health effects (Stone et al., 2014).

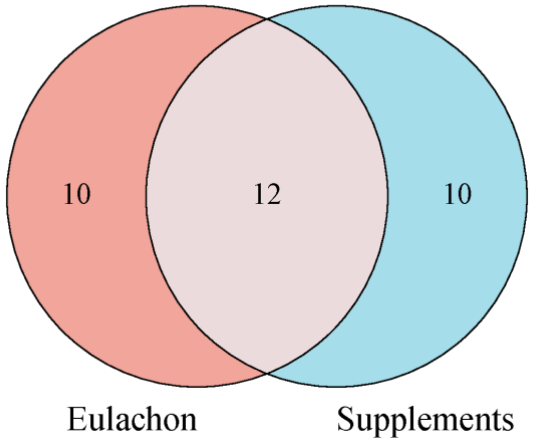

Figure 3.8 – Venn diagram comparing the number of FAs that are unique to eulachon grease samples (10), unique to supplement samples (10), and those that they have in common (12).

The eulachon grease samples had five PUFAs, three MUFAs, and two SFAs that were not present in the supplement samples, whereas the supplements had one PUFA, three MUFAs, and six SFAs that were not identified in eulachon grease samples. The additional PUFAs in eulachon grease samples and SFAs in supplement samples are apparent when the AVG of each FA group (i.e., PUFA, MUFA, and SFA) for all eulachon grease and supplement samples, respectively, are compared (Figure 3.9). Eulachon grease samples are made up of an AVG of 42.55% PUFA, while supplements only AVG 12.96% PUFA. As covered in section 3.4.1, PUFAs are beneficial for cardio-protection and reduced CVD risk (i.e., the favourable effects on lipid and lipoprotein metabolism, blood pressure, platelet function, etc.) (Kapoor et al., 2021;

Shibabaw, 2021; Shahidi & Ambigaipalan, 2018; Fleming & Kris-Etherton, 2014; Siriwardhana et al., 2012). In contrast, supplement samples are made up of an AVG of 65.67% SFA, while eulachon grease samples only AVG 33.03% SFA. As covered in section 3.4.2, diets high in SFAs are associated with an increased risk for CVD (Stone et al., 2014).

While the eulachon grease samples appear to contain more PUFAs and less SFAs than the ω-3/fish oil supplements available on the Canadian market, the results of the statistical tests were insignificant (df = 10, p = 0.352 and df = 11p = 0.424, respectively). The pie charts in Figure 3.9 also show that the amount of MUFAs in eulachon grease and supplement samples is similar, with 24.42% and 21.37%, respectively. The t-test to compare the MUFA levels between eulachon grease and supplement samples was also not statistically significant ($\alpha < 0.05$, df = 6, p = 0.469).

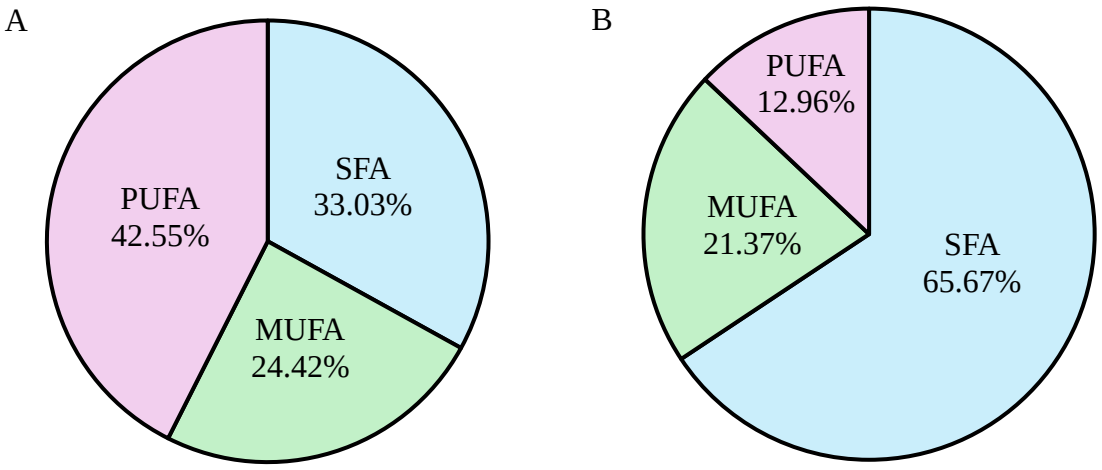

Figure 3.9 – (A) Percentage of each group of FAs (SFA, MUFA, and PUFA) in eulachon grease samples using GC-MS methodologies; (B) Percentage of each group of FAs (SFA, MUFA, and PUFA) in supplement samples using GC-MS methodologies.

Moreover, PCA results (Figure 3.10) show that eulachon grease samples 2 and 5 are grouped together in the middle-bottom of the chart, whereas the other eulachon grease samples and their replicates are grouped on the top right of the chart. In contrast, the supplement samples

are grouped in the bottom right except samples 1, 8, 9, and 10, which were identified as outliers in the individual results. Hence, the eulachon grease samples are more similar to one another than the supplement samples and the supplement samples are more similar to one another than the eulachon grease samples.

The heatmap (Figure 3.11) shows similar results to those of the PCA. The top bar of the heatmap shows that the eulachon grease samples are all grouped together on the left in blue and the supplement samples are grouped together on the right in red. Within the eulachon grease samples, most of the replicates from samples 2 and 5 are grouped on the end of the right side of the heatmap. Within the supplement samples, samples 1, 8, 9, and 10 are grouped at the opposite end on the left side of the heatmap. Using the right-hand y-axis on the heatmap to explore which FAs are present in higher or lower concentrations between eulachon grease and supplement samples is possible. Thus, what distinguishes eulachon grease samples from supplement samples and vice-versa is the higher concentrations of the FAs that are unique to each sample type. For example, the eulachon grease samples contain FAs like C16:1, C17:0 (br), and C24:1 in high quantities, which are not present in supplement samples and the supplement samples contain FAs like C13:0, C16:3, and C21:0 in high quantities that are not present in eulachon grease samples.

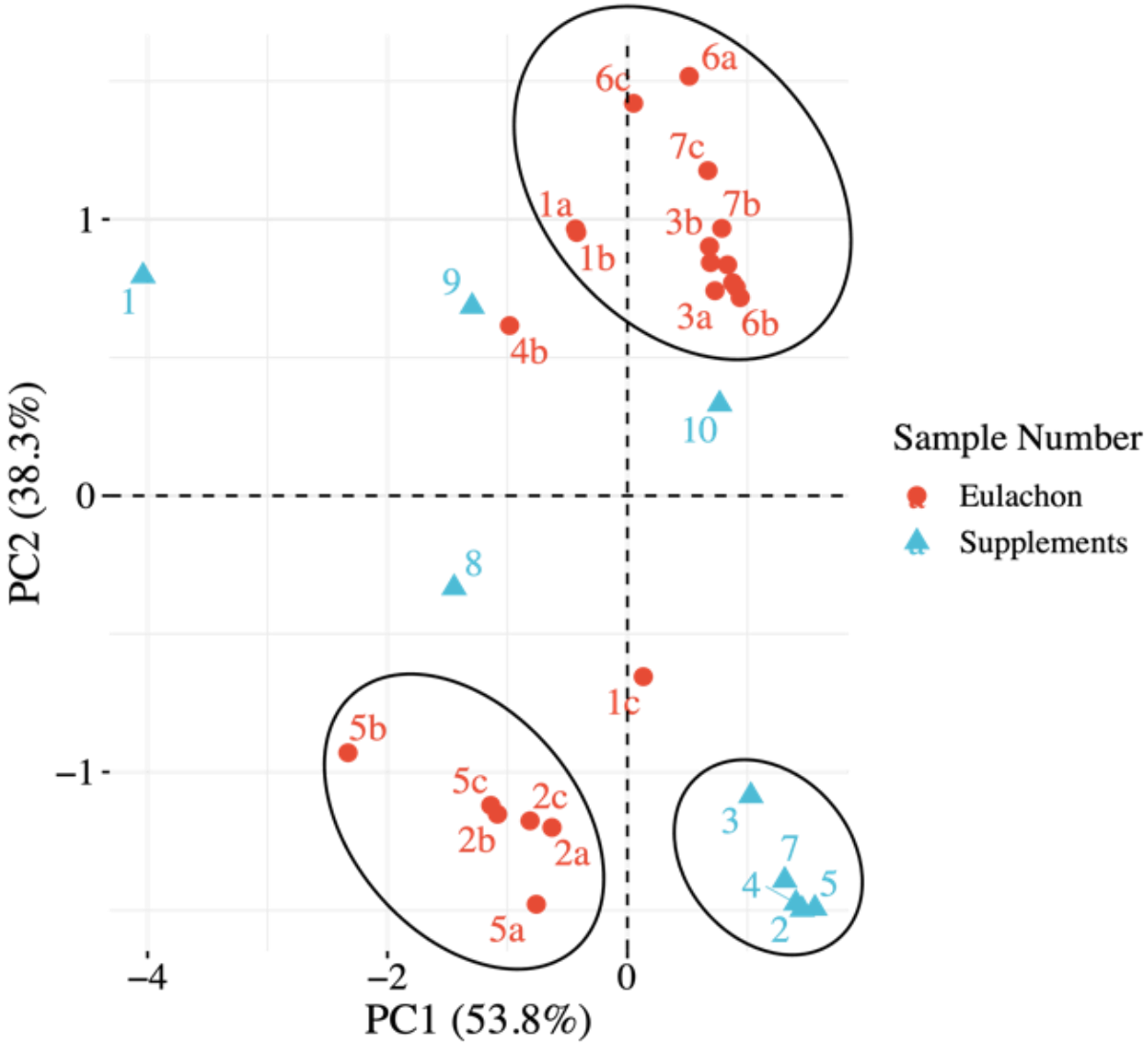

Figure 3.10 – PCA that shows the three replicates (a, b, c) for each of the seven eulachon grease samples (1, 2, 3…) (n=21) (red) as well as the single replicate for the nine supplement samples (1, 2, 3…) (n=9) (blue) using the variable peak area and GC-MS methodologies. (Supplement sample 6 omitted due to it not being fish oil.)

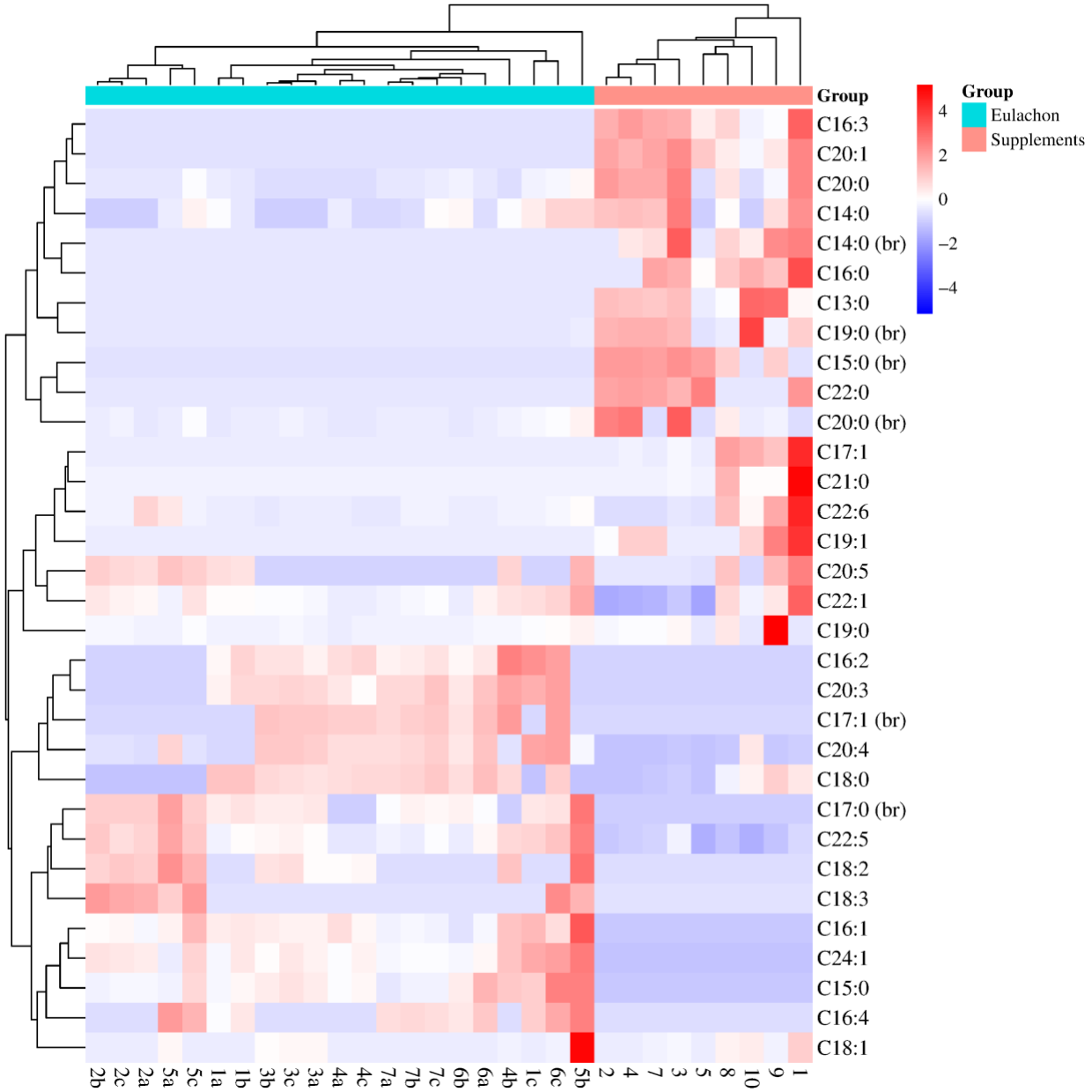

Figure 3.11 – Heatmap that compares the concentration of each FA (n=32) identified across the three replicates (a, b, c) for each of the seven eulachon grease samples (1, 2, 3…) (n=21) (blue) as well as the single replicate for the nine supplement samples (1, 2, 3…) (n=9) (red) using the variable peak area and GC-MS methodologies, ((br): branched). (Supplement sample 6 omitted due to it not being fish oil.)

Additionally, a two-way ANOVA was conducted on the eulachon grease and supplement samples' peak areas (Table 3.8). The two-way ANOVA tested 1) the effect of peak area on the amount of individual FAs (n=32) across eulachon grease and supplement samples and 2) the effect of peak area on the overall FA composition of eulachon grease and supplement samples (n=16). The first part of the two-way ANOVA had a statistically significant result ($\alpha<0.05$), meaning that there is a difference in the amount of individual FAs across eulachon grease and supplement samples. The second part of the two-way ANOVA also had a statistically significant result ($\alpha<0.05$), meaning that there is a difference in the total amount of FAs between eulachon grease and supplement samples.

Table 3.8 – The results of a two-way ANOVA which tests 1) The effect of peak area on the amount of individual FAs (n=32) across eulachon grease and supplement samples and 2) The effect of peak area on the overall FA composition of eulachon grease and supplement samples (n=16) (supplement sample 6 omitted due to it not being fish oil). This was calculated using the %FA values determined from using GC-MS methods. There is a significant difference in the amount of individual FAs present in eulachon grease and supplement samples as well as in the number/total amount of FAs present between eulachon grease and supplement samples (p<0.05).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Fatty Acid	31	3.670e+16	1.184e+15	16.365	< 2e-16 ***
Sample	15	2.393e+15	1.595e+14	2.205	0.00673 **
Residuals	251	1.816e+16	7.235e+13		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
214 observations deleted due to missingness.

Two Tukey tests (Figure 3.12 and 3.13) were conducted to explore which FAs were contributing to the statistically significant results in the two-way ANOVA between eulachon grease and supplement samples. The scatter plot in Figure 3.12 depicts the results of the first Tukey test; there were 496 possible FA combinations, 120 of which were contributing significantly ($\alpha<0.05$) to the first positive ANOVA result. Of the 120 significant results, every possible combination contained at least one of six FAs, C15:0 (br) (pentadecylic acid), C18:0 (stearic acid), C18:3 (ALA), C20:5 (EPA), C22:0 (behenic acid), and C22:1 (cetolic acid). Of those six FAs, C18:0, C18:3, and C22:1 are in the topmost abundant FAs for eulachon grease samples, and C15:0 (br), C18:0, C20:5, and C22:0 are in the topmost abundant FAs for supplement samples. Furthermore, the scatter plot in Figure 3.13 depicts the results of the second Tukey test; there were 120 possible sample combinations, five of which contributed significantly ($\alpha<0.05$) to the second positive ANOVA result. Of the 5 significant results, every combination contained supplement sample 1. Supplement sample 1 was paired against supplement samples 2, 3, 4, 5, and 7 in the five significant combinations which means that supplement samples 8, 9 and 10 as well as all eulachon grease samples were relatively comparable to each other and supplement sample 1 in terms of their total FA concentration (i.e., not significantly different).

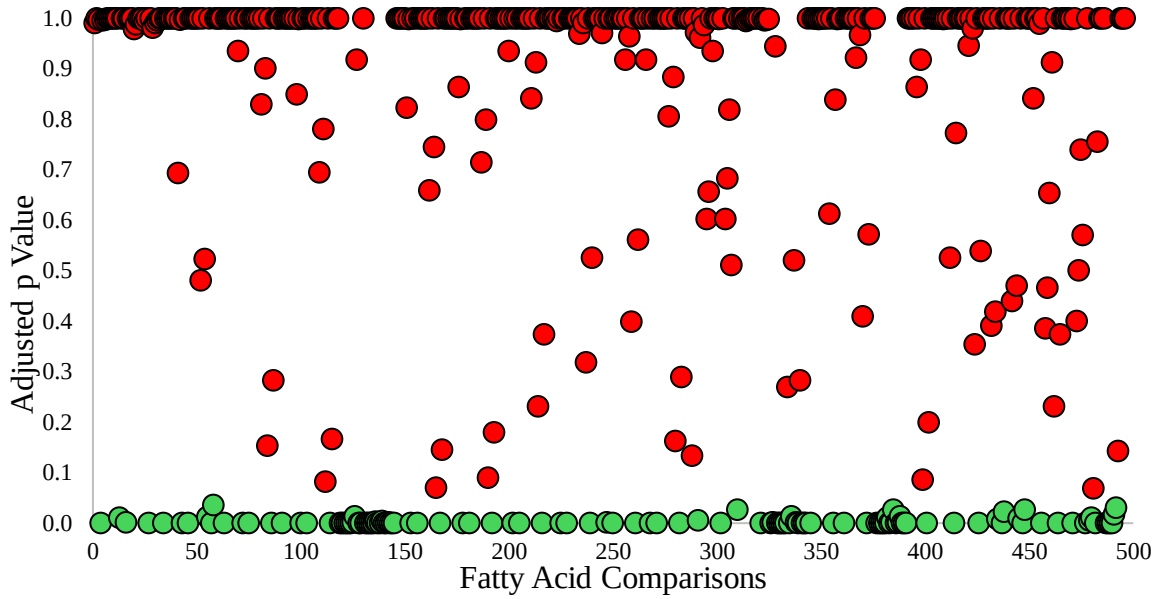

Figure 3.12 – Tukey test results following the significant the two-way ANOVA ($p<0.05$) comparing the effect of peak area on the amount of individual FAs ($n=32$) across eulachon grease and supplement samples. (Supplement sample 6 omitted due to it not being fish oil.) This was calculated using the %FA values determined from using GC-MS methods. There were 496 possible sample combinations, 120 of which were significant ($p<0.05$) (green points).

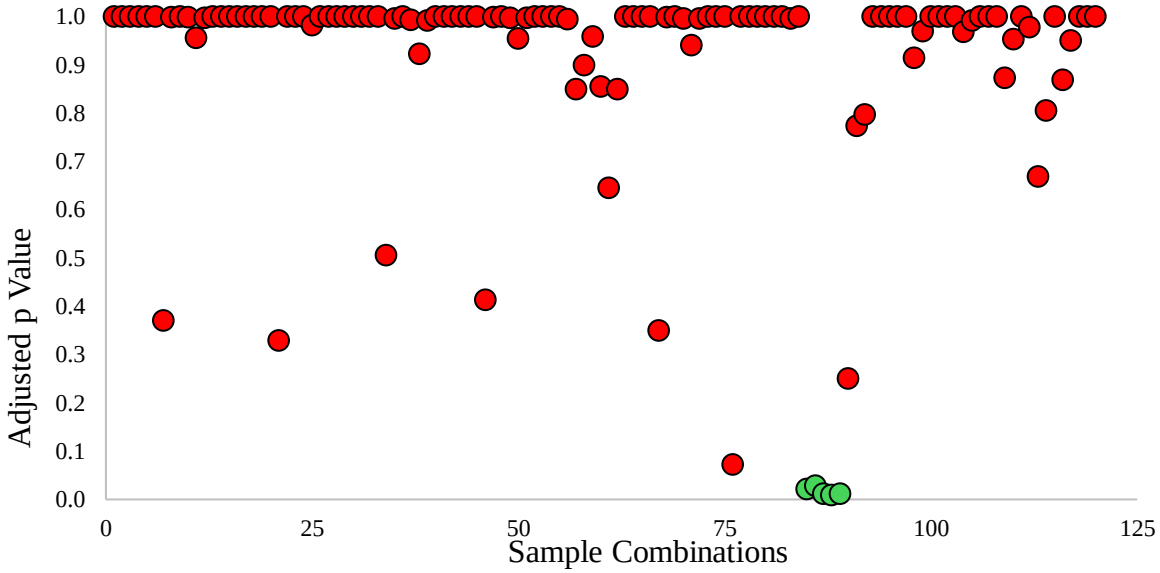

Figure 3.13 – Tukey test results following the significant two-way ANOVA ($p<0.05$) comparing the effect of peak area on the overall FA composition of eulachon grease and supplement samples ($n=16$). (Supplement sample 6 omitted due to it not being fish oil.) This scatter plot shows the adjusted p values for the sample combinations. This was calculated using the %FA values determined from using GC-MS methods. There were 120 possible sample combinations, 5 of which were significant ($p<0.05$) (green points).

Moreover, the P:S ratio was calculated for each individual eulachon grease and supplement sample. The P:S ratio is the ratio of PUFAs to SFAs and is the most used index for evaluating the nutritional value of dietary foods (Chen & Liu, 2020). The P:S ratio is normally used to assess the impact of diet on cardiovascular health (CVH) (Chen & Liu, 2020). The P:S ratio hypothesizes that all PUFAs in the diet can depress low-density lipoprotein cholesterol (LDL-C) and lower levels of serum cholesterol, whereas all SFAs contribute to high levels of serum cholesterol (Chen & Liu, 2020). Thus, the higher this ratio, the more positive the effect. In Western diets, the AVG ratio is 0.6; it is suggested that increasing it to near 1.0 would reduce the risk of atherosclerosis and coronary heart disease (Bender, 2009). The results of the P:S ratio calculations for individual eulachon grease and supplement samples are presented in Figures 3.14 and 3.15, respectively.

Eulachon grease samples 2 and 5 have very high P:S ratios (i.e., 33.62 and 20.54, respectively) as they were outliers with their high concentrations of PUFAs and low concentrations of SFAs compared to the other eulachon grease samples. In comparison, the P:S ratio of the other eulachon grease samples falls around an AVG of 0.7. Overall, eulachon grease samples have high P:S ratio and would likely contribute to benefits in CVH such as depressing LDL-C and lowering levels of serum cholesterol (Chen & Liu, 2020). Alternatively, there are no outliers in the supplement samples regarding the P:S ratio. The AVG P:S ratio across all nine supplement samples is 0.25. This low P:S ratio in supplement samples could increase serum cholesterol levels and increase the risk of heart disease (Chen & Liu, 2020; Bender, 2009). A t-test was conducted to determine if there was a statistically significant difference between the P:S ratios of eulachon grease and supplement samples. The result was non-significant ($\alpha < 0.05$, df = 6, p = 1.943).

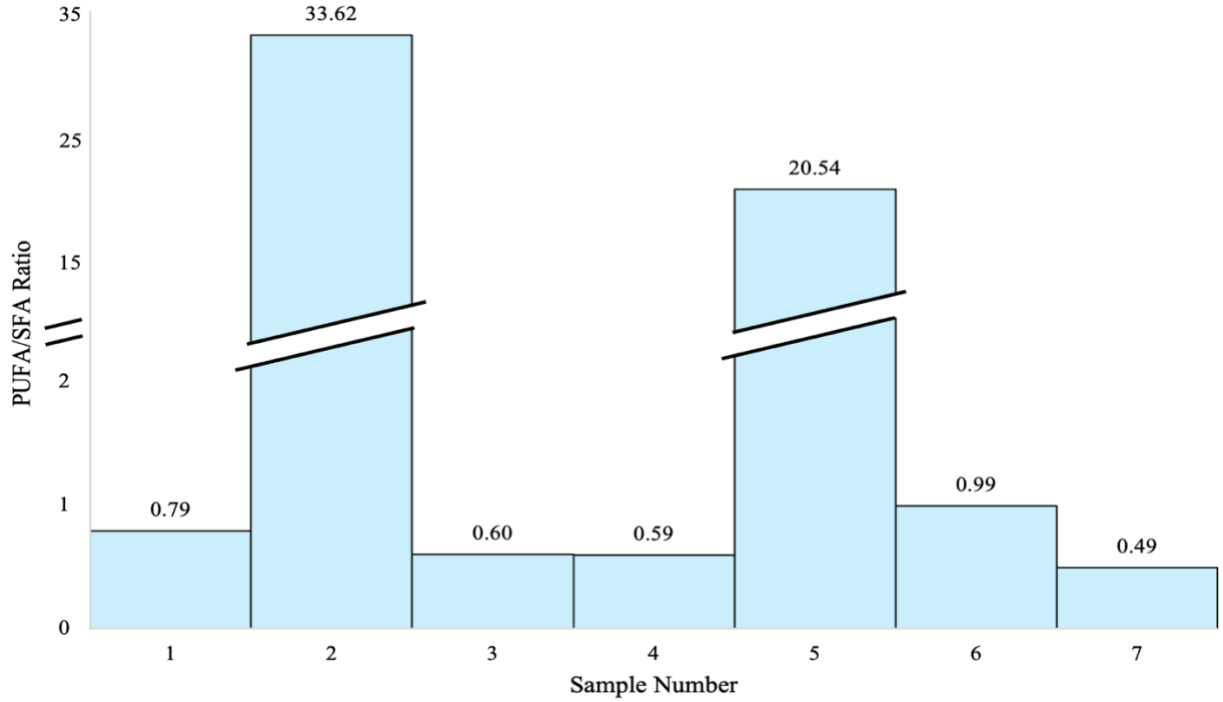

Figure 3.14 – The P:S ratio for eulachon grease samples (n=7) using GC-MS methodologies. The P:S ratio is the ratio between polyunsaturated and saturated FAs, calculated by dividing the %PUFA by the %SFA and multiplying the quotient by 100.

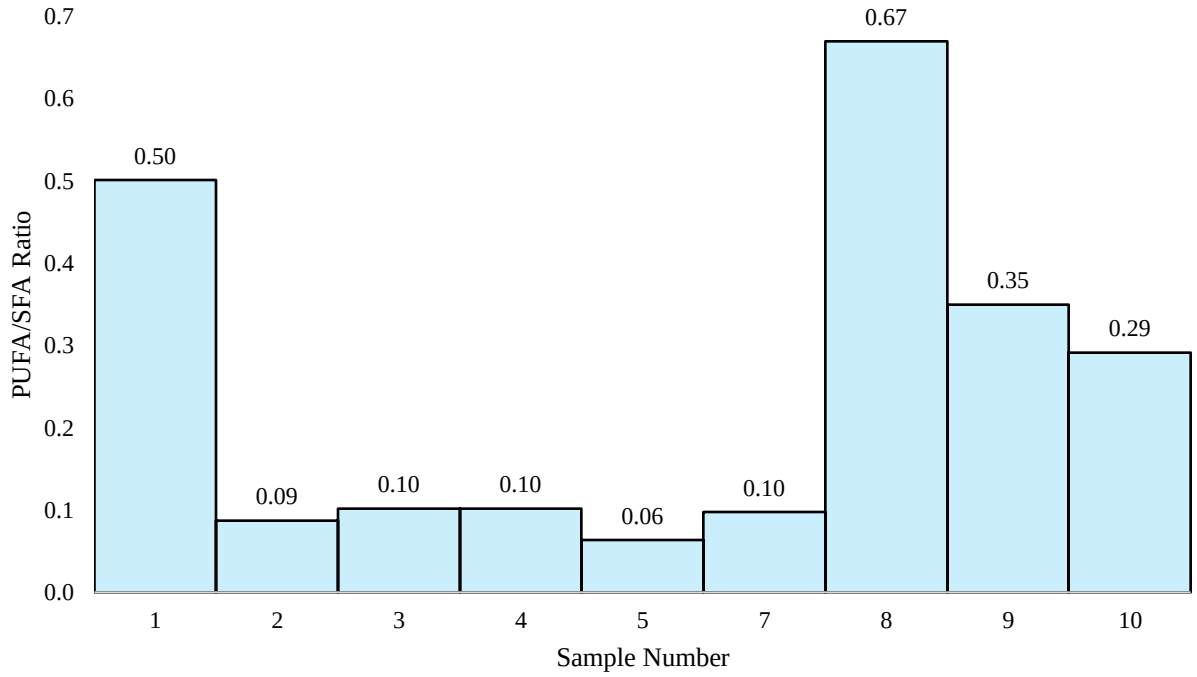

Figure 3.15 – The P:S ratio for supplement samples (n=9) using GC-MS methodologies. The P:S ratio is the ratio between polyunsaturated and saturated FAs, calculated by dividing the %PUFA by the %SFA and multiplying the quotient by 100. (Supplement sample 6 omitted due to it not being fish oil.)

Three previous studies (i.e., Phinney et al., 2009, Iverson et al., 2002, and Kuhnlein et al., 1996) have used GC-MS techniques to report on the FA concentrations of either eulachon fish (i.e., Iverson et al., 2002) or eulachon grease (i.e., Phinney et al., 2009 and Kuhnlein et al., 1996). In the most recent study by Phinney et al., 2009, four eulachon grease samples were collected in either 2005 or 2006 from the Nass River region and analyzed. In the 2002 study by Iverson et al., 20 eulachon fish samples from the Prince William Sound embayment in the Gulf of Alaska were caught between 1994-1998 and analyzed. The third and oldest study by Kuhnlein et al. in 1996, analyzed several eulachon grease samples but there were three samples of interest to the current study as they were collected in Knight Inlet in 1994. Two of the studies (i.e., Phinney et al., 2009 and Iverson et al., 2002) quantified their results using the unit %FA for the FAs identified, and the other study (i.e., Kuhnlein et al., 1996) quantified their results using the unit g/100g. Since eulachon grease contains close to 100% fats (Kuhnlein et al.., 1996; Kuhnlein et al., 1982), the unit g/100g is comparable to the unit %FA in this case.

The three previous studies (i.e., Phinney et al., 2009, Iverson et al., 2002, and Kuhnlein et al., 1996) and the current study have 10 FAs that were identified and quantified in common (i.e., C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:1, C20:4, C20:5, and C22:6). Unlike the current study, which couldn't distinguish individual FA isomers, some of the previous studies had measured the FA concentrations of the different isomers (i.e., C16:1 n-7, C16:1 n-9, C16:1 n-11, etc.). In this case, all the measurements of the isomers for a given FA were added together to represent one value for that individual FA (i.e., C16:1). Table 3.9 summarizes and compares the results for the 10 common FAs from eulachon grease and supplement samples in this study to the eulachon fish/grease results from the three previous studies.

Table 3.9 – Comparing the FA composition (in g/100g or %FA) of 10 FAs identified in either eulachon fish or grease reported by three different studies (i.e., Phinney et al., 2009, Iverson et al., 2002, and Kuhnlein et al., 1996) to the FA composition measured for those same 10 FAs in the current study in eulachon grease and supplement samples. All studies used GC-MS methodologies to identify and quantify the FAs. Significant variations in C16:0, C18:0, C18:1, C20:4, and C20:5, are observed across the studies.

FA	Study 1 – Phinney et al., 2009 (%FA)	Study 2 – Iverson et al., 2002 (%FA)	Study 3 – Kuhnlein et al., 1996 (g/100g)	This study – Eulachon grease (%FA)	This study – Supplements (%FA)
C14:0	5.4	8.6	5.4	0.2	0.4
C16:0	20.0	16.8	12.0	NA	0.04
C16:1	6.7	8.7	5.0	0.4	NA
C18:0	3.8	2.4	2.6	30.7	8.9
C18:1	45.0	37.3	38.0	4.3	2.7
C18:2	0.7	0.9	0.5	0.05	NA
C20:1	0.2	6.0	0.4	NA	7.0
C20:4	0.3	0.4	0.3	16.6	1.9
C20:5	0.6	1.5	0.9	9.9	8.6
C22:6	1.7	2.5	11.0	0.3	0.4

NA: Not Identified/Measured

In sum, the results from the previous studies (i.e., Phinney et al., 2009, Iverson et al., 2002, and Kuhnlein et al., 1996) do not corroborate strongly with the results of the current study. More specifically, C16:0 and C18:1 were identified and measured consistently high (20.0%, 16.8%, 12g/100g and 45.0%, 37.3%, 38.0g/100g, respectively) in the three previous studies, whereas they are present in very low quantities or entirely absent from the current study for eulachon grease and supplement samples (0.0%, 0.04% and 4.3%, 2.7%, respectively). Additionally, C18:0 and C20:4 measured quite high in the current study's eulachon grease samples (30.7% and 16.6%, respectively) (not the supplements with 8.9% and 1.9%) compared to the C18:0 and C20:4 levels in the previous studies (3.8%, 2.4%, 2.6% and 0.3%, 0.4%, 0.3%, respectively). The level of C20:5 measured in this study in both eulachon grease and supplement samples (9.9% and 8.6%, respectively) were much higher than the levels in previous studies (0.6%, 1.5%, and 0.9 g/100g, respectively). C18:2 is one of the only FAs that recorded similar measurements across all studies (0.7%, 0.9%, 0.5g/100g, 0.05% (not identified in supplement samples)). The current study agrees with the results from Phinney et al., 2009 (1.7%) and Iverson et al., 2002 (2.5%), regarding the level of C22:6 quantified as in the current study, we measured 0.3% C22:6 for eulachon grease samples and 0.4% for supplement samples while Kuhnlein et al., 1996 recorded a much higher amount (11.0g/100g) in eulachon grease samples.

There are several potential explanations for the discrepancies in the FA concentrations recorded in this study compared to the previous studies (i.e., Phinney et al., 2009, Iverson et al., 2002, and Kuhnlein et al., 1996). Firstly, the eulachon grease samples analyzed in this study are 15+ years newer than the samples collected in the previous studies. Over the last 15+ years, many changes have occurred in the marine environment (e.g., increased pollution levels), climate change impacts have worsened (e.g., rising water temperatures), and marine biodiversity has

shifted (e.g., changes in prey availability or in species migration patterns) (Talloni-Álvarez et al., 2019 Weatherdon et al., 2016; Hollowed et al., 2013). All the aforementioned factors could alter the behaviour and/or diet of eulachon and, consequently, their FA composition. Secondly, although we couldn't make direct links between preparation methods and the FA profile of the eulachon grease samples in this study, it is possible that there are considerable differences in the techniques used to make eulachon grease now compared to 15-20 years ago (e.g., variation in the number of fermentation days, different cooking temperatures, changes in the straining technique or in the storage conditions). These differences could lead to changes in the FA profile of eulachon grease between the current and previous studies. Thirdly, changes in sensitivity and the advancements in accuracy that have occurred with GC-MS technologies in the last 15-20 years (Xu et al., 2020; Hu & Zhang, 2018), could lead to differences in the methods (i.e., sample prep, solvents, machine type, etc.) used to analyze the FA profiles across studies and could contribute to variations in reported levels. Overall, the discrepancies in the FA concentrations recorded in this study compared to previous studies (i.e., Phinney et al., 2009, Iverson et al., 2002, and Kuhnlein et al., 1996) are likely a consequence of a combination of environmental, methodological, and technological factors.

3.5 Conclusion

This chapter summarizes the results from the GC-MS analyses on the eulachon grease samples from 'Namgis First Nation and the supplement samples from the Canadian market. Ultimately, 22 FAs were identified in eulachon grease samples, and 22 FAs were identified in supplement samples. However, they were not the exact same 22 FAs, eulachon grease and supplement samples had 12 FAs in common and 10 unique FAs, respectively. It was determined that both eulachon grease and supplement samples contained beneficial FAs, including BCFAs.

The FA profiles of eulachon grease and supplement samples appear to be different but were not statistically different, likely due to the small sample size and the high variability between samples. For example, there were considerable differences among eulachon grease samples, with samples 2 and 5 being outliers and among supplement samples such as samples 1, 8, 9, and 10. The discrepancies between supplement samples were explained based on their format (i.e., liquid or softgel), their reported EPA and DHA concentrations on the label, and their ingredients (i.e., similar or different fish species used between samples). However, it was more difficult to form direct links between the eulachon grease samples and the respective preparation techniques. Most Knowledge Holders interviewed in Chapter 2 described very similar eulachon grease preparation techniques across families and stated that the current climate change impacts are what affect their eulachon harvest and grease-making process the most. Further studies will require a larger sample size. The results suggest that the differences within eulachon grease samples are determined by the individual eulachons that make up each jar of eulachon grease. Namely, where the eulachon lived in the ocean (habitat), what the eulachon ate (diet), and potentially their sex or age at harvest (Kuhnlein et al., 1982). This consensus was echoed as the results from this study were compared to the results of previous studies (i.e., Phinney et al., 2009, Iverson et al., 2002, and Kuhnlein et al., 1996). The results from the current study did not fully corroborate with the results from previous studies; this is a consequence of the time that has passed between the collection of the samples for the previous studies and the collection of the samples for this study which could have had effects on the environmental, methodological, and technological factors between this and previous studies.

3.6 References

Abdelhamid, A. S., Brown, T. J., Brainard, J. S., Biswas, P., Thorpe, G. C., Moore, H. J., Deane, K. H., AlAbdulghafoor, F. K., Summerbell, C. D., Worthington, H. V., Song, F., & Hooper, L. (2018). Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. The Cochrane database of systematic reviews, 11(11), CD003177.

Bailey, R. L., Gahche, J. J., Miller, P. E., Thomas, P. R., & Dwyer, J. T. (2013). Why US Adults Use Dietary Supplements. JAMA Internal Medicine, 173(5), 355–361.

Barnes, P. M., Bloom, B., & Nahin, R. L. (2008). Complementary and alternative medicine use among adults and children: United States, 2007. National Health Statistics Reports, 12.

Bender, D. A. (2009). *A Dictionary of Food and Nutrition* (3rd ed.). Oxford University Press.

Burger, J., & Gochfeld, M. (2009). Perceptions of the risks and benefits of fish consumption: Individual choices to reduce risk and increase health benefits. Environmental Research, 109(3), 343-349.

Chen, J., & Liu, H. (2020). Nutritional Indices for Assessing Fatty Acids: A Mini-Review. International Journal of Molecular Sciences, 21(16), 5695.

Clarke, T. C., Black, L. I., Stussman, B. J., Barnes, P. M., & Nahin, R. L. (2015). Trends in the use of complementary health approaches among adults: United States, 2002-2012. National health statistics reports, (79), 1–16.

Dingess, K. A., Valentine, C. J., Ollberding, N. J., Davidson, B. S., Woo, J. G., Summer, S., ... Morrow, A. L. (2017). Branched-chain fatty acid composition of human milk and the impact of maternal diet: The Global Exploration of Human Milk (GEHM) Study. The American Journal of Clinical Nutrition, 105(1), 177–184.

Fleming, J. A., & Kris-Etherton, P. M. (2014). The Evidence for α-Linolenic Acid and Cardiovascular Disease Benefits: Comparisons with Eicosapentaenoic Acid and Docosahexaenoic Acid. Advances in Nutrition, 5(6), 863S-876S.

Gozdzik, P., Magkos, F., Sledzinski, T., & Mika, A. (2023). Monomethyl branched-chain fatty acids: Health effects and biological mechanisms. Progress in Lipid Research, 90, 101226.

Hollowed, A. B., Barange, M., Beamish, R. J., Brander, K., Cochrane, K., Drinkwater, K., Foreman, M. G. G., Hare, J. A., Holt, J., Ito, S., Kim, S., King, J. R., Loeng, H., MacKenzie, B. R., Mueter, F. J., Okey, T. A., Peck, M. A., Radchenko, V. I., Rice, J. C., … Yamanaka, Y. (2013). Projected impacts of climate change on marine fish and fisheries. ICES Journal of Marine Science, 70(5), 1023–1037.

Hu, T., & Zhang, J.-L. (2018). Mass-Spectrometry-Based Lipidomics. Journal of Separation Science, 41, 351–372.

Iverson, S., Frost, K., & Lang, S. (2002). Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: Factors contributing to among and within species variability. Marine Ecology Progress Series, 241, 161–181.

Kapoor, B., Kapoor, D., Gautam, S., Singh, R., & Bhardwaj, S. (2021). Dietary Polyunsaturated Fatty Acids (PUFAs): Uses and Potential Health Benefits. Current Nutrition Reports, 10(3), 232–242.

Kuhnlein, H. V., & Chan, H. M. (1998). Ooligan grease: A traditional food fat of western Canada and Alaska. International Journal of Circumpolar Health, 57, 211–214.

Kuhnlein, H. V., Yeboah, F., Sedgemore, M., Sedgemore, S., & Chan, H. M. (1996). Nutritional qualities of ooligan grease: A traditional food fat of British Columbia First Nations. Journal of Food Composition and Analysis, 9, 18–31.

Kuhnlein, H. V., Chan, A. C., Thompson, J. N., & Nakai, S. (1982). Ooligan Grease: A Nutritious Fat Used by Native People of Coastal British Columbia. Journal of Ethnobiology, 2(2), 154–161.

Li, Q., Chen, J., Yu, X., & Gao, J.-M. (2019). A mini review of nervonic acid: Source, production, and biological functions. Food Chemistry, 301, 125286.

Lu, H., Wang, Z., Cao, B., Cong, F., Wang, X., & Wei, W. (2024). Dietary sources of branched-chain fatty acids and their biosynthesis, distribution, and nutritional properties. Food Chemistry, 431, 137158.

Mason, R. P., & Sherratt, S. C. R. (2017). Omega-3 fatty acid fish oil dietary supplements contain saturated fats and oxidized lipids that may interfere with their intended biological benefits. Biochemical and Biophysical Research Communications, 483(1), 425–429.

Microsoft Corporation. (2021). Microsoft Excel. Retrieved from https://office.microsoft.com/excel .

Nevigato, T., Masci, M., & Caproni, R. (2021). Quality of Fish-Oil-Based Dietary Supplements Available on the Italian Market: A Preliminary Study. Molecules, 26(16), 5015.

Patton, A. K., Martindale, A., Orchard, T. J., Vanier, S., & Coupland, G. (2019). Finding eulachon: The use and cultural importance of Thaleichthys pacificus on the northern Northwest Coast of North America. Journal of Archaeological Science: Reports, 23, 687–699.

Phinney, S. D., Wortman, J. A., & Bibus, D. (2009). Oolichan Grease: A Unique Marine Lipid and Dietary Staple of the North Pacific Coast. Lipids, 44(1), 47–51.

Ran-Ressler, R. R., Bae, S., Lawrence, P., Wang, D. H., & Brenna, J. T. (2014). Branched-chain fatty acid content of foods and estimated intake in the USA. The British Journal of Nutrition, 112(4), 565–572.

RStudio Team. (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston. Retrieved from http://www.rstudio.com/ .

Shahidi, F., & Ambigaipalan, P. (2018). Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits. Annual Review of Food Science and Technology, 9(1), 345–381.

Shibabaw, T. (2021). Omega-3 polyunsaturated fatty acids: Anti-inflammatory and anti-hypertriglyceridemia mechanisms in cardiovascular disease. Molecular and Cellular Biochemistry, 476(2), 993–1003.

Siriwardhana, N., Kalupahana, N. S., & Moustaid-Moussa, N. (2012). Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. Advances in food and nutrition research, 65, 211-222.

Stone, N. J., Robinson, J. G., Lichtenstein, A. H., Bairey Merz, C. N., Blum, C. B., Eckel, R. H., … Wilson, P. W. F. (2014). 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology, 63(25 Part B), 2889-2934.

Talloni-Álvarez, N. E., Sumaila, U. R., Le Billon, P., & Cheung, W. W. L. (2019). Climate change impact on Canada's Pacific marine ecosystem: The current state of knowledge. Marine Policy, 104, 163–176.

Tang, D., Chen, M., Huang, X., Zhang, G., Zeng, L., Zhang, G., Wu, S., & Wang, Y. (2023). SRplot: A free online platform for data visualization and graphing. PLoS One, 9, 18(11), e0294236.

U.S. Department of Agriculture (USDA), Agricultural Research Service, Beltsville Human Nutrition Research Center. (2019). FoodData Central, Fish, pollock, Alaska, cooked. Retrieved from https://fdc.nal.usda.gov/ .

Venn-Watson, S., & Schork, N. J. (2023). Pentadecanoic Acid (C15:0), an Essential Fatty Acid, Shares Clinically Relevant Cell-Based Activities with Leading Longevity-Enhancing Compounds. Nutrients, 15(21), 4607.

Wang, Z., Park, H. G., Wang, D. H., Kitano, R., Kothapalli, K. S. D., & Brenna, J. T. (2020). Fatty acid desaturase 2 (FADS2) but not FADS1 desaturates branched chain and odd chain saturated fatty acids. Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids, 1865(3), Article 158572.

Weatherdon, L. V., Ota, Y., Jones, M. C., Close, D. A., & Cheung, W. W. L. (2016). Projected Scenarios for Coastal First Nations' Fisheries Catch Potential under Climate Change: Management Challenges and Opportunities. PLOS ONE, 11(1), e0145285.

Xu, T., Hu, C., Xuan, Q., & Xu, G. (2020). Recent Advances in Analytical Strategies for Mass Spectrometry-Based Lipidomics. Analytica Chimica Acta, 1137, 156–169.

Yang, X., Karrar, E., Cong, F., Lu, H., Jin, Q., Xu, X., ... Wang, X. (2023). Identification and quantification of branched-chain fatty acids and odd-chain fatty acids of mammalian milk, dairy products, and vegetable oils using GC/TOF-MS. International Dairy Journal, 140, Article 105587.

4. Supplementary Fatty Acid Profile of Eulachon Grease Determined Using Liquid Chromatography-Mass Spectrometry

4.1 Introduction

The previous chapters of this thesis extensively detail the cultural, nutritional, and socioeconomic value of eulachon grease as a vital traditional food and medicine for BC coastal First Nations communities. Despite its significance, comprehensive studies on the FA profile of eulachon grease remain limited. Previous studies exploring the FA composition of eulachon grease use GC-MS methodologies and are more than 15 years old (e.g., Phinney et al., 2009; Iverson et al., 2002; Kuhnlein et al., 1996). In this thesis, the conventional technique (i.e., GC-MS) was used to evaluate the FA profile of eulachon grease (see Chapter 3). However, other chemical analysis techniques should be explored. Previous studies documenting the FA profile of eulachon grease did not use LC-MS techniques. Thus, this chapter uses LC-MS methodologies to investigate the FA profile of eulachon grease samples.

The eulachon grease samples analyzed in this chapter were collected from Knowledge Holders in Alert Bay, BC, in July 2023. The FA profile of ω-3/fish oil supplements available on the Canadian market will also be determined using the same LC-MS methodologies as the eulachon grease samples. To my knowledge, there are no studies that have measured the FA profile of ω-3/fish oil supplements available on the Canadian market methodologies despite their promotion for health and frequent public use (Bailey et al., 2013; Barnes et al., 2008).

This chapter describes the analysis of the FA profile of eulachon grease and commercially available ω-3 supplements using LC-MS methods. The identified FAs in eulachon grease and supplement samples will be presented as %FAs, and the three FA groups (i.e., PUFA,

MUFA, and SFA) levels will be stated and compared. The results of this chapter not only update the scientific understanding of eulachon grease and ω-3/fish oil supplement samples but also reinforce the cultural, nutritional, and ecological importance of preserving eulachon grease as an invaluable traditional food and medicine source.

4.2 Materials

4.2.1 Solvents and Reagents

Solvents such as MS-grade isopropanol, chloroform, and methanol, plus MS-water and ammonium acetate (eluent additive for LC-MS, 1 M) were purchased from Sigma-Aldrich (St. Louis, USA). Additionally, external FA standards such as caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid (LA), ALA, EPA, and DHA were purchased from Sigma-Aldrich (St. Louis, USA).

4.2.2 Eulachon Grease Samples

The same seven eulachon grease samples that had been collected from local Knowledge Holders in Alert Bay on the traditional territory of ‘Namgis First Nation on the coast of BC, in July 2023 (described in Table 2.1) and shipped frozen to Ottawa, Ontario were used again for the procedures outlined in this chapter. Each grease sample represents extract from approximately 600 lbs of fish (Kuhnlein & Chan, 1998; Kuhnlein et al., 1982). Duplicate analyses were completed on all the eulachon grease samples.

4.2.3 Supplement Samples

The same 10 bottles of ω-3/fish oil supplements from individual brands bought from the Canadian market in January 2023, as used in Chapter 3 (and described in Table 3.1), were used again for the procedures outlined in this chapter. All supplement samples were still within their stated shelf lives. Triplicate analyses were completed on all the supplement samples.

4.3 Methods

4.3.1 Sample Preparation

To prepare the supplements for lipid extraction, a sub-sample of 10 softgels were randomly taken out of the bottle with the intention of representing the contents of each bottle of ω-3/fish oil supplements. From each of the 10 softgels, 0.1 mL of fish/algal oil was extracted using Hamilton syringes and combined in a 2 mL amber glass vial. The samples were vortexed for 30 sec. This was repeated for each of the 10 supplement brands, except for sample 1, which is in liquid form. In this case, two sub-samples of 0.5 mL were removed from the bottle, placed in a 2 mL amber glass vial, and vortexed for 30 sec. From these sub-samples, 50 µL (4.25 mg) of each was transferred to its own 15 mL glass centrifuge vial.

For lipid extraction, the grease was thawed under controlled conditions and mixed well. Two sub-samples of 0.5 mL of each participant's preparation were removed from the container and combined into their own 2 mL amber glass vial. The samples were vortexed for 30 sec. Then, from these sub-samples of eulachon grease, 50 µL (4.25 mg) of each was transferred to its own 15 mL glass centrifuge vial.

Lipid extraction of the ω-3/fish oil supplements and eulachon grease was performed using the method described by Gowda with minor modifications (Gowda et al., 2022). First, 600 µL of chloroform was added to each of the 15 mL glass centrifuge vials containing the ω-3/fish oil supplements and the eulachon grease samples, and were vortexed for 2 min. This step was repeated, adding another 600 µL of chloroform to the 15 mL glass centrifuge vials containing the lipid extracts, and they were vortexed for another 2 min. Subsequently, 150 µL of MS-water was added to each lipid extract, and the samples were vortexed for 30 sec. The lipid extracts were centrifuged at 2500 rpm for 15 min. After centrifugation, 900 µL of the chloroform layer was

transferred to a new 2 mL amber glass vial. The aqueous layer was re-extracted with an additional 600 µL of chloroform. Again, after centrifugation, 400 µL of the chloroform layer containing the lipids was collected. The combined 1300 µL of lipid extraction and chloroform was placed in a centrifuge evaporator. Once the chloroform was evaporated (i.e., 36+ hrs), the remaining lipid extraction was dissolved in 100 µL of methanol, centrifuged, and transferred to an LC-MS vial.

4.3.2 Data Acquisition

LC-MS analysis was completed by the John L. Holmes Mass Spectrometry (JLHMS) Facility at the University of Ottawa, Ottawa, Ontario. All samples were analyzed by nanoLC coupled to the Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific, Waltham, USA). Chromatographic separation of metabolites was performed on a Proxeon EASY nLC 1000 System (Thermo Fisher Scientific, Waltham, USA) equipped with a Thermo Scientific™ Acclaim™ PepMap™ RSLC C18 column (P/N ES800A), 15 cm x 75 µm ID, 3 µm, 100 Å employing a water/methanol/isopropanol in presence of 1 mM ammonium acetate gradient. 1 µL of samples were loaded onto the column for 60 min at a flow rate of 0.16 µl/min. Compounds were separated using a linear gradient from 0 to 100 % of solvent A (35:35:30 % H2O:MeOH:isopropanol) and B (1:4:95 % H2O:MeOH:isopropanol) for 35 min, followed by washing 15 min with 100 % of solvent B, then using a gradient from 100 to 0 % of solvent B for 5 min and washing for 5 min at 100 % of solvent A. Eluted compounds were directly sprayed into mass spectrometer using negative electrospray ionization (ESI) at an ion source temperature of 250 °C and an ionspray (Thermo Scientific™ EASY spray, Waltham, USA) voltage of 2.1 kV. The FTMS scan type was full MS/data dependent (dd)-MS2. The parameters of the full mass scan were as follows: a resolution of 70,000, an auto gain control target under 3 × 106, a

maximum isolation time of 100 ms, and an m/z range of 110–1100. The parameters of the dd-MS2 scan were as follows: a resolution of 17,500, an auto gain control target under 1 × 105, a maximum isolation time of 100 ms, a loop count of top 10 peaks, an isolation window of m/z 2, a normalized collision energy of 35 and dynamic exclusion duration of 10 s. The LC-FTMS system was controlled using Xcalibur 4 software (Thermo Fisher Scientific, Waltham, USA), and data was collected with the same software.

4.3.3 Data Processing

Ten external FA standards (i.e., C6:0 (caproic acid), C8:0 (caprylic acid), C10:0 (capric acid), C12:0 (lauric acid), C14:0 (myristic acid), C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C18:3 (ALA), C20:5 (EPA), and C22:6 (DHA)) at 7 different concentrations (i.e., 0.2 µM, 1 µM, 2 µM, 5 µM, 10 µM, 25 µM, and 50 µM) were analysed via LC-MS. Based on these results, 4-7 points that best fit the linear regression were chosen to create calibration curves for each FA standard. In some cases, the higher concentrations resulted in a breakdown in the MS spectra, and these points were rejected. The calibration curves will be used as external FA standards in the identification of FAs in eulachon grease and supplement samples.

Targeted and untargeted analyses were used to identify the FAs present in the supplement and eulachon grease samples using LC-MS. For targeted analyses, a list of potential FAs was compiled based on their exact mass value using the LIPID MAPS® Structure Database (LMSD) (Fahy et al., 2007). Then, using Xcalibur 4 software (Thermo Fisher Scientific, Waltham, USA), that mass was searched, and the extracted ion chromatograms and MS spectra were illustrated. To be considered a "real" peak, the intensity signal of the peak had to read above 10^4, couldn't be too thin or too wide, and had to be substantially above the baseline. For peaks determined to

be “real”, the peak area and retention time was recorded in a Microsoft Excel spreadsheet for each FA of interest (when present) for the 10 supplement samples and 7 eulachon grease samples (and their replicates). The identification of lipid molecular species was confirmed by comparing their MS2 spectra with known MS2 spectra available from online databases (e.g., the MassBank (Horai et al., 2010), the NIST Chemistry WebBook (Linstrom & Mallard, 2024), and the Human Metabolome Database (Wishart et al., 2022)) and (when possible) the external standards. For untargeted analyses, the raw MS data obtained was processed for peak-picking, peak alignment, noise subtraction, and identification of FA structures using MSDial software (Tsugawa et al., 2015).

After the targeted and untargeted analyses were complete and the Excel spreadsheet was compiled, the same data clean-up protocols explained in Chapter 3 for the GC-MS data were followed. Namely, if one of the two peak areas was recorded for the duplicate runs of the eulachon grease samples, the second replicate was changed to a zero for that FA identification. Plus, if three of seven eulachon grease samples had zeros as their peak areas for a given FA, it was determined that that FA did not actually exist in the samples, and it was removed from the dataset. Again, a similar process was followed for the supplement samples, if two of three replicates had peak areas of zero, the third replicate was changed to a zero for that FA identification. Additionally, if five (or more) of ten supplement samples had zeros as their peak areas, it was determined that that FA did not actually exist in the samples, and it was removed from the dataset.

Unfortunately, through the untargeted and targeted analyses, it became apparent that some level of carryover from one sample to the next had occurred. In essence, before any samples were run in the LC-MS machine, a blank sample was run, then the eulachon or

supplement samples were run through the machine, and at the end, a second blank sample was run. In the end blank, often, there was a peak present, and in some cases, it was of a greater intensity than what was present in the samples. To assess the magnitude of this carryover, the peak area measured for each FA in the final blank sample was divided by the peak area that had been measured for each FA in each eulachon grease or supplement sample. The quotient was multiplied by 100 to obtain a "percent carryover" (%C) for each FA measurement of each sample ran.

4.3.4 Statistical Analyses

The respective FA concentrations for each supplement sample was presented as the mean of the peak areas from the triplicate runs. Subsequently, using the mean of the peak areas from the triplicate runs for supplements, the sum of all means for a given sample was calculated. Then, the %FA was calculated for each FA in each sample by dividing the mean peak area by the sum of all the peak areas for a given sample. The quotient was then multiplied by 100 to obtain the %FA for each FA in each sample. The same process was followed with the duplicate runs for the eulachon grease samples, to obtain the %FA for eulachon grease samples.

Once the %FA was determined for each FA and each sample for eulachon grease and supplements, the FAs identified were separated into three groups: MUFAs, PUFAs, and SFAs for eulachon grease and supplement samples, respectively. The sum of all the %FAs categorized as PUFA and SFA was calculated for each for eulachon grease and supplement samples, respectively. Then, the sum of the PUFAs was divided by the sum of the SFAs to determine the P:S ratio for eulachon grease and supplement sample.

To study the differences within and between the supplement and eulachon grease samples, the peak area data was subjected to PCA and cluster heatmap analyses using SRplot

(Tang et al., 2023). Venn diagrams were made using SRplot (Tang et al., 2023) and any graphical visualizations (e.g., pie charts, scatter plots, and bar charts) were made in Microsoft Excel (Microsoft Corporation, 2021) using the %FA data.

4.4 Results and Discussion

4.4.1 LC-MS Carryover Contamination (%C)

As mentioned in the Data processing section (4.3.3), through the LC-MS analyses, there was a level of carryover that occurred between eulachon grease samples and supplement samples, respectively. Thus, Table 4.1 depicts the %C for eulachon grease samples and Table 4.2 states the %C for supplement samples.

In Table 4.1, there are 12 FAs across the seven eulachon grease samples that have an acceptable amount of crossover (i.e., <5%) and 17 FAs across the seven eulachon grease samples that have an unacceptable amount of crossover (i.e., >5%). Evidently, over half (17/29) of the eulachon grease samples were contaminated with crossover from previous samples. Furthermore, in Table 4.2, there are 20 FAs across the 10 supplement samples that have an acceptable amount of carryover (<5%) and 7 FAs across the 10 supplement samples that have an unacceptable amount of carryover (>5%).

Table 4.1 – LC-MS crossover contamination table (%C) for eulachon grease samples. This table shows the amount of crossover in the control (or blank), which was run after all eulachon grease samples. This was calculated by dividing the peak area of the control for each FA by the corresponding FA's peak area in each eulachon grease sample, then multiplying the quotient by 100. It was determined that 5% would be an acceptable/negligeable amount of contamination. Cells in the table with N/A mean that there was no peak in the control for that FA.

FAs	1	2	3	4	5	6	7
C14:0	419.50	237.00	817.91	529.71	346.98	448.69	150.05
C14:1	239.37	135.42	299.99	229.37	322.71	429.51	166.38
C15:0	1826.75	876.45	1997.96	2157.49	2526.71	2738.85	1708.35
C16:0	206.79	173.44	362.79	333.86	1720.39	305.39	243.16
C16:1	68.40	45.95	92.66	62.65	81.59	113.85	54.43
C16:2	10.08	5.96	15.42	8.57	12.97	22.29	7.41
C16:3	0.76	0.53	1.53	0.64	0.98	2.23	0.59
C16:4	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C17:0	1207.82	769.71	1418.49	1431.99	1735.46	1740.65	1446.02
C17:1	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C18:0	534.01	544.93	937.83	841.62	1266.29	1074.12	1016.41
C18:1	12.24	9.22	16.41	12.24	16.91	19.72	12.37
C18:2	40.96	21.31	46.45	30.92	34.62	42.07	27.96
C18:3	8.33	5.52	10.61	7.47	8.15	9.63	8.15
C18:4	1.72	1.11	2.43	1.40	1.99	2.28	1.57
C19:0	127.55	41.65	85.94	117.40	144.22	92.21	66.06
C19:1	181.67	108.65	198.76	173.19	228.40	232.83	141.32
C20:1	18.04	12.41	20.59	14.94	21.87	23.87	11.82
C20:2	15.60	14.40	14.69	11.69	13.87	26.14	20.76
C20:3	0.98	0.74	1.06	0.97	0.99	1.93	1.74
C20:4	3.07	1.55	1.83	1.71	1.68	1.56	2.23
C20:5	0.22	0.18	0.18	0.20	0.22	0.16	0.38
C20:6	3.55	3.06	4.50	4.89	6.72	8.25	7.06
C21:5	1.03	0.59	0.69	0.61	0.74	0.58	0.99
C22:1	7.59	5.29	9.87	5.70	8.12	9.28	3.22
C22:4	0.96	0.68	1.39	0.71	0.83	1.54	1.21
C22:5	0.02	0.01	0.02	0.02	0.02	0.03	0.03
C22:6	0.18	0.17	0.14	0.16	0.13	0.10	0.24
C24:1	4.56	3.01	7.29	4.16	5.12	6.97	4.29

Table 4.2 – LC-MS crossover contamination table (%C) for supplement samples. This table shows the amount of crossover in the control (or blank), which was run after all supplement samples. This was calculated by dividing the peak area of the control for each FA by the corresponding FA's peak area in each supplement sample, then multiplying the quotient by 100. It was determined that 5% would be an acceptable/negligeable amount of contamination. Cells in the table with N/A mean that there was either no peak in the control or no peak in the sample for that FA.

FAs	1	2	3	4	5	6	7	8	9	10
C9:0	1.67	2.62	2.56	5.24	1.97	1.93	1.68	2.47	1.38	1.87
C11:0	246.23	278.05	185.45	134.82	239.69	559.80	121.64	139.29	172.40	140.01
C14:0	145.78	33.99	169.92	170.87	114.19	55.56	62.19	147.93	9.98	104.91
C14:1	16.10	0.41	1.36	5.19	4.17	3.00	2.20	4.15	0.38	4.51
C15:0	104.39	12.37	35.44	54.48	57.02	80.71	32.83	61.22	17.24	58.49
C16:0	112.45	52.29	71.28	68.07	121.24	140.93	43.00	99.64	63.56	117.20
C16:1	4.12	0.41	0.40	2.98	6.10	6.27	1.38	2.32	0.14	3.71
C16:2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C16:3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C16:4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C17:0	45.73	16.82	23.40	28.73	31.72	38.45	18.30	34.82	10.89	29.14
C17:1	9.66	0.86	1.86	5.53	9.02	7.88	3.57	6.85	0.18	7.82
C18:0	101.82	67.13	72.90	63.70	103.02	106.92	47.56	100.94	86.67	92.29
C18:1	7.92	1.35	1.41	2.41	11.49	2.97	2.04	6.88	0.30	6.72
C18:2	3.75	0.69	0.65	1.59	7.40	0.16	0.98	5.58	0.20	5.71
C18:3	0.03	0.01	0.01	0.01	0.22	0.00	0.01	0.03	0.00	0.05
C18:4	0.04	0.02	0.01	0.01	N/A	N/A	0.01	0.03	0.00	0.10
C19:1	6.50	0.94	1.10	2.58	9.22	7.41	1.91	6.09	0.23	5.16
C20:0	11.75	4.50	8.91	6.40	11.69	10.81	5.03	13.39	10.75	12.62
C20:1	0.30	0.06	0.05	0.12	3.51	1.32	0.09	0.51	0.01	0.08
C20:2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C20:4	0.10	0.04	0.03	0.04	5.88	4.57	0.05	0.34	0.01	0.43
C20:5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C21:5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C22:1	0.68	0.29	0.14	0.84	N/A	7.75	0.39	1.02	0.04	0.20
C22:5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C22:6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

20 of 27 FAs with an acceptable amount of carryover in the supplement samples is a better result than with the eulachon grease samples. This can be explained by the differences in processing between eulachon grease and supplement samples. Eulachon grease is extracted by cooking the eulachon in moderately high-temperature water, while fish oils for supplements are extracted using acids, enzymes, bacteria or other solvents (Bonilla-Méndez & Hoyos-Concha, 2018). Additionally, eulachon grease is purified using various types of cloths (i.e., towels, bed sheets, cheesecloth, etc.), whereas supplement samples are purified using industrial techniques (e.g., degumming, neutralization, bleaching, and deodorization) (Bonilla-Méndez & Hoyos-Concha, 2018). These differences in processing techniques result in eulachon grease that is thick (viscous) and slightly cloudy with small pieces of fish while the fish oil in supplements is very thin (non-viscous) and clear. Thus, the non-viscous fish oil in supplements likely flowed better through the column of the LC-MS machine, getting caught up less often, while the opposite was true for eulachon grease. Nevertheless, in both the eulachon grease and supplement samples, some FAs recorded a higher area in the control/blank sample than in the eulachon grease or supplement samples (i.e., 100%+ carryover). For these reasons, we couldn't confidently accept these results.

There are several explanations for the high level of carryover; changes to the methodologies could be made to make the LC-MS analysis of fish oils more successful. For example, 1 μL of each eulachon grease and supplement sample was injected into the nanoLC; considering the complex mix of highly lipophilic FAs, triglycerides, and other nonpolar compounds that are present in eulachon grease and supplement samples, it is possible that the samples should have been further diluted before being injected into the column. The column could have been overloaded with FAs, leading to the incomplete separation and retention of FAs

on the C18 stationary phase, which can result in the incomplete elution of these compounds during a 60-minute run, causing carryover into subsequent injections. Furthermore, FAs and other lipophilic compounds could adsorb into parts of the LC system (e.g., tubing, injection needle, autosampler), especially in larger volumes, causing contamination between runs. Cleaning the autosampler and needle more frequently could help prevent carryover between injections.

Another factor that could have led to the high level of carryover is the gradient solvent system used in the methods. The gradient solvent system was a combination of water, methanol, and isopropanol with 1 mM ammonium acetate. This combination is generally suitable for FA analysis however, long-chain unsaturated FAs may require stronger elution conditions. In this case, isopropanol may still have left residual FAs or complex lipids on the column if the gradient wasn't optimized for complete desorption. Plus, The use of 35:35:30 water:methanol:isopropanol may not have been aggressive enough at the start of the run, causing some FAs to elute too slowly or persist on the column, leading to carryover. Additionally, the length of the gradient (i.e., 35 minutes) may not have been sufficient to fully elute all FAs and the re-equilibration phase (i.e., the 5-minute gradient from 100% solvent B back to 0% B (solvent A) and the 5-minute wash at 100% solvent A) may have also been insufficient to re-equilibrate the column which could leave residual FAs trapped on the column, contributing to carryover. The gradient solvent system should be optimized to include more aggressive elution and longer gradient/re-equilibration phases to reduce the level of carryover between samples. These changes would particularly benefit the more hydrophobic FAs that were present in the eulachon grease and supplement samples.

Lastly, while the method includes a 15-minute wash phase with 100% solvent B (i.e., 95% isopropanol), followed by re-equilibration, the wash phase might not have been long enough or aggressive enough to completely clean the column. More time and/or a stronger wash solvent (e.g., use higher concentrations of isopropanol in the wash solvent or use higher concentrations of chloroform during the sample preparation) might be necessary to fully remove residual FAs, especially those with longer chains or higher degrees of unsaturation, to avoid carryover.

4.4.2 Identification and Quantification of the Fatty Acids in Eulachon Grease and Supplement Samples

Initially, there were 59 FAs identified in the eulachon grease samples using LC-MS methodologies. However, many of these FAs were present in very small quantities and were likely misidentified or not actually present in the samples. Thus, any FAs that were present in less than 0.10% were omitted. In the end, 29 FAs were identified in the eulachon grease samples which are listed in Table 4.3, along with the %FA of each FA for each sample and the AVG %FA of all samples. Overall, the five most abundant FAs were C18:1 (oleic acid) (28.87%), C16:0 (palmitic acid) (15.69%), C16:1 (10.94%) (palmitoleic acid), C22:6 (DHA) (7.35%), and C20:5 (EPA) (7.08%). Neither BCFAs nor isomers could be distinguished in this case, using the LC-MS methodologies.

Of the 29 FAs, 15 were PUFAs, eight were MUFAs, and six were SFAs; the percentage of each group (i.e., PUFA, MUFA, and SFA) for each sample as well as the AVG of all samples is listed in Table 4.4. Based on the overall AVGs, eulachon grease is made up mostly of MUFAs (42.03%), then SFAs (29.00%), and followed very closely by PUFAs (28.97%). Diets high in MUFAs can provide anti-inflammatory effects (Farag & Gad, 2022; Yang et al., 2020; García,

2019). Unlike with the GC-MS methodologies, there are no outliers in the eulachon grease samples in this case. The carryover may have masked any potential differences that could have existed/been reported among eulachon grease samples. Due to the lack of differentiation in the FA profiles across eulachon grease samples using LC-MS methods, it is impossible to draw meaningful conclusions between the preparation techniques and the FA profiles.

Table 4.3 – Percent fatty acid (%FA) for eulachon grease samples. Each sample is the AVG of the duplicate runs. The %FA was calculated by dividing the peak area of each FA from each sample by the total of the peak area for each sample, respectively. The quotient was then multiplied by 100. The first column (ID) contains FA identification, and the final column (AVG) contains the average %FA for each FA from all samples. The peak areas were obtained using LC-MS methodologies. Eulachon grease samples were collected in Alert Bay, BC from participating Knowledge Holders from 'Namgis First Nation in July 2023.

ID	1	2	3	4	5	6	7	AVG
C14:0	5.08	7.33	2.97	3.65	6.86	5.25	11.91	6.15
C14:1	0.39	0.48	0.41	0.41	0.36	0.27	0.53	0.41
C15:0	0.77	1.16	0.98	0.69	0.75	0.66	0.80	0.83
C16:0	17.76	18.54	15.17	13.55	10.05	17.84	16.94	15.69
C16:1	11.01	11.26	10.15	12.00	11.35	8.06	12.74	10.94
C16:2	1.53	1.57	1.10	1.59	1.29	0.75	1.71	1.36
C16:3	0.75	0.73	0.46	0.89	0.71	0.31	0.88	0.67
C16:4	0.36	0.33	0.21	0.45	0.40	0.16	0.60	0.36
C17:0	0.47	0.58	0.57	0.46	0.48	0.46	0.44	0.49
C17:1	0.44	0.60	0.36	0.47	0.68	0.60	0.54	0.53
C18:0	7.90	5.97	6.12	5.68	4.62	5.57	4.31	5.74
C18:1	28.89	29.26	28.86	31.95	28.62	24.31	30.18	28.87
C18:2	1.69	2.08	1.70	2.07	2.28	1.91	2.16	1.98
C18:3	0.69	0.78	0.71	0.82	0.93	0.77	0.69	0.77
C18:4	0.71	0.67	0.55	0.77	0.67	0.58	0.64	0.66
C19:0	0.06	0.14	0.12	0.07	0.07	0.11	0.13	0.10
C19:1	0.12	0.15	0.14	0.14	0.13	0.12	0.17	0.14
C20:1	0.66	0.68	0.72	0.82	0.72	0.63	0.99	0.74
C20:2	0.58	0.44	0.77	0.80	0.89	0.44	0.50	0.63
C20:3	2.52	2.48	3.04	2.75	3.32	1.69	1.47	2.47
C20:4	1.76	1.65	2.45	2.12	2.64	2.82	1.52	2.14
C20:5	6.47	5.18	9.23	6.95	7.74	10.67	3.33	7.08
C20:6	0.27	0.26	0.31	0.24	0.21	0.18	0.15	0.23
C21:5	0.28	0.25	0.39	0.36	0.37	0.46	0.21	0.33
C22:1	0.21	0.22	0.21	0.30	0.27	0.22	0.51	0.28
C22:4	0.75	0.73	0.63	1.03	1.10	0.58	0.60	0.77
C22:5	2.13	1.92	2.75	2.32	3.01	1.79	1.20	2.16
C22:6	5.64	4.43	8.81	6.51	9.34	12.69	4.00	7.35
C24:1	0.12	0.14	0.10	0.14	0.15	0.10	0.14	0.13

Table 4.4 – Percentage of each FA group (i.e., PUFA, MUFA, and SFA) in each eulachon grease sample as well as the total average of each group for all samples (final column - AVG). This was calculated by summing the individual %FAs based on the group they belong to for each sample. Eulachon grease samples were collected in Alert Bay, BC from participating Knowledge Holders from 'Namgis First Nation in July 2023.

FA Group	1	2	3	4	5	6	7	AVG
PUFA	26.13	23.51	33.13	29.67	34.89	35.79	19.67	28.97
MUFA	41.84	42.78	40.94	46.23	42.28	34.32	45.79	42.03
SFA	32.03	33.71	25.93	24.10	22.83	29.89	34.54	29.00

Initially, there were 42 FAs identified in the supplement samples using LC-MS methodologies. However, many of these FAs were present in very small quantities, making the identification of these FAs in the samples difficult. Therefore, any FAs that were present in less than 0.10% were omitted. In the end, 27 FAs were identified in the supplement samples, which are listed in Table 4.5, along with the %FA of each FA for each sample and the AVG %FA of all samples. Overall, the five most abundant FAs were C16:0 (palmitic acid) (51.52%), C18:0 (stearic acid) (15.61%), C20:5 (EPA) (7.62%), C18:1 (oleic acid) (4.98%), and C22:6 (DHA) (3.97%). Again, BCFAs and isomers could not be distinguished with the current LC-MS methodologies.

Of the 27 FAs, 12 were PUFAs, seven were MUFAs, and eight were SFAs; the percentage of each group (i.e., PUFA, MUFA, and SFA) for each sample as well as the AVG of all samples is listed in Table 4.6. Based on the overall AVGs, the ω-3/fish oil supplement samples are made up mostly of SFAs (73.69%), followed by PUFAs (16.29%), and lastly MUFAs (10.02%). Diets high in SFAs are associated with an increased risk for CVD (Stone et al., 2014).

Unlike the results when using GC-MS methodologies, sample 6 was not an outlier in this case despite it being made of algal oil, not fish oil. Thus, it was included in all the following analyses/results. The supplement samples all follow a similar distribution of PUFAs, MUFAs,

and SFAs (i.e., no outliers) except for sample 9. Sample 9 has a relatively high concentration of PUFAs (41.80%) and low amount of SFA (35.72%) compared to the overall AVG (16.29% and 73.69%, respectively) or the other samples. There is no logical explanation for sample 9 being an outlier in this case, but one potential reason is that it is the only supplement sample made from salmon. The problem with the carryover is that it may have masked potential differences that could have existed/been reported among the supplement samples.

Table 4.5 – Percent fatty acid (%FA) in supplement samples, each sample is the AVG of the triplicate runs. The %FA was calculated by dividing the peak area of each FA from each sample by the total of the peak area for each sample, respectively. The quotient was then multiplied by 100. The first column (ID) contains the FA identification, and the final column (AVG) contains the average %FA for each FA from all samples. The peak areas were obtained using LC-MS methodologies. Supplement samples were bought from common Canadian retailers, and all were within their stated shelf lives.

IDs	1	2	3	4	5	6	7	8	9	10	AVG
C9:0	3.44	1.01	1.90	0.67	2.79	2.77	1.58	1.42	1.28	3.58	2.04
C11:0	0.25	0.10	0.14	0.26	0.29	0.27	0.19	0.28	0.08	0.43	0.23
C14:0	1.18	3.04	0.97	0.65	1.64	2.74	1.26	2.65	4.08	1.80	2.00
C14:1	0.03	0.70	0.19	0.06	0.11	0.08	0.09	1.28	0.16	0.06	0.27
C15:0	0.54	3.08	0.82	0.76	1.19	0.66	0.82	4.39	0.77	0.97	1.40
C16:0	56.54	46.90	42.31	59.27	66.42	52.28	60.41	47.93	23.83	59.33	51.52
C16:1	0.81	6.67	4.11	0.96	1.29	0.57	1.41	10.78	6.16	0.99	3.37
C16:2	0.07	0.17	0.35	0.06	0.00	0.00	0.09	0.27	0.31	0.01	0.13
C16:3	0.17	0.19	0.40	0.04	0.00	0.03	0.11	0.10	0.18	0.00	0.12
C16:4	0.23	0.20	0.67	0.11	0.00	0.00	0.24	0.28	0.34	0.02	0.21
C17:0	0.51	0.69	0.43	0.58	0.86	0.59	0.62	1.31	0.52	0.84	0.70
C17:1	0.11	1.36	0.52	0.21	0.31	0.14	0.25	1.64	1.46	0.17	0.62
C18:0	17.85	10.49	13.12	18.32	21.44	18.54	15.91	13.76	5.10	21.54	15.61
C18:1	1.87	6.05	4.91	3.86	1.99	4.67	4.29	7.53	12.22	2.38	4.98
C18:2	0.42	1.24	1.11	0.63	0.38	9.64	1.00	0.88	1.91	0.29	1.75
C18:3	0.20	0.28	0.38	0.24	0.06	2.19	0.29	0.20	1.28	0.11	0.52
C18:4	0.32	0.31	0.77	0.69	0.00	0.00	0.39	0.28	1.58	0.15	0.45
C19:1	0.03	0.15	0.10	0.05	0.04	0.03	0.06	0.25	0.22	0.04	0.10
C20:0	0.20	0.21	0.15	0.23	0.23	0.21	0.20	0.20	0.05	0.20	0.19
C20:1	0.23	0.51	0.57	0.36	0.04	0.05	0.32	0.30	1.68	0.97	0.50
C20:2	0.13	0.33	0.33	0.16	0.05	0.03	0.15	0.37	0.39	0.08	0.20
C20:4	0.75	0.81	1.28	1.08	0.01	0.02	0.61	0.15	2.71	0.19	0.76
C20:5	8.78	11.71	16.37	8.00	0.69	0.07	6.55	2.36	18.52	3.12	7.62
C21:5	0.52	0.65	1.34	0.25	0.02	0.00	0.38	0.09	0.38	0.14	0.38
C22:1	0.12	0.12	0.27	0.06	0.00	0.01	0.09	0.08	0.57	0.48	0.18
C22:5	0.18	0.08	0.21	0.10	0.00	0.77	0.09	0.05	0.22	0.04	0.17
C22:6	4.54	2.98	6.28	2.34	0.16	3.65	2.59	1.15	13.98	2.06	3.97

Table 4.6 – Percentage of each FA group (i.e., PUFA, MUFA, and SFA) in each supplement sample as well as the total AVG of each group for all samples (final column – AVG). This was calculated by summing the individual %FAs based on the group they belong to for each sample. Supplement samples were bought from common Canadian retailers, and all were within their stated shelf lives.

FA Group	1	2	3	4	5	6	7	8	9	10	AVG
PUFA	16.30	18.92	29.48	13.68	1.38	16.40	12.49	6.20	41.80	6.21	16.29
MUFA	3.20	15.56	10.67	5.57	3.77	5.54	6.51	21.87	22.48	5.09	10.02
SFA	80.50	65.52	59.85	80.75	94.85	78.06	81.00	71.73	35.72	88.70	73.69

4.4.3 Comparing the LC-MS Results of Eulachon Grease and Supplement Samples

Using LC-MS methodologies, the FAs identified in eulachon grease and supplement samples were mostly the same. Eulachon grease and supplement samples had 24 FAs in common, five FAs were unique to eulachon grease samples and three FAs were unique to supplement samples. The 24 FAs they have in common are: C14:0, C14:1, C15:0, C16:0, C16:1, C16:2, C16:3, C16:4, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C18:4, C19:1, C20:1, C20:2, C20:4, C20:5, C21:5, C22:1, C22:5, C22:6. The five FAs unique to eulachon grease samples are: C19:0, C20:3, C20:6, C22:4, and C24:1. The three FAs that were unique to supplement samples are: C9:0, C11:0, and C20:0.

The 5 FAs identified in this study that are unique to eulachon grease samples could provide exclusive health benefits. For example, C20:6 or gamma-linoleic acid (GLA). C20:6 is used mainly for its anti-inflammatory effects and a reduction in inflammation can have positive effects on several diseases/conditions (e.g., rheumatoid arthritis, eczema, xeroderma, etc.) ((Van Hoorn et al., 2008). C20:6 is also shown to stimulate apoptosis of cancer cells (without affecting healthy cells) and to increase the efficacy of anticancer agents (Van Hoorn et al., 2008). Furthermore, as mentioned in section 3.4.3, C24:1 was only found in eulachon grease samples, not the supplement samples (using both GC-MS and LC-MS). C24:1 is closely associated with the development and maintenance of the brain and the biosynthesis and improvement of nerve

cells thus, it can be effective in the treatment of neurological diseases (Li et al., 2019). Evidently, the FAs identified in this study that are unique to the supplement samples may also provide exclusive health benefits. However, the three FAs unique to supplement samples are SFAs which typically result in negative health effects (Stone et al., 2014).

The eulachon grease samples had three PUFAs, one MUFA, and one SFA that were not present in the supplement samples whereas, the supplements had simply three SFAs that were not identified in eulachon grease samples. The additional PUFAs and MUFA in eulachon grease samples and SFAs in supplement samples are apparent when the AVG of each FA group (i.e., PUFA, MUFA, and SFA) for all eulachon grease and supplement samples, respectively are compared (Figure 4.1). Eulachon grease samples are made up of an AVG of 28.97% PUFA and 42.03% MUFA while supplements AVG 16.29% PUFA and 10.02% MUFA. In contrast, supplement samples are made up of an AVG of 73.69% SFA while eulachon grease samples AVG 29.00% SFA.

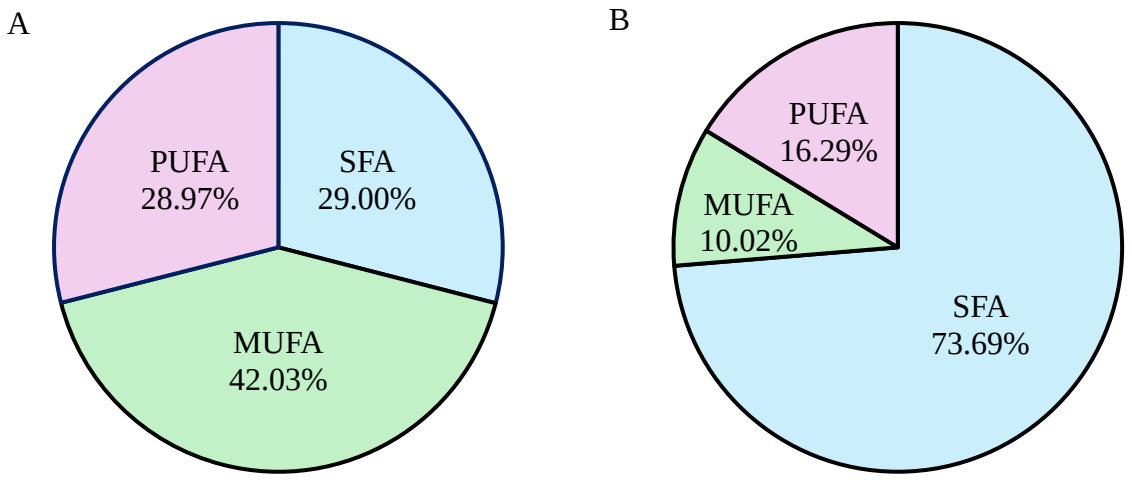

Figure 4.1 – (A) Percentage of each group of FAs (SFA, MUFA, and PUFA) in eulachon grease samples using LC-MS methodologies; (B) Percentage of each group of FAs (SFA, MUFA, and PUFA) in supplement samples using LC-MS methodologies.

PCA results (Figure 4.2) (PC1-58.4% and PC2-16.4%) help to better visualize the differences between the eulachon grease and supplement samples. Similar to the individual results, there are no outliers with the eulachon grease samples, all samples and their replicates are grouped quite closely together in the middle-bottom of the chart in red. In contrast, the supplement samples and their replicates are grouped in the middle right in blue, except sample 9 and its replicates which are grouped in the bottom right corner and were identified as outliers in the individual results. Despite supplement sample 9 being an outlier, the rest of the supplement samples and all eulachon grease samples form distinct groups.

The heatmap (Figure 4.3) echoes the results of the PCA. The top bar of the heatmap shows that the eulachon grease samples are all grouped together on the right in blue, and the supplement samples are grouped together on the left in red, with sample 9 on the far right of the supplement sample group. It is possible to use the right-hand y-axis on the heatmap to explore which FAs are present in higher or lower concentrations between eulachon grease and supplement samples. Thus, what seems to distinguish the eulachon grease samples from the supplement samples is their higher concentrations of PUFAs such as C20:3, C20:6, and C22:5 and MUFAs like C16:1, C18:1 and C24:1. Whereas, the supplement samples seem to distinguish themselves from the eulachon grease samples due to their high concentrations of C9:0, C11:0, and C20:0 which were not identified in eulachon grease samples. Additionally, supplement sample 9 distinguishes itself from the rest of the supplement samples due to its high concentrations of FAs such as C17:1, C20:1, and C22:6. Supplement sample 9 is also distinguishable from the eulachon grease samples due to its low concentrations of FAs like C20:3, C20:6, and C22:5.

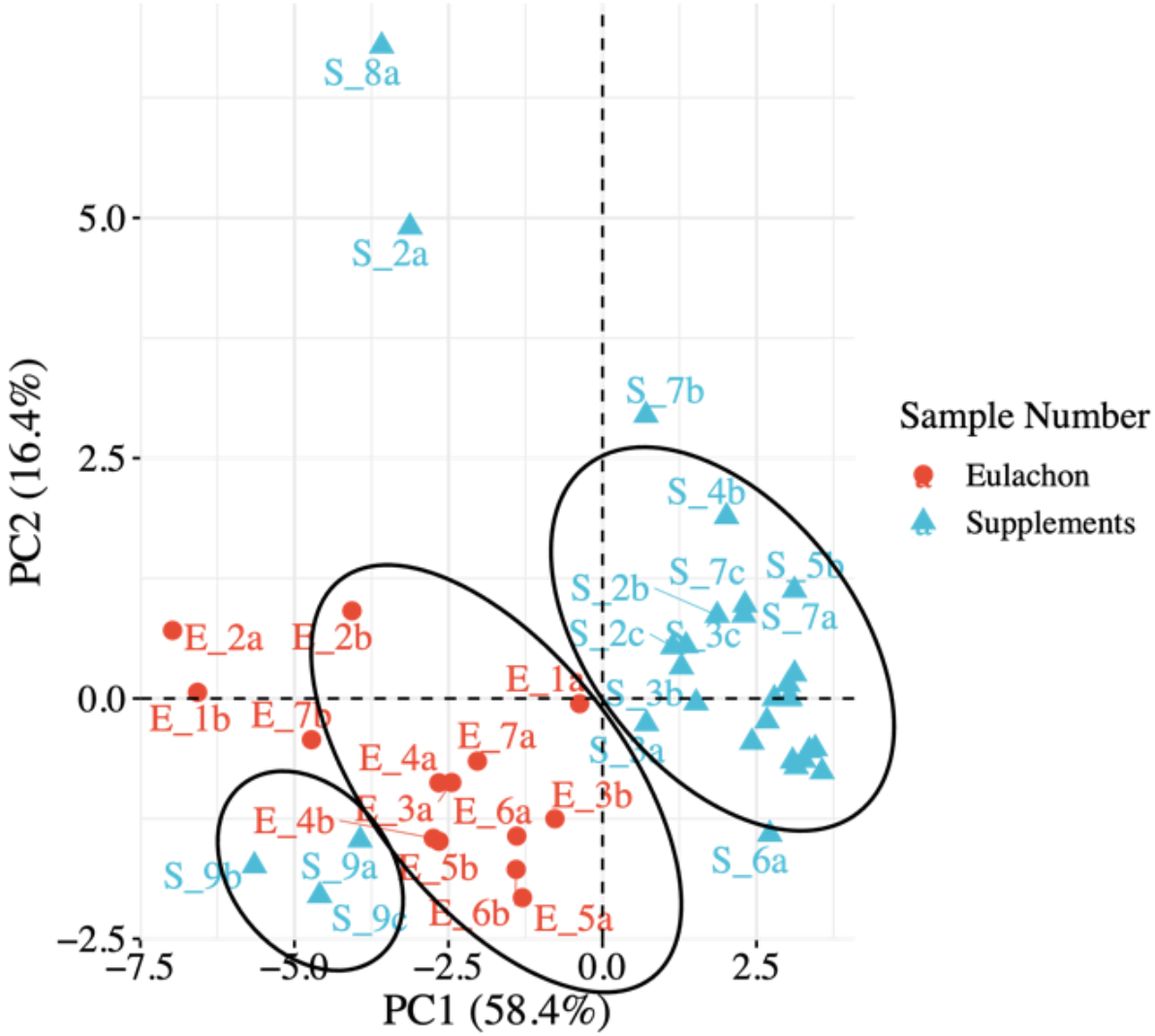

Figure 4.2 – PCA that shows the two replicates (a and b) for each eulachon grease sample (1, 2, 3…) (n=14) (red) as well as the three replicates (a, b, c) for each supplement sample (1, 2, 3…) (n=30) (blue) using the variable peak area and LC methodologies. (Supplement sample 6 included as it wasn't an outlier using LC methodologies, despite it being algal and not fish oil.)

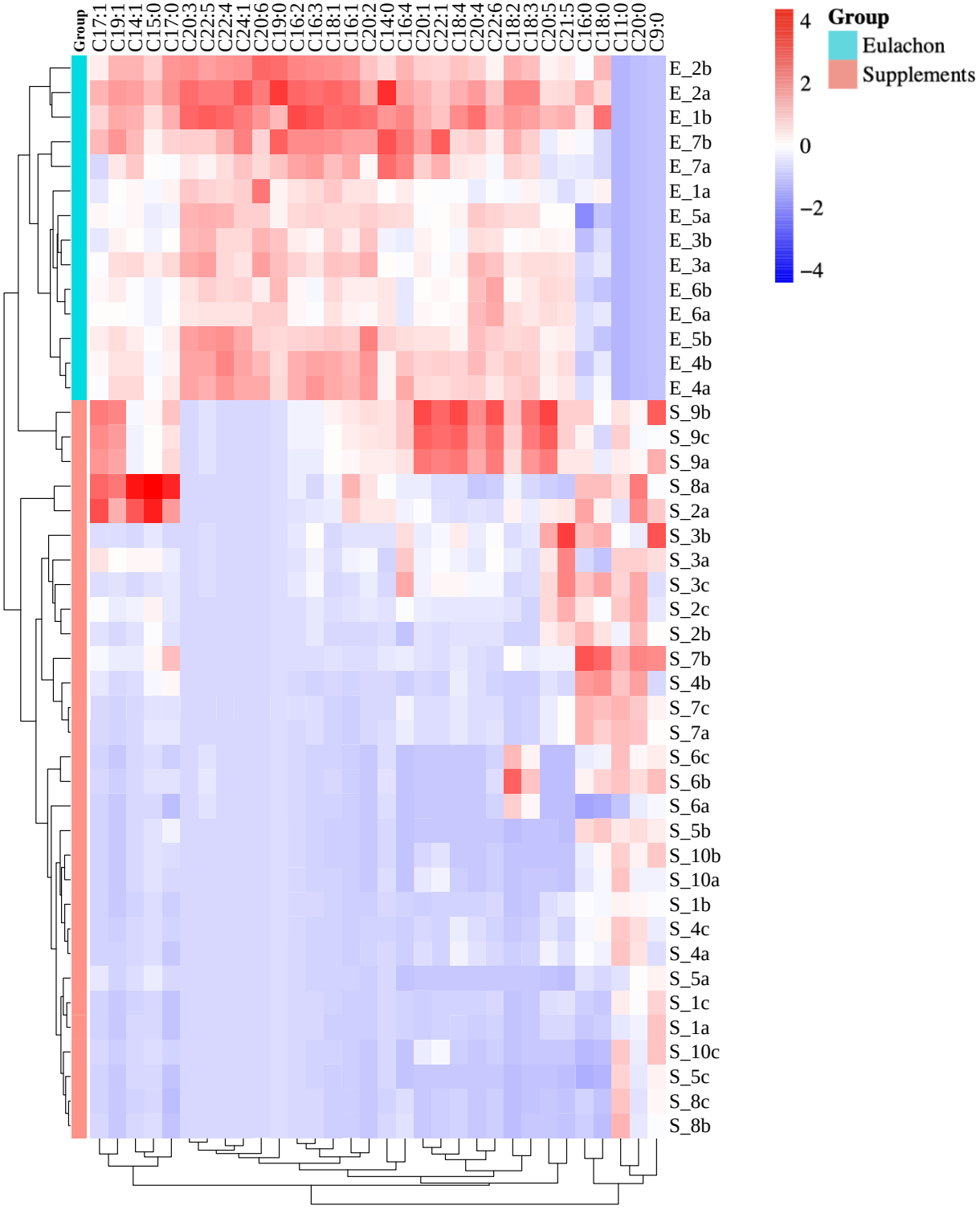

Figure 4.3 – Heatmap that compares the concentration of each FA (n=32) identified across the two replicates (a and b) for each of the seven eulachon grease samples (1, 2, 3…) (n=14) (blue) as well as the three replicates for the ten supplement samples (1, 2, 3…) (n=30) (red) using the variable peak area and LC-MS methodologies. (Supplement sample 6 included despite it not being fish oil as it was not an outlier when using LC-MS methodologies.)

Lastly, the P:S ratio for each individual eulachon grease and supplement sample was calculated. The P:S ratio is the ratio of PUFAs to SFAs and is the most used index for evaluating the nutritional value of dietary foods (Chen & Liu, 2020). The P:S ratio is normally used to assess the impact of diet on CVH (Chen & Liu, 2020). The P:S ratio hypothesizes that all PUFAs in the diet can depress LDL-C and lower levels of serum cholesterol, whereas all SFAs contribute to high levels of serum cholesterol (Chen & Liu, 2020). Thus, the higher this ratio, the more positive the effect. In Western diets, the AVG ratio is 0.6; it is suggested that increasing it to near 1.0 would reduce the risk of atherosclerosis and coronary heart disease (Bender, 2009). The results of the P:S ratio calculations for individual eulachon grease and supplement samples are presented in Figures 4.4 and 4.5, respectively. The P:S ratio of the seven eulachon grease samples averages 1.05. Eulachon grease samples have a higher P:S ratio and would likely contribute to benefits in CVH, such as depressing LDL-C and lowering levels of serum cholesterol (Chen & Liu, 2020). In comparison, the P:S ratio of supplement samples is lower, averaging 0.29 across the 10 samples. Apart from sample 9, which inexplicably recorded a high P:S ratio of 1.17, the generally low P:S ratio across the other 9 supplement samples (even the vegan sample 6) could increase serum cholesterol levels and increase the risk of heart disease (Chen & Liu, 2020; Bender, 2009).

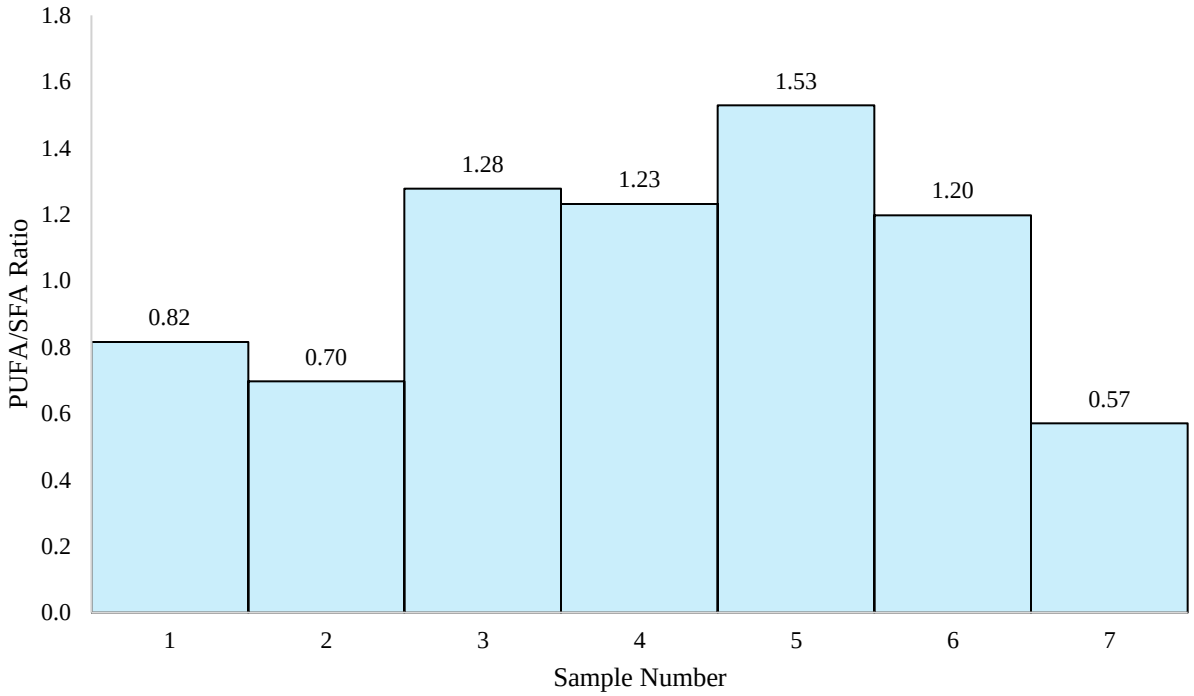

Figure 4.4 – The P:S ratio for eulachon grease samples (n=7) using LC-MS methodologies. The P:S ratio is the ratio between polyunsaturated and saturated fatty acids, calculated by dividing the %PUFA by the %SFA and multiplying the quotient by 100.

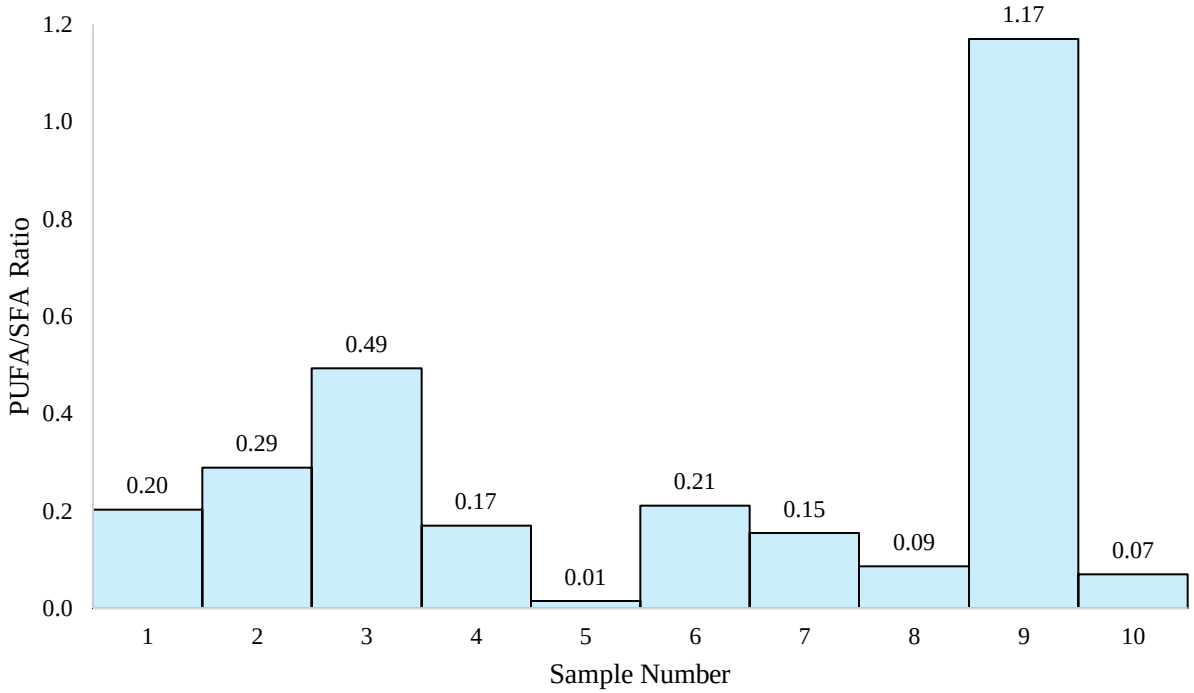

Figure 4.5 – The P:S ratio for supplement samples (n=10) using LC methodologies. The P:S ratio is the ratio between polyunsaturated and saturated fatty acids, calculated by dividing the %PUFA by the %SFA and multiplying the quotient by 100. (Supplement sample 6 included as it wasn't an outlier using LC methodologies, despite it being algal and not fish oil.)

4.5 Conclusion

This chapter summarizes the results from the LC-MS analyses on the eulachon grease samples from ‘Namgis First Nation and the supplement samples from the Canadian market. Due to the problem of carryover between samples, the quantification of the FAs may not be accurate. However, the relative abundance of the FAs between samples should be reliable. Ultimately, 29 FAs were identified in eulachon grease samples, and 27 FAs were identified in supplement samples. Evidently, the eulachon grease and supplement samples differed in the FAs identified. In fact, eulachon grease and supplement samples had 24 FAs in common, there were 5 FAs unique to eulachon grease samples and 3 FAs unique to supplement samples. It was determined that both eulachon grease and supplement samples contained beneficial FAs, including C15:0 (Venn-Watson & Schork, 2023), C18:3 (Abdelhamid et al., 2018), C20:5, and C22:6 (Siriwardhara et al., 2012).

On AVG, eulachon grease was found to have higher levels of PUFAs and MUFAs (28.97% and 42.03%, respectively) and lower levels of SFAs (29.00%), compared to supplement samples (16.29%, 10.02%, and 73.69%, respectively). The difference in the level of PUFAs and SFAs between eulachon grease and supplement samples led eulachon grease samples to have a much higher AVG P:S ratio (1.05) compared to supplement samples (0.29). These differences could also be visualized in the PCA (Figure 4.2) and heatmap (Figure 4.3), where eulachon grease and supplement samples are grouped together, respectively (with the exception of sample 9).

Unlike the GC-MS results, within the eulachon grease samples, there were no distinguishable outliers. The carryover that occurred between eulachon grease samples using LC-MS may have masked any differences that would have been reported between samples.

Since all the eulachon grease samples presented relatively similar FA profiles to one another, it was impossible to draw meaningful conclusions between the different preparation techniques and the differences in the FA profiles. Moreover, the outliers present in the GC-MS results for supplement samples were not present in the LC-MS results. The high level of carryover between supplement samples may have masked the differences that should have been seen across samples. The only supplement sample of note was sample 9 which inexplicably had a high level of PUFAs (41.80%) and low level of SFAs (35.72%). A potential reason for this spike in sample 9 would be that it was the only supplement sample made with salmon oil.

Evidently, the high level of carryover that occurred across eulachon grease and supplement samples using LC-MS methodologies is problematic, and the results are less reliable for quantification. One explanation for this high level of carryover could be the volume of the sample injected into the nanoLC which could have overwhelmed the column and led to the incomplete separation and retention of FAs in the column and contributed to carryover. As a solution, the samples should be further diluted prior to injection. Moreover, FAs could absorb into parts of the LC system (e.g., tubing, injection needle, autosampler) causing contamination between runs. Cleaning the autosampler and needle more frequently could help prevent carryover between injections. Additionally, the gradient solvent system used in the methods was insufficient. The solvents (i.e., 35:35:30 water:methanol:isopropanol) were not aggressive enough, causing FAs to persist in the column, leading to carryover. Also, the gradient and re-equilibration phases were not applied for enough time, causing residual FAs to be trapped in the column and contribute to carryover. The gradient solvent system could be optimized to include more aggressive elution and longer phases to reduce the amount of carryover between sample runs. Thirdly, the wash phase was not long or aggressive enough to completely clean the

column. More time and/or a stronger wash solvent (e.g., including higher concentrations of isopropanol or chloroform in the sample preparation) would help fully remove residual FAs and reduce the level of carryover.

Another limitation of this study is that it was not possible to identify/distinguish FAs that are branched nor isomers of FAs in the profile. As there were no previous studies using LC-MS techniques to explore the FA profile of eulachon grease and there are no studies, to my knowledge, on the FA profile of Canadian supplement brands, this was an exploration of this technique, and now there are areas of improvement to make LC-MS methodologies successful in assessing the FA profiles of eulachon grease and fish oil supplement samples in the future. A comparison between the GC-MS and LC-MS results from Chapter 3 and Chapter 4, will be presented in the next chapter.

4.6 References

Abdelhamid, A. S., Brown, T. J., Brainard, J. S., Biswas, P., Thorpe, G. C., Moore, H. J., Deane, K. H., AlAbdulghafoor, F. K., Summerbell, C. D., Worthington, H. V., Song, F., & Hooper, L. (2018). Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. The Cochrane database of systematic reviews, 11(11), CD003177.

Bender, D. A. (2009). *A Dictionary of Food and Nutrition* (3rd ed.). Oxford University Press.

Bonilla-Méndez, J. R., & Hoyos-Concha, J. L. (2018). Methods of extraction, refining and concentration of fish oil as a source of omega-3 fatty acids. Corpoica Ciencia y Tecnología Agropecuaria, 19(3).

Chen, J., & Liu, H. (2020). Nutritional Indices for Assessing Fatty Acids: A Mini-Review. International Journal of Molecular Sciences, 21(16), 5695.

Fahy, E., Sud, M., Cotter, D., & Subramaniam, S. (2007). LIPID MAPS® online tools for lipid research. Nucleic Acids Research, 35, W606-12.

Farag, M. A., & Gad, M. Z. (2022). Omega-9 fatty acids: Potential roles in inflammation and cancer management. Journal of Genetic Engineering and Biotechnology, 20(1), 48.

García, V. L. (2019). The Omega 7 as a Health Strategy for the Skin and Mucous Membranes. EC Nutrition, 14.6, 484-489.

Gowda, S. G. B., Minami, Y., Gowda, D., Chiba, H., & Hui, S. P. (2022). Detection and characterization of lipids in eleven species of fish by non-targeted liquid chromatography/mass spectrometry. *Food Chemistry*, *393*, 133402.

Horai, H., Arita, M., Kanaya, S., Nihei, Y., Ikeda, T., Suwa, K., Ojima, Y., Tanaka, K., Tanaka, S., Aoshima, K., Oda, Y., Kakazu, Y., Kusano, M., Tohge, T., Matsuda, F., Sawada, Y., Hirai, M. Y., Nakanishi, H., Ikeda, K., Akimoto, N., … Nishioka, T. (2010). MassBank: a public repository for sharing mass spectral data for life sciences. Journal of mass spectrometry, 45(7), 703–714.

Kuhnlein, H. V., & Chan, H. M. (1998). Ooligan grease: A traditional food fat of western Canada and Alaska. International Journal of Circumpolar Health, 57, 211–214.

Kuhnlein, H. V., Chan, A. C., Thompson, J. N., & Nakai, S. (1982). Ooligan Grease: A Nutritious Fat Used by Native People of Coastal British Columbia. Journal of Ethnobiology, 2(2), 154–161.

Li, Q., Chen, J., Yu, X., & Gao, J.-M. (2019). A mini review of nervonic acid: Source, production, and biological functions. Food Chemistry, 301, 125286.

Linstrom, P. J., & Mallard, W. G., Eds. (2024). NIST Chemistry WebBook, NIST Standard Reference Database Number 69, National Institute of Standards and Technology, Gaithersburg MD, 20899.

Microsoft Corporation. (2021). Microsoft Excel. Retrieved from https://office.microsoft.com/excel .

RStudio Team. (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston. Retrieved from http://www.rstudio.com/ .

Siriwardhana, N., Kalupahana, N. S., & Moustaid-Moussa, N. (2012). Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. Advances in food and nutrition research, 65, 211-222.

Stone, N. J., Robinson, J. G., Lichtenstein, A. H., Bairey Merz, C. N., Blum, C. B., Eckel, R. H., … Wilson, P. W. F. (2014). 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology, 63(25 Part B), 2889-2934.

Tang, D., Chen, M., Huang, X., Zhang, G., Zeng, L., Zhang, G., Wu, S., & Wang, Y. (2023). SRplot: A free online platform for data visualization and graphing. PLoS One, 9, 18(11), e0294236.

Tsugawa, H., Cajka, T., Kind, T., Ma, Y., Higgins, B., Ikeda, K., Kanazawa, M., VanderGheynst, J., Fiehn, O., & Arita, M. (2015). MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. Nature methods, 12(6), 523–526.

Van Hoorn, R., Kapoor, R., & Kamphuis, J. (2008). A short review on sources and health benefits of GLA, The GOOD omega-6. Oilseeds & Fats Crops and Lipids, 15(4), 262–264.

Venn-Watson, S., & Schork, N. J. (2023). Pentadecanoic Acid (C15:0), an Essential Fatty Acid, Shares Clinically Relevant Cell-Based Activities with Leading Longevity-Enhancing Compounds. Nutrients, 15(21), 4607.

Wishart, D. S., Guo, A., Oler, E., Wang, F., Anjum, A., Peters, H., Dizon, R., Sayeeda, Z., Tian, S., Lee, B. L., Berjanskii, M., Mah, R., Yamamoto, M., Jovel, J., Torres-Calzada, C., Hiebert-Giesbrecht, M., Lui, V. W., Varshavi, D., Varshavi, D., Allen, D., … Gautam, V. (2022). HMDB 5.0: the Human Metabolome Database for 2022. Nucleic acids research, 50(D1), D622–D631.

Yang, Z.-H., Amar, M., Sorokin, A. V., Troendle, J., Courville, A. B., Sampson, M., Playford, M. P., Yang, S., Stagliano, M., Ling, C., Donkor, K., Shamburek, R. D., Mehta, N. N., & Remaley, A. T. (2020). Supplementation with saury oil, a fish oil high in omega-11 monounsaturated fatty acids, improves plasma lipids in healthy subjects. Journal of Clinical Lipidology, 14(1), 53-65.

5. Summary of Results and Conclusion

5.1 Comparing the GC-MS and LC-MS Results for Eulachon Grease and Supplement Samples

This thesis identified and quantified the FA profile of eulachon grease and supplement samples using GC-MS and LC-MS methodologies. Across the two methods and all samples, 41 individual FAs were identified. The Venn diagram in Figure 5.1 visualizes the breakdown of where those 41 FAs were found, whether in eulachon grease or supplement samples (or both) or using GC-MS or LC-MS (or both). Of these 41 FAs, nine were uniquely found using GC-MS techniques and nine were uniquely found using LC-MS methods. There were six FAs that were only present in eulachon grease samples across GC-MS and LC-MS techniques and seven FAs that were only present in supplement samples across GC-MS and LC-MS methods.

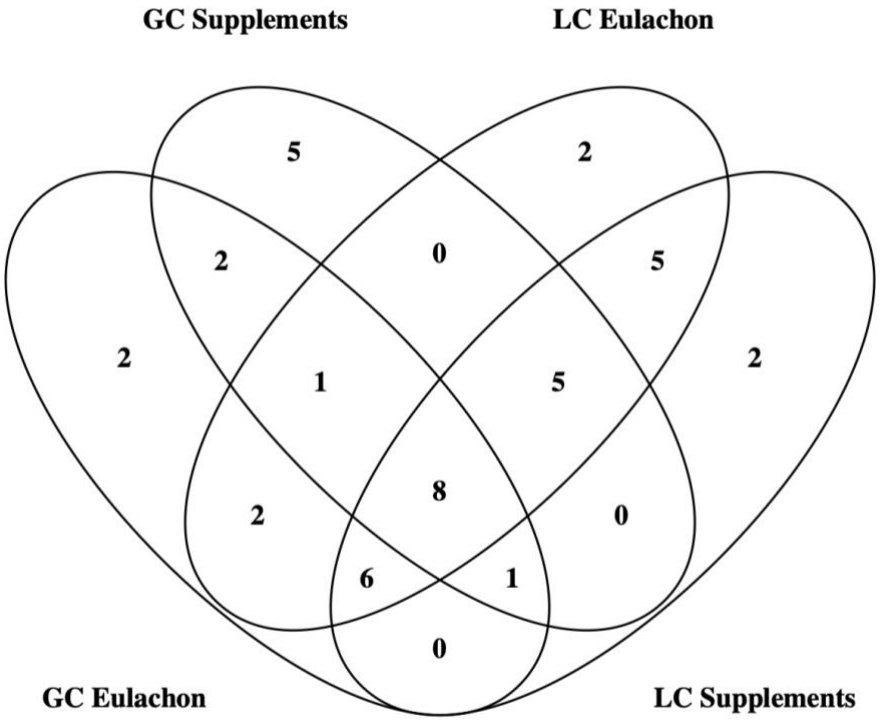

Figure 5.1 – Venn diagram depicting which FAs are unique or similar between eulachon and supplement samples using either/both GC-MS and LC-MS methodologies.

The six FAs that were unique to eulachon grease samples included: C17:0 (br), C17:1 (br), C20:3, C20:6, C22:4, and C24:1. Within these 6 FAs, only one is a SFA but it is also a BCFA, two FAs are MUFAs, and three FAs are PUFAs. Alternatively, the seven FAs that were unique to supplement samples included: C9:0, C11:0, C13:0, C14:0(br), C15:0(br), C21:0, and C22:0. These seven FAs are all SFAs. Evidently, eulachon grease samples contained additional, beneficial PUFAs and MUFAs (Kuhnlein et al., 1996; Kuhnlein et al., 1982), and less harmful SFAs (Stone et al., 2014), compared to supplement samples.

The elevated levels of PUFAs/MUFAs and low SFAs levels claimed in eulachon grease are further validated when comparing the amount of PUFAs, MUFAs, and SFAs measured in eulachon grease samples compared to supplement samples. For example, using GC-MS methods, eulachon grease samples were made of 42.55% PUFAs, 33.03% MUFAs, and 33.03% SFAs while supplement samples had 12.96% PUFAs, 21.37% MUFAs, and 65.67% SFAs on AVG. Plus, when using LC-MS methods, eulachon grease samples were made of 28.97% PUFAs, 42.03% MUFAs, and 29.00% SFAs while supplement samples had 16.29% PUFAs, 10.02% MUFAs, and 73.69% SFAs, on AVG. Thus, when using GC-MS techniques, PUFAs were considerably more elevated in eulachon grease samples and when using LC-MS MUFAs were considerably more elevated in eulachon grease samples. Meanwhile, when using either method (i.e., GC-MS or LC-MS), the amounts of SFAs are considerably higher in supplement samples. Due to this pattern of high PUFA and low SFA in eulachon grease, the P:S ratios calculated for eulachon grease samples was considerably higher than the P:S ratio calculated for supplement samples. The P:S ratio is the ratio of PUFAs to SFAs, in western diets, the AVG ratio is 0.6; it is suggested that increasing it to near 1.0 would reduce the risk of atherosclerosis and coronary heart disease (Bender, 2009). The AVG P:S ratios for eulachon grease samples

using GC-MS and LC-MS were 8.23 (n=7) and 1.05 (n=7), respectively. Alternatively, the AVG P:S ratios for supplement samples using GC-MS and LC-MS were 0.25 (n=9) and 0.29 (n=10), respectively. Evidently the AVG P:S ratios for eulachon grease samples were well above and the AVG P:S ratios for supplement samples were well below the AVG P:S ratio of 0.6 in western diets.

Ultimately, there are clear differences in the composition of the FA profiles of eulachon grease and supplement samples when using both GC-MS and LC-MS methodologies. These differences were confirmed when comparing the FAs identified, the %PUFA, %MUFA, %SFA, the P:S ratios, and could even be visualized in the PCAs and heatmaps. Eulachon grease is composed of additional beneficial FAs that would likely contribute to benefits in CVH (Chen & Liu, 2020), especially regarding cardio-protection and lowered CVD risk (Kapoor et al., 2021; Shibabaw, 2021), as well as anti-inflammation properties (Van Hoorn et al., 2008), immunoregulatory effects, and neuroprotective actions (Lu et al., 2024; Gozdzik et al., 2023). Whereas supplements are composed of additional potentially harmful SFAs, that could contribute to an increased risk of CVD and inflammation (Stone et al., 2014).

Moreover, despite the consensus that eulachon grease is higher in PUFAs and MUFAs while fish oil supplements are higher in SFAs, there were disparities in the FAs identified, the %PUFA, %MUFA, %SFA, and the P:S ratios among eulachon grease samples and supplement samples, respectively using the two different methods (i.e., GC-MS vs. LC-MS). In other words, the results found for eulachon grease and supplement samples, respectively, using GC-MS did not corroborate with the same results using LC-MS techniques. The differences in individual sample results for eulachon grease and supplement samples using GC-MS and LC-MS can be visualized with the pie charts in Figure 5.2. The most egregious examples of the mismatch in

results between GC-MS and LC-MS methods are eulachon grease samples 2 and 5 and supplement samples 6 and 9. When using GC-MS techniques, samples 2 and 5 were major outliers with very high concentrations of PUFAs and very low concentrations of SFAs compared to the other eulachon grease samples. However, when using LC-MS, there were no outliers across the eulachon grease samples; the 7 eulachon grease samples all followed a very similar composition of the FA groups and samples 2 and 5 did not have significantly higher PUFA or lower SFA levels. Alternatively, with the supplement samples, when using GC-MS, sample 6 was a major outlier with a very high percentage of PUFAs and a low amount of SFAs compared to the other samples. When using LC-MS, supplement sample 6 was no longer an outlier, rather sample 9 had an elevated amount of PUFAs and reduced SFA levels which was not observed in the GC-MS methods.

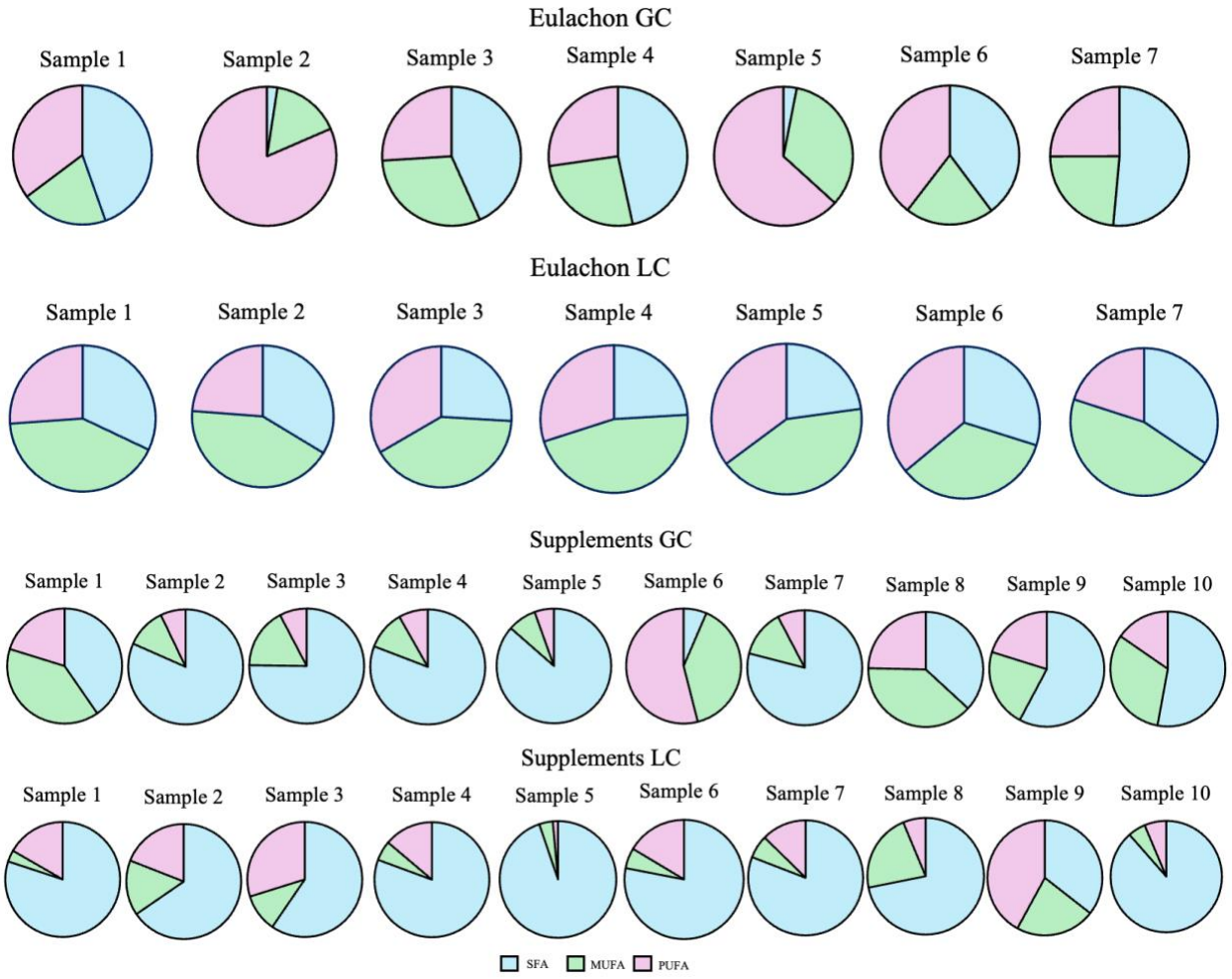

Figure 5.2 – Pie charts with the percentage of SFA, MUFA, and PUFAs for each individual sample of eulachon grease (n=7) and supplements (n=10) using both GC-MS and LC-MS methodologies.

The main explanation for the discrepancies in the FA profiles of eulachon grease and supplement samples when comparing the results from GC-MS to the results from LC-MS is the carryover contamination that occurred throughout the LC-MS methodologies. The high level of carryover that occurred across eulachon grease and supplement samples using LC-MS methodologies is problematic and consequently, the results are less reliable. The carryover that occurred between samples during the LC-MS runs may have masked the differences that should have been observed between samples. There were a few explanations for the high level of carryover. Firstly, the sample volume injected into the nanoLC was likely too high and overwhelmed the column; this volume could have also caused FAs to absorb into parts of the LC system, both causing contamination between runs. Secondly, the gradient solvent system used in the methods was insufficient. The solvents were not aggressive enough and the gradient and re-equilibration phases were not applied for enough time, causing residual FAs to be trapped in the column and contributing to carryover. Thirdly, the wash phase was not long or aggressive enough to completely clean the column, again, allowing residual FAs to remain in the column and persist across runs. Aside from the high levels of carryover between samples when using LC-MS techniques, there were a few additional drawbacks of using this method in the timeframe of this project. Namely, isomers (e.g., -cis/-trans or omega number) and BCFAs were unable to be identified/distinguished. Plus, the absolute values (e.g., mg/g or g/100g) of each FA were not able to be quantified.

Similarly to the drawbacks of using LC-MS, the main drawbacks of using GC-MS in this case was that FA isomers (e.g., -cis/-trans or omega number) were unable to be identified or distinguished. Additionally, due to time and budget constraints, the absolute values (e.g., mg/g or g/100g) of each FA were not able to be quantified. Moreover, the results from this study

using GC-MS methodologies did not corroborate with the results from previous studies quantifying the FA profile of eulachon grease samples (i.e., Phinney et al., 2009, Iverson et al., 2002, and Kuhnlein et al., 1996). There are several explanations for the discrepancies between this study and previous studies, at the crux of it, the eulachon grease samples analyzed in this study are 15+ years newer than the samples analyzed in previous studies. Over the last 15+ years, many changes have occurred in the marine environment (e.g., increased pollution levels), climate change impacts have worsened (e.g., rising water temperatures), and marine biodiversity has shifted (e.g., changes in prey availability or in species migration patterns) (Talloni-Álvarez et al., 2019 Weatherdon et al., 2016; Hollowed et al., 2013). All the aforementioned factors could alter the behaviour and/or diet of eulachon and, consequently, the FA composition of eulachon grease. Additionally, the grease processing techniques used by First Nations have varied over the last 20 years as climate change has worsened the environmental conditions during the eulachon harvest and grease processing period. Plus, not only are there changes in the methodology used in this study compared to previous studies but changes in sensitivity and advancements in accuracy have occurred with GC-MS technologies in the last 15-20 years (Xu et al., 2020; Hu & Zhang, 2018). All these factors could lead to differences in the reported FA profiles of eulachon grease samples between this study and previous studies.

5.2 Review of Research Objectives, Hypotheses, and Predictions

5.2.1 Research objectives

The first objective of this study was to employ lipidomic techniques to identify and quantify individual FA levels in eulachon grease samples and compare them to those of fish oil supplements from the Canadian market. This objective was attained as lipidomic techniques were employed to two different methodologies (i.e., GC-MS and LC-MS) to identify FAs in

eulachon grease and supplement samples using untargeted and targeted searches and quantified them based on %FA. The second objective of this study was to compare the findings of Western science (i.e., the FA profile of eulachon grease and fish oil supplements from the Canadian market) to TK (i.e., the preparation techniques). This objective was partially obtained as the transcripts from the interviews with Knowledge Holders were analyzed to learn about specific preparation techniques of eulachon grease and compared that information to differences in the FA profiles determined using GC-MS and LC-MS for eulachon grease. However, there were no conclusive links between the preparation techniques of eulachon grease and the FA profiles (as explained in sections 3.4.1 and 4.4.2). In this case, the changes in the FA profile of eulachon grease are not related to the preparation methods but rather, determined by the individual eulachons that make up each jar of eulachon grease. Namely, where the eulachon lived in the ocean (habitat), what the eulachon ate (diet), and potentially their sex or age at harvest (Kuhnlein et al., 1982).

The ultimate purpose of this study was to support First Nations TK with the chemical analyses to determine which preparation methods of eulachon grease provide optimal nutritional and/or medicinal effects. Evidently, the lack of correlation between traditional preparation methods and the FA profile of eulachon grease in this study did not allow for the determination of which preparation methods provide optimal nutritional/medicinal effects. However, it was determined that eulachon grease contains several beneficial FAs in relatively high concentrations that would promote CVH (Chen & Liu, 2020), reduce the risk of CVDs (Kapoor et al., 2021; Shibabaw, 2021), and provide anti-inflammatory, immunoregulatory, and neuroprotective effects (Lu et al., 2024; Gozdzik et al., 2023; Van Hoorn et al., 2008). These health benefits could be obtained through the ingestion or dermal application of eulachon grease. The health benefits of

eulachon grease determined using western science techniques substantiate the traditional uses of eulachon grease as a food or medicine regardless of preparation technique. Eulachon grease is critical to the diet, health, and well-being of coastal BC First Nations people.

5.2.2 Hypotheses and Predictions

The first hypothesis of this study is that there would be characteristics in the FA profile of eulachon grease that are unique from that of the fish oil supplements. This hypothesis was true as it was determined that eulachon grease samples had unique FAs that were not present in the FA profile of supplement samples. Supplement samples also had unique FAs that were not identified in the FA profile of eulachon grease. However, using both GC-MS and LC-MS methods the additional FAs in eulachon grease were mostly beneficial PUFAs and MUFAs whereas the FAs unique to supplement samples were mostly SFAs. Moreover, the prediction that eulachon grease would have lower levels of PUFAs and higher levels of MUFAs than supplement samples is not true. It was determined that eulachon grease has higher levels of PUFAs than supplements and that they are more comparable in their amounts of MUFAs identified using GC-MS methodologies.

The second hypothesis of this study stated that different preparation techniques will result in significant changes in the FA profile of eulachon grease. This hypothesis had inconclusive results as the preparation methods could not be corroborated with specific changes in the FA profile of eulachon grease samples. The preparation techniques of eulachon grease described by several Knowledge Holders differed very little from one another. Plus, many explained how climate change is impacting the eulachon grease harvest and preparation period more than anything else, year after year. Specifically, that the warmer conditions during the eulachon grease harvest and preparation period limits the amount of time that the eulachon can ferment.

Thus, the length of eulachon fermentation/ripening period is not a variable that currently changes significantly from year to year or from family to family. Where the eulachon lived in the ocean (habitat), what the eulachon eat (diet), and potentially their sex or age at harvest have more of an impact on the FA profiles than the preparation techniques (e.g., length of fermentation period). In any case, all eulachon grease samples, regardless of preparation method were found to contain FAs that are beneficial to health.

The third and final hypothesis of this study was that the unique FA composition of eulachon grease and the differences in the preparation methods will substantiate the traditional uses of eulachon grease by First Nations people. Although the preparation techniques could not be linked to specific differences in the FA profiles of eulachon grease samples, all eulachon grease samples contained beneficial FAs (e.g., PUFAs and MIUFAs) that would substantiate the traditional uses of eulachon grease as a food and medicine for BC coastal First Nations peoples.

In summary, this study provides the most up-to-date FA profile of eulachon grease and links unconventional FAs to health benefits. The results yielded from the Western science used in this study support the TK of First Nations Peoples regarding the benefits of using eulachon grease as a food source and for medicinal purposes. This study demonstrates that eulachon grease fills an important niche in the diet of BC coastal First Nations Peoples while highlighting the cultural/social importance of the traditional eulachon harvest and grease-making process. In this thesis, concerns about conservation and climate change regarding the eulachon harvest were emphasized. Consequently, this study will help strengthen First Nation management authority in protecting First Nation fisheries and advancing First Nation food independence and well-being in coastal BC. This study is transdisciplinary and collaborative and contributes to the growing body of knowledge in the fields of ethnobiology, analytical chemistry, and public health.

5.3 Future Directions

Future research on the FA profiles of eulachon grease and fish oil supplements could expand in several important directions. Firstly, collecting additional eulachon grease samples from Knight Inlet and having more detailed conversations about the eulachon harvest and processing techniques for the specific samples being collected would be beneficial. In the study, find direct connections between the TK shared about the preparation methods of eulachon grease and its effect on the FA profile of eulachon grease determined using western science could not be identified. If there were additional samples and fewer "unknowns" in the points of interest about the processing techniques the link between the preparation methods and its impacts on the FA profiles (i.e., nutritional value) could be more definitive. Thus, this additional data would further substantiate the traditional uses (e.g., food, medicine) of eulachon grease among coastal First Nations communities in BC.

Moreover, while relative values of FAs (%FA) provide important insights, the additional quantification of the FA profiles of eulachon grease and supplement samples via absolute values (i.e., mg or g of FA per g or 100g of sample) could be beneficial. The absolute value would provide a clearer understanding of the total intake of specific FAs which is crucial for evaluating the nutritional contribution of eulachon grease and fish oil supplements in the diet. With more time, money, and additional steps to the GC-MS methodologies or using high-performance liquid chromatography (HPLC), one could quantify the exact amounts of each FA in eulachon grease and supplement samples. These exact values could then be compared to dietary recommendations or limits. In the case of the supplement samples, these absolute values could also be compared to the amount of EPA, DHA, total ω-3s, and fish oil claimed on each manufacturer's label in a form of quality assessment. This would be beneficial as such high

levels of SFAs were detected in supplement samples in this study. Additionally, with the techniques necessary to determine the absolute values of the FAs in eulachon grease and supplement samples, it would also be possible to identify the isomers of FAs such as whether it is the -cis or -trans form, and the different types of omega FAs (i.e., ω-3, ω-6, ω-9, etc.). Specifying the type of isomer would be a very important distinction as they can all have drastically different health impacts. This would equally provide a more refined FA profile for eulachon grease and supplement samples.

As climate change impacts intensify Canada-wide, it would be valuable to investigate the potential environmental contaminants in eulachon grease or supplement samples. Evidently, both eulachon grease and fish oil supplements are made from fish species that lived in aquatic ecosystems. Here, they were potentially exposed to industrial pollutants, heavy metals, or persistent organic pollutants (POPs). It is important to assess whether there are residual contaminants in the grease after traditional preparation techniques or in the supplement samples after industrial processing. The presence of contaminants in eulachon grease or supplement samples could impact the safety and long-term health effects of consuming/using eulachon grease or fish oil supplements. Techniques like inductively coupled mass spectrometry (ICP/MS) can be used to detect contaminants like mercury and lead and GC-MS could be used to detect POPs to ensure levels are within safe consumption limits.

While FAs are the major focus of this thesis, other nutritional components such as vitamin D or E, retinol (vitamin A), protein, cholesterol, and minerals like calcium and iron would also be important to quantify to understand the full scope of health benefits of eulachon grease and fish oil supplements. Measuring these additional nutrients could further validate the traditional uses of eulachon grease and could also provide consumers and health professional

with more complete information of evaluate the safety of fish oil supplements on the Canadian market. Several methods exist to quantify nutritional components. For example, vitamins D, E, and A could be measured with HPLC, proteins can be measured by determining the total nitrogen content in a sample with the Kjeldahl method, and cholesterol and minerals can be measured using various colorimetric assays.

In conclusion, expanding on the analysis of eulachon grease and fish oil supplements through the directions proposed above, will provide a more comprehensive understanding of the nutritional composition, safety, and health benefits of eulachon grease and fish oil supplements. By integrating additional TK with modern scientific techniques, investigating potential contaminants, and analyzing key nutritional components, future research will offer valuable insights for both BC coastal First Nations communities and consumers of fish oil supplements. Future studies will help ensure the safe and informed use of fish oil supplements while highlighting the cultural, nutritional, and socioeconomic significance of eulachon grease.

5.4 References

Bender, D. A. (2009). *A Dictionary of Food and Nutrition* (3rd ed.). Oxford University Press.

Chen, J., & Liu, H. (2020). Nutritional Indices for Assessing Fatty Acids: A Mini-Review. International Journal of Molecular Sciences, 21(16), 5695.

Gozdzik, P., Magkos, F., Sledzinski, T., & Mika, A. (2023). Monomethyl branched-chain fatty acids: Health effects and biological mechanisms. Progress in Lipid Research, 90, 101226.

Hollowed, A. B., Barange, M., Beamish, R. J., Brander, K., Cochrane, K., Drinkwater, K., Foreman, M. G. G., Hare, J. A., Holt, J., Ito, S., Kim, S., King, J. R., Loeng, H., MacKenzie, B. R., Mueter, F. J., Okey, T. A., Peck, M. A., Radchenko, V. I., Rice, J. C.,

… Yamanaka, Y. (2013). Projected impacts of climate change on marine fish and fisheries. ICES Journal of Marine Science, 70(5), 1023–1037.

Hu, T., & Zhang, J.-L. (2018). Mass-Spectrometry-Based Lipidomics. Journal of Separation Science, 41, 351–372.

Iverson, S., Frost, K., & Lang, S. (2002). Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: Factors contributing to among and within species variability. Marine Ecology Progress Series, 241, 161–181.

Kapoor, B., Kapoor, D., Gautam, S., Singh, R., & Bhardwaj, S. (2021). Dietary Polyunsaturated Fatty Acids (PUFAs): Uses and Potential Health Benefits. Current Nutrition Reports, 10(3), 232–242.

Kuhnlein, H. V., Chan, A. C., Thompson, J. N., & Nakai, S. (1982). Ooligan Grease: A Nutritious Fat Used by Native People of Coastal British Columbia. Journal of Ethnobiology, 2(2), 154–161.

Kuhnlein, H. V., Yeboah, F., Sedgemore, M., Sedgemore, S., & Chan, H. M. (1996). Nutritional qualities of ooligan grease: A traditional food fat of British Columbia First Nations. Journal of Food Composition and Analysis, 9, 18–31.

Lu, H., Wang, Z., Cao, B., Cong, F., Wang, X., & Wei, W. (2024). Dietary sources of branched-chain fatty acids and their biosynthesis, distribution, and nutritional properties. Food Chemistry, 431, 137158.

Phinney, S. D., Wortman, J. A., & Bibus, D. (2009). Oolichan Grease: A Unique Marine Lipid and Dietary Staple of the North Pacific Coast. Lipids, 44(1), 47–51.

Shibabaw, T. (2021). Omega-3 polyunsaturated fatty acids: Anti-inflammatory and anti-hypertriglyceridemia mechanisms in cardiovascular disease. Molecular and Cellular Biochemistry, 476(2), 993–1003.

Stone, N. J., Robinson, J. G., Lichtenstein, A. H., Bairey Merz, C. N., Blum, C. B., Eckel, R. H., … Wilson, P. W. F. (2014). 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology, 63(25 Part B), 2889-2934.

Talloni-Álvarez, N. E., Sumaila, U. R., Le Billon, P., & Cheung, W. W. L. (2019). Climate change impact on Canada's Pacific marine ecosystem: The current state of knowledge. Marine Policy, 104, 163–176.

Van Hoorn, R., Kapoor, R., & Kamphuis, J. (2008). A short review on sources and health benefits of GLA, The GOOD omega-6. Oilseeds & Fats Crops and Lipids, 15(4), 262–264.

Weatherdon, L. V., Ota, Y., Jones, M. C., Close, D. A., & Cheung, W. W. L. (2016). Projected Scenarios for Coastal First Nations' Fisheries Catch Potential under Climate Change: Management Challenges and Opportunities. PLOS ONE, 11(1), e0145285.

Xu, T., Hu, C., Xuan, Q., & Xu, G. (2020). Recent Advances in Analytical Strategies for Mass Spectrometry-Based Lipidomics. Analytica Chimica Acta, 1137, 156–169.

Appendices

Appendix 1

Copy of the UOttawa Research Ethics Board Certificate of Approval.

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

CERTIFICAT D'APPROBATION ÉTHIQUE | CERTIFICATE OF ETHICS APPROVAL

Numéro du dossier / Ethics File Number	H-04-23-9108
Titre du projet / Project Title	Nutritional Profiles of Eulachon Grease
Type de projet / Project Type	Thèse de maîtrise / Master's thesis
Statut du projet / Project Status	Approuvé / Approved
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	11/04/2023
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	10/04/2024

Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
Anik MARTIN	Département de biologie / Department of Biology	Chercheur Principal / Principal Investigator
Laurie CHAN	Département de biologie / Department of Biology	Superviseur / Supervisor

Appendix 2

Template of the information sheet and consent form signed by local Knowledge Holders who participated in the interviews conducted in Alert Bay, BC, July 2023.

Information Sheet and Consent Form

This information sheet presents the research project in which you are invited to participate. We invite you to send any questions you might have to the researchers.

1. Title of the Study: Nutritional Profiles of Eulachon Grease

2. Project Contact Information

Researcher: Anik Martin
Department of Biology
Faculty of Science
University of Ottawa

Supervisor: Dr. Laurie Chan
Department of Biology
Faculty of Science
University of Ottawa

3. Project Funding

This project is funded by the University of Ottawa and the Canadian Institutes of Health Research.

4. Purpose of the Study

First Nations in coastal British Columbia want to document the importance of the nutritional benefits of eulachon grease. Within each Nation, each family has their own traditional techniques for preparing eulachon grease, and the preparation may help to promote certain nutritional quality of the grease.

The study will help better understand the nutritional quality of eulachon grease and how the different preparation methods used by individual families will change the quality and use of the grease. The purpose is to use scientific data to support the importance of eulachon grease among First Nations in British Columbia.

5. Participation

Knowledge holder(s) within each family (age: 18+) that participated in the community-organized trip to Knight Inlet for the eulachon harvest, will be asked to participate in 1-2 approx. 30-minute-long interviews to discuss the quality of eulachon and eulachon grease (e.g., colour, taste, nutritional value) as well as the procedures used to reduce eulachon into eulachon grease. Prior to this interview, consent forms will be explained, and verbal or written consent will be given by willing participants.

During the discussion, participants will share information about traditional knowledge and how this knowledge is used to prepare and assess eulachon grease. At the end of the interview, the researcher will collect a small sample (i.e., < 10g) of the participants' eulachon grease preparation which will be later analysed at the University of Ottawa. At this time, participants

will be invited to share their contact information with the researcher if they wish to be informed about project results and news.

6. Benefits

Participating in this study will be useful to you, your community, and other First Nation communities to incorporate First Nation traditional knowledge with western science evidence to understand which preparation methods of eulachon will provide the best health promotion effects and/or taste.

7. Risks

As a participant in this study, you will share significant information on intergenerational traditional knowledge with non-Indigenous individuals. This may cause you to feel that you have shared confidential information. There is a risk of misusing this information (e.g., for commercial use). You are given assurance from the research team that this information will not be misinterpreted or misused in the study and will not be used for commercial gains. Moreover, there may be a risk of COVID-19 or other infectious diseases during the interviews. To minimize health risks, you can request that safety measures be followed during the meeting, such as wearing masks and social distancing.

8. Participant compensation

Each family will receive an honorarium of $300 to compensate for the time involved in this project (i.e., $300 will be given to each family, even if more than one member participates). If you choose to withdraw from the project at any point in time, you will still be able to receive full compensation.

9. Confidentiality and anonymity

The conversation during the interview will be recorded for data analysis. A copy of the anonymized interview transcript will be returned to the ‘Namgis First Nation to keep. The information will be integrated and presented as a general practice in the community. If the method used by your family is described, the identity of your family or you will be kept confidential. Your name will only be attached to the information collected if you choose to disclose your identity (by selecting the box below). However, due to the group nature of the study, e.g., other community members may know that you have talked to us and can associate the results with your family. Therefore, your full confidentiality/anonymity cannot be guaranteed.

The information you share will only be used for the development of dietary advice for your community and to advance research in this area.

☐ By selecting this box, you consent to having your identity (name) published/mentioned in academic presentations and publications (e.g., in the use of quotes recalling personal/lived experiences).

Furthermore, the researcher will be taking photographs and video recordings of eulachon and eulachon related information/environments. With your consent (by selecting the box below), these photographs and video recordings will be used in presentations, conferences, and/or publications.

☐ By selecting this box, you consent to having pictures and video recordings of your methods of eulachon and eulachon grease preparation published in academic presentations and publications.

In any case, if you choose to withdraw from the study for any reason, all your data will also be withdrawn, unless you give permission for its use.

10. Conservation of data

The data collected (anonymized transcripts) will be kept on a password-protected computer at Dr. Laurie Chan's laboratory in the Department of Biology at the University of Ottawa for five years following the completion of the project in August 2024 (approximately 6 years following data collection). Thereafter they will be securely destroyed. The stored transcript will only be used to confirm the results of this study.

11. Identification of Research Contacts

If you have questions about the interviews or the research project, you can contact the principal investigator of the project:

- Dr. Laurie Chan, Principal Investigator

12. Ethical Conduct of Research

If you have any questions regarding the ethical conduct of this study, you may contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall: 550 Cumberland Street, Room 154, Ottawa, ON K1N 6N5; Tel: (613)-562-5387; Email: ethics@uottawa.ca

Participant Consent

Acceptance: I, _____ (Name), agree to participate in the above research study conducted by Anik Martin and Dr. Laurie Chan (Dept. of Biology) of the University of Ottawa.

By signing below, I confirm the following:

- Full details of the study, including the risks associated with my participation, have been explained to me.
- I have read the information sheet and consent form and understand what is involved in this study.
- My questions about the study have been answered.
- I understand that I can choose to stop participating at any time and that I can choose not to answer any specific questions.
- I understand that if I have questions about the study in the future, I may contact the researchers.
- I understand that I can indicate how I will be identified in the study and if I choose to have photos/videos of my eulachon/eulachon grease preparation used in this study by indicating my preference in the checkboxes above.
- I understand that the information I share may be shared with the research team for the development of nutritional advice related to eulachon grease.
- I understand that the discussion will be audio-recorded, and I will have access to the transcript of the recording to ensure that accurate information is analyzed, presented, and published by the research team.
- I understand that if I choose to withdraw from this study I will still be compensated and that my data will be withdrawn.
- I understand that there are two copies of the consent form, one of which is mine to keep.

Participant's Name, Email Address: _____

Date of Birth (dd/mm/yyyy): _____

Signature for Consent Date (dd/mm/yyyy)

_____ _____

Researcher's Signature Date (dd/mm/yyyy)

_____ _____

Appendix 3

Template of the interview questions given and asked to local Knowledge Holders during the interviews conducted in Alert Bay, BC, in July 2023.

Questionnaire For Families – ‘Namgis

1. Email address (to receive a copy of the transcript of this interview):

2. Participant (Family) Identity Number (match to label on bottle):

3. Location of eulachon collection, if known:

4. Date of eulachon collection, if known:

5. In your experience, was it a plentiful or scarce year for eulachon harvest? Please explain.

6. Date of eulachon grease preparation, if known:

7. Mode of preparation:
    a. Fish:
    b. Grease:

8. Date frozen, if applicable:
    a. Fish:
    b. Grease:

9. Main use of eulachon grease (i.e., condiment, ingredient, preservative, medicine, other), please explain:

10. Describe the quality of the taste of this year’s catch/batch.
    a. Fish:
    b. Grease:

11. Describe the colour compared to previous years.
    a. Fish:
    b. Grease:

12. What do you think/how do you feel about the nutritional composition of this batch, please explain?

13. How do you feel climate change is impacting your ability to harvest eulachon and make eulachon grease?