View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Munin - Open Research Archive

UiT The Arctic University of Norway

Faculty of Biosciences, Fisheries and Economics

Biochemical composition and antioxidant capacity of two sea cucumbers (*Cucumaria frondosa* and *Parastichopus tremulus*)

Celine Edvardsen

Master's thesis in Seafood Science FSK-3960 May 2020

ACKNOWLEDGEMENTS

This assignment marks the end of a five-year study at the Norwegian School of Fisheries, at the Seafood Science group. In this regard, I would like to thank my main supervisor Edel Oddny Elvevoll for all the support and wise input along the way. Thanks to my co-supervisor Ida-Johanne Jensen, for all the knowledge about antioxidant capacity. Thanks to Guro Kristine Edvinsen for all the help in the lab and with my results, plus big and small questions that have emerged along the way.

A big thank you to my partner Adrian Rinaldo for everything you do for me in everyday life, and for all the help with statistics and guidance on this thesis, I couldn´t have done this without you. Thanks to my Mum and sisters for all the support and help, it has been invaluable. And thanks to my Dad (1963-2017), for always being so proud of me, I miss you more than anything.

I would also like to thank Cassandra Granlund, Agneta Hansen and Thea Kristine Benjaminsen for all the fun and enjoyable moments these last five years. Thanks to Simon Skålsvik Hansen for dragging me through most of the submissions we have had in recent years and all help when preparing the sea cucumbers. And lastly, thanks to Maria Nilsen Heimland and Ronja Amalie Gabrielsen for all the hours in the lab, they would not have been the same without you.

Celine Edvardsen

May 2020

SUMMARY

Sea cucumber is one of the most expensive seafood products in the world and dried sea cucumbers can be valued at more than USD 300 per kilogram for *Astichopus japonicus*, which is one of the highest priced species. Both market and consumption are centred in East- Asia. Sea cucumbers are sold in various forms, but the majority are distributed to the '*bêche- de-mer*' market (gutted, boiled and dried body wall of sea cucumber). Norway has a long history as a seafood nation and has set new export records every year since 2012, but has no commercial harvest of sea cucumbers. They mostly occur as by-catch during shrimp trawling and crayfish fishing in the fjords. The Norwegian Government has set a goal to increase economic growth for new marine species. Out of the 31 species of sea cucumber along the Norwegian coast, Norwegian red sea cucumber (*Parastichopus tremulus*) and Orange-footed sea cucumber (*Cucumaria frondosa*) are considered to be the species of interest for the Asian market. Especially *P. tremulus*, as it belongs to the same family as preferred species *A. japonicus*. Based on market feedback, the estimated price potential for dried Norwegian sea cucumber is NOK 1000-2000 per kilogram. The analyses in this study were conducted on freeze-dried material and were recalculated to wet weight. The amount of fat, protein, water and ash was analysed, including analyses of amino acid composition and fatty acid composition. Analyses were also made of antioxidant capacity in the sea cucumber species. Significant differences were found for the proximate composition between the sea cucumber species in this study, for example, the fat content was 0.4% for *P. tremulus* and 2.0% for *C. frondosa*. The composition of fatty acids were similar for both species, with the highest content of PUFA followed by MUFA and SFA respectively. Both species had a favourable ratio of n-6:n-3 fatty acids, but only *C. frondosa* complied with the daily recommendation of EPA and DPA by consuming 100 grams of sea cucumber. The protein content was higher in *C. frondosa* than in *P. tremulus* with 6.6% and 3.2%, respectively. Both total amino acid content and essential amino acid content were significantly different between the species and significant differences were found for all amino acids except glycine, methionine and proline. In general, *C. frondosa* had a lower antioxidant capacity than *P. tremulus* demonstrated by both ferric reducing antioxidant power (FRAP) and oxygen radical antioxidant capacity (ORAC-) assay. In conclusion, *P. tremulus* and *C. frondosa* appear to have a beneficial biochemical composition for human consumption.

SAMMENDRAG

Sjøpølser er et av de dyreste sjømatproduktene i verden og tørkede sjøpølser omsettes for mer enn 300 amerikanske dollar per kilogram for *Astichopus japonicus*, som er en av de dyreste artene. Både markedet og forbruket er sentrert rundt Øst-Asia. Sjøpølsene selges i forskjellige former (fersk, frossen eller levende), men det meste distribueres til "*bêche-de-mer*" markedet (sløyd, kokt og tørket kroppsvegg av sjøpølse). Norge har en lang historie som sjømatnasjon og har satt nye eksportrekorder hvert år siden 2012, men har ingen kommersiell høsting av sjøpølser. Sjøpølsene forekommer for det meste som bifangst under reketråling samt kreps- og hummerfiske i fjordene. Den norske regjeringen har satt et mål om å øke verdiskapingen for nye marine arter og av de 31 ulike artene av sjøpølser langs norskekysten anses rødpølsen (*Parastichopus tremulus*) og brunpølsen (*Cucumaria frondosa*) å være artene av størst interesse for det asiatiske markedet. Spesielt *P. tremulus* ettersom den er i samme familie som den foretrukne arten *A. Japonicus*. Basert på tilbakemeldinger fra markedet er prispotensialet for tørket rødpølse estimert til 1000-2000 norske kroner per kilogram. Analysene i denne studien ble utført på frysetørket materiale og resultatene er omregnet og presentert på våtvektsbasis. Mengden fett, protein, vann og aske ble analysert, i tillegg ble analyser av aminosyre- og fettsyresammensetning gjennomført. Antioksidativ kapasitet ble også målt på frysetørkede materiale av sjøpølsene. Det ble funnet signifikante forskjeller i den biokjemiske sammensetningen mellom sjøpølseartene i denne studien, for eksempel var fettinnholdet $0,4\%$ for *P. tremulus* og $2,0\%$ for *C. frondosa*. Sammensetningen av fettsyrer var svært lik, med det høyeste innholdet for PUFA etterfulgt av henholdsvis MUFA og SFA. Begge artene hadde et gunstig forhold mellom n-6:n-3, men bare C. frondosa oppfylte den daglige anbefalingen for inntak av EPA og DPA ved konsum av 100 gram sjøpølse. Proteininnholdet var høyere i *C. frondosa* enn i *P. tremulus* med henholdsvis $6,6\%$ og $3,2\%$.Både totalt aminosyreinnhold og innhold av essensielle aminosyrer var forskjellig mellom artene og det ble funnet signifikante forskjeller for alle aminosyrer bortsett fra for glysin, metionin og prolin. Generelt hadde *C. frondosa* en lavere antioksidantkapasitet enn *P. tremulus* vist både med ≪ferric reducing antioxidant power≫ (FRAP) og ≪oxygen radical antioxidant capacity≫ (ORAC) analyse. Avslutningsvis ser *P. tremulus* og *C. frondosa* ut til å ha en gunstig biokjemisk sammensetning for menneskelig forbruk.

Table of Contents

List of Figures x

List of Tables x

1 Introduction 1

2 Materials and Methods 6
- 2.1 Freeze-drying . . . 6
- 2.2 Lipid extraction . . . 6
- 2.3 Fatty acids composition and gas chromatography . . . 7
- 2.4 Water and ash content . . . 8
- 2.5 Amino acid analysis . . . 8
- 2.6 Ferric reducing antioxidant power-assay . . . 9
- 2.7 Oxygen radical absorbance capacity-assay . . . 9
- 2.8 Statistical analysis . . . 10

3 Results 11
- 3.1 Proximate composition . . . 11
- 3.2 Amino acid composition . . . 11
- 3.3 Fatty acid composition . . . 13
- 3.4 Antioxidant capacity . . . 15

4 Discussion 17
- 4.1 Proximate composition . . . 17
- 4.2 Amino acid composition . . . 19
- 4.3 Fatty acid composition . . . 22
- 4.4 Antioxidant capacity . . . 24
- 4.5 Limitations . . . 25

5 Conclusion 26

List of Figures

1.1 An overview of enzymes responsible of conversion of linoleic acid and alfa-linoleic acid into arachidonic acid and eicosapentaenoic acid 3

3.1 Antioxidant capacity of *P. tremulus* and *C. frondosa* 16

4.1 Estimate intake of essential amino acids from *C. frondosa* and *P. tremulus* . . . 20

4.2 Ability of *P. tremulus* and *C.frondosa* to cover EPA, DPA and DHA 23

List of Tables

3.1 Proximate composition: water, lipid, ash and protein of *P. tremulus* and *C. frondosa* 11

3.2 Amino acid profile of *P. tremulus* and *C. frondosa* 12

3.3 Fatty acid composition of *P. tremulus* and *C. frondosa* 14

Acronyms

AA Amino acid. 4, 11

AAPH 2,2'azobis(isobuttersaüreamidin) dihydrochlorid. 9, 15

ALA Alfalinolenic acid. 3, 4

AOC Antioxidant capacity. 4, 24

ARA Arachidonic acid. 3, 4, 13, 22

DCM Dichlormetane. 6, 7

DCM:MeOH Dichlormetane methanol. 6, 7

DHA Docosahexaenoic acid. 3, 13, 22, 23

DPA Docosapentaenoic acid. 13, 22, 23

DW Dry weight. 11, 24

EAA Essential amino acid. 4, 11, 12, 19, 21

EPA Eicosapentaenoic acid. 3, 13

FA Fatty acid. 13, 22

FRAP Ferric reducing antioxidant power. 4, 5, 9, 10, 15, 16, 24

LA Linoleic acid. 3, 4, 13

MUFA Monounsaturated fatty acid. 3, 4, 13, 22

n-3 Omega-3 fatty acid. 3, 13, 22

n-6 Omega-6 fatty acid. 3, 13, 22

NCFS Norwegian School of Fishery Science. 18

NFH Norges Fiskerihøyskole. 18

ORAC Oxygen radical absorbance capacity. 4, 5, 9, 10, 16, 24

PUFA Polyunsaturated fatty acid. 3, 4, 13, 22

SFA Saturated fatty acid. 3, 4, 13, 22

TAA Total amino acid. 11, 12, 19

TE Trolox equivalents. 5

TPTZ 2,4,6-Tri(2-pyridyl)-2-triazine. 9

Trolox 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. 5, 9, 10

WW Wet weight. 17, 19

1 | INTRODUCTION

Sea cucumber is one of the most expensive seafood products in the world, and the high-priced Japanese spiky sea cucumber (*Apostichopus japonicus*, Selenka, 1867) can be valued at more than USD 300 per kilogram (dried) (Purcell et al. 2010). The market for sea cucumber is centred around East-Asia, where the consumption is also the highest. In Norway, human consumption of sea cucumber is rare, and the class of *Holothuroidea* (Blainville, 1834) is not commercially harvested. However, they often occur as by-catch from the Norwegian fishing fleet, especially during shrimp trawling- and crayfish fishing in the fjords (Kjerstad, Bjørkevoll, and Christophersen 2020).

Norway has exported seafood for millennia, and since 2012 the nation has set new export records every year (Norwegian Seafood Council 2020). Hitherto, the record for total turnover for Norwegian seafood export was NOK 100 billion, which was set in 2019 (Norwegian Seafood Council 2020). The largest importers of Norwegian seafood are Poland and Denmark, with NOK 10.63 and 9.22 billion, respectively. However, in 2019 Norway exported seafood to more than 149 countries, which illustrates Norway's position as a seafood exporter (Norwegian Seafood Council 2020). By species, Atlantic salmon (*Salmo salar*, Linnaeus 1758) generates the highest revenue with a total of NOK 72.41 billion in 2019 (Norwegian Seafood Council 2020). In comparison, for wild fish; cod (*Gadus morhua*, Linnaeus 1758) had the highest total export value with NOK 14.85 billion.

Sea cucumbers are cylindrical echinoderms in the class *Holothuroidea* occurring on various depths on the seafloor. There are more than 1400 species worldwide, and approximately 50-60 of these are edible (Bechtel et al. 2013; Han, Keesing, and Liu 2016). The use of sea cucumber as food and as a food-ingredient resource is traditional in China and other Asian countries, such as Hong Kong (SAR), Singapore and Taiwan (ROC). During the Ming Dynasty (1368-1644 BC), sea cucumber was considered as 'tonic food' (Chen 2004). According to Chinese philosophy, food should serve as medicine for 'prevention and treatment of disease' (Chen et al., 2004) and sea cucumbers as traditional medicine was regularly used to treat everything from anaemia, asthma and rheumatism to hypertension and sinus congestion (Fredalina et al. 1999).

Sea cucumbers are sold fresh, frozen or alive, but the vast majority is distributed for the

'*bêche-de-mer*' market, which is gutted, boiled and dried body wall of sea cucumber (Toral-Granda et al. 2008; Purcell et al. 2010). The demand for sea cucumber in the eastern world is increasing and has caused populations of sea cucumbers to decline (Conand 2004; Uthicke et al. 2004). As such, aquaculture has become a way of supporting this growing demand (Chen 2004). The Japanese spiky sea cucumber has been considered the most economically valuable species for a long time (Han, Keesing, and Liu 2016), and is one of the few species that are cultivated.

Madsen and Hansen (1994) estimated that there are around 31 species of sea cucumber along the Norwegian coast, of which Norwegian red sea cucumber (*Parastichopus tremulus*, Gunnerus, 1767) and Orange-footed sea cucumber (*Cucumaria frondosa*, Gunnerus, 1767) are considered to be the species of interest for the Asian market (Kjerstad et al. 2015). Especially, *P. tremulus* appears to be the most profitable species as it belongs to the same family as preferred species *A. japonicus* (Kjerstad et al. 2015), and based on market feedback (Kjerstad, Bjørkevoll, and Christophersen 2020). The Norwegian Government has set a goal to increase value creation for new marine species, both through capture and aquaculture (Nærings og Fiskeridepartementet 2015; AkvaplanNIVA 2019). The estimated price potential for dried Norwegian *P. tremulus* is NOK 1000-2000 per kilogram (Kjerstad and Rønneberg 2005).

Norwegian red sea cucumber, commonly known as "rødpølse" in Norwegian, occurs in the northeast Atlantic (Kjerstad et al. 2015; Kjerstad and Rønneberg 2005). Characteristics of *P. tremulus* is a cylindrical thick body wall with dotted small brown spots on the entire body. It has a different colour on the dorsal and ventral side, which is red and white, respectively. According to Madsen and Hansen (1994), *P. tremulus* is found on muddy or sandy bottoms. They noted that it is rarely longer than 50 cm long and 10 cm wide (Madsen and Hansen 1994), and is found at depths from 20 down to 2000 meters (Madsen and Hansen 1994).

Orange-footed sea cucumber, commonly known as "brunpølse" in Norwegian, occurs in the northwest Atlantic and the northeast Atlantic and is typically found at depths between 20 to 200 meters (Madsen and Hansen 1994). Most are found with a shade of brown, but some individuals have pale tones of orange. Unlike *P. tremulus*, it has a rounder football-like shape and does not exceed 20-30 cm in length and is primarily found on pebbly or rocky bottoms (Hamel and Mercier 1996b). There is reason to believe that there is seasonal variation in the quality of sea cucumber. For example, fishing for *C. frondosa* is stopped for about a month during summer due to spawning and subsequent poor quality (Kjerstad, Bjørkevoll, and Christophersen 2020).

Previous studies on these species show that values between 0.4%-0.9% can be expected for fat, 3.3%-5.3% for protein content, 3.5%-3.8% for ash content and water content between 88%-91% for *P. tremulus* (Kjerstad, Bjørkevoll, and Christophersen 2020). Whereas for *C. frondosa* one can expect the water and ash content to be slightly lower at 86.2% and 1.9%, respectively. While the protein and fat content for *C. frondosa* are expected to be higher with $9,3\%$ and

0.91%, respectively (Zhong, Khan, and Shahidi 2007).

Fatty acids are the simplest form of lipids consisting of carbon chains with a methyl group at one end and a carboxylic acid at the other (Olsen 2017). The length is normally between 12 and 22 carbon atoms. They can be divided into two main groups; saturated fatty acids (SFA) without carbon-carbon double bonds and unsaturated fatty acids. The unsaturated fatty acids are again divided into monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). MUFA got one double bond, whilst PUFA can have between 2 to 6 double bonds. The position of the double bound closest to the methyl end is also important as it distinguishes the two main groups of PUFA. When the first double bond is located at the third or sixth carbon atom from the methyl end, they are known as omega-3 (n-3) or omega-6 (n-6) fatty acids, respectively.

Essential fatty acids are fatty acids the human body cannot synthesise by itself and thus must be supplied through the diet. Phytoplankton and plants are the only ones with enzymes capable of placing double bonds in the carbon chain of n-3 and n-6 fatty acids, as humans lack the enzymes Δ12desaturase and Δ15desaturase. This makes linoleic acid (LA, 18:2 n-6) and alfalinolenic acid (ALA 18:3 n-3) essential fatty acids for all animals, including humans and fish (Rustan and Drevon 2005). LA and ALA are necessary also as precursors for important biological active fatty acids that can be synthesized from these namely arachidonic acid (ARA, 20:4 n-6) from LA, eicosapentaenoic acid (EPA 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) from ALA (see Figure 1). Animals and fish generally have an inefficient ability to synthesize EPA and DHA from ALA, but fish and other seafood (including sea cucumber) acquire ample amounts of marine phytoplankton through their normal diet.

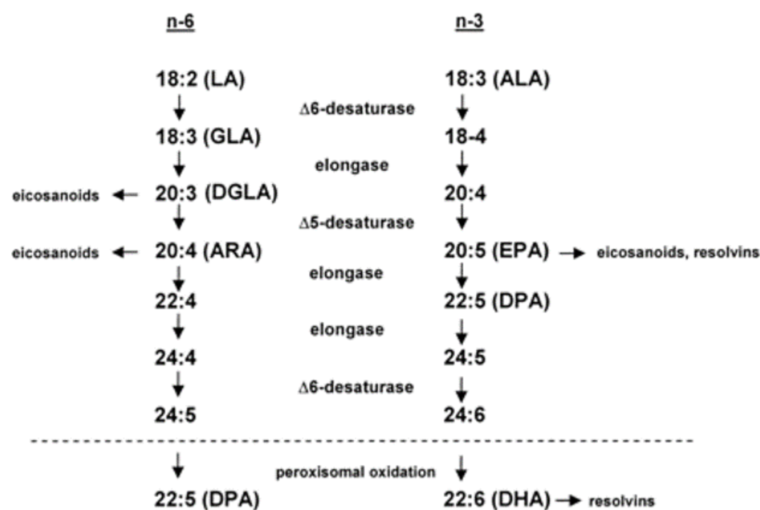


Figure 1.1: An overview of the enzymes responsible for the conversion of linoleic acid and alfa-linoleic acid into arachidonic acid (ARA, 20:4 n-6) and eicosapentaenoic acid/docosahexaenoic acid (EPA,20:5 n-3 and DHA,22:6 n-3) respectively (Arterburn, Hall, and Oken 2006).

The relative amount of ARA (from 18:2 n-6) and EPA (20:5 n-3), both synthesised by the

same enzymes, will be determined by the relative amount of these essential C-18 fatty acids available in the diet. A Western diet by today´s standard favours ARA, where there is often around 20x more 18:2 n-6 than 18:3 n-3. In addition, ALA is also more readily oxidised to energy than LA (Arterburn, Hall, and Oken 2006). *P. tremulus* has, from prior studies, a known fatty acid composition of 19% of SFA, 19% of MUFA and 32% PUFA (Kjerstad and Rønneberg 2005), while *C. frondosa* has a fatty acid composition ranging from 8% to 12% for SFA, from 20% to 34% for MUFA and from 57% to 68% for PUFA (Zhong, Khan, and Shahidi 2007).

Proteins have a number of functions in the body and are necessary for growth, enzyme activity, maintenance and transport of various nutrients and other compounds across the cell membrane. They are large molecules that consist of one or more chains of amino acids (AA) bound to each other. There are about 20 different amino acids that act as building materials for proteins in living organisms (Wu 2009). The amino acids are divided into essential and non-essential, where the essential amino acids (EAA) are amino acids the body cannot synthesize. Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are the 9 amino acids the human body needs to get through diet (Wu 2009). The body´s largest protein store is in the muscles and it is important to provide the body with sufficient amounts of protein through the diet to maintain important functions. Insufficient amounts can lead to increased muscle protein burn and thus to reduced muscle growth and loss. This, in turn, will have an effect on the immune system and reduce enzymatic and hormonal activity in the body.

The amino acid composition of *P. tremulus* is known from previous studies and contains all essential amino acids and the amino acids glycine (8.6 mg/g), glutamic acid (4.8 mg/g) and aspargine (3.5 mg/g) are found in highest quantities (Kjerstad and Rønneberg 2005). The amino acid composition in *C. frondosa* has also been shown to be dominated glutamic acid (57.7-89.9 mg/g), glycine (29.8-77.9 mg/g) and aspartic acid (27.8-51.3 mg/g) (Zhong, Khan, and Shahidi 2007).

The role of antioxidants is to donate an electron to a free radical while remaining stable, which in turn stops the free radical chain reactions and reduces the capacity of the radical to damage cells (Lobo et al. 2010). A free radical is defined by Lobo et al. (2010) to be molecular species containing an unpaired electron in an atomic orbit. They act as oxidants or reducing agents by either donating or accepting an electron from other molecules. Radicals are often very reactive and unstable. Oxidative stress is a condition that can arise from an unfavourable balance between free radicals and the antioxidant defence (Lobo et al. 2010). Oxidative stress is thought to cause significant damage to several molecular species such as nucleic acid, lipids and proteins. Common methods for measuring antioxidant capacity (*i.e.,* AOC) are ORAC- and FRAP-assays. ORAC measures the AOC of tissue by fluorescent spectroscopy after initiating the breakdown of a fluorescent molecule (*e.g.,* fluorescein) by peroxyl radicals. By comparison

of decay curves, standard known antioxidants (*i.e.,* Trolox) versus sample, the antioxidant capacity is calculated and, thus, is often expressed as Trolox equivalents. In essence, the ferric reducing ability of plasma or ferric ion reducing antioxidant power (FRAP) utilises the sample principle of redox-reactions as ORAC, but in reverse. Instead of fluorescence spectroscopy, the FRAP-assay uses absorption spectroscopy. When the Fe^{III}-TPTZ complex is reduced to FE^{II} in solutions with low pH the solution gradually becomes blue with an absorption maximum of 593 nm (Benzie and Strain 1996). Again, by comparison to a standard curve based on Trolox, the ferric reducing ability of the sample is measured and then expressed in Trolox equivalents (TE). Zhong, Khan, and Shahidi (2007) have previously reported ORAC values between 2.09-2.60 $mmol$ of Trolox equivalents per g of dry sample for *C. frondosa*.

Few studies have been performed on the nutritional quality and biochemical composition of sea cucumber species in Norwegian waters. Therefore, the purpose of this study is to analyse nutritional quality, the biochemical composition, and the antioxidant capacity of whole *P. tremulus* and *C. frondosa*.

2 | MATERIALS AND METHODS

Two different species of sea cucumber are analysed in this paper. They were harvested at different geographical locations and depths. *P. tremulus* was collected at about 200 meters depth in Western Norway in June 2018 and stored at -18°C. *C. frondosa* was collected at 10-15 meters depth in Northern Norway outside Tromsø in January 2020 and was stored only a few hours before further preparation for analysis. *P. tremulus* had a total number of ten batches, while *C.frondosa* only had three. Both species of sea cucumber consisted of three individuals of sea cucumber which were ground, frozen and freeze-dried. Each analysis was performed in triplicates.

2.1 | FREEZE-DRYING

The freeze-drying was done with a Genesis EL35 freeze dryer (VirTis SP Scientific, NY, USA). About 100 g of sea cucumber was weighed and completely frozen before the freeze-drying could begin. The samples were freeze-dried for approximately 48 hours.

2.2 | LIPID EXTRACTION

Total lipids were extracted from ground and freeze-dried sea cucumbers and determined gravimetrically using a modified Folchs method (Folch, Lees, and Sloane 1957). Heptadecanoic acid (Supelco Analytical, Bellefonte, PA, USA) was used as the internal standard and dichloromethane (DCM) was substituting chloroform. The analysis was performed in triplicates. Approximately 19 mL of DCM:MeOH and 1 mL of Internal standard (IS, 10 mg/mL) was added to 1 g of freeze-dried samples in Teflon tubes and mixed in a Multi Reax automatic homogenizer (Heidolph Instruments, Germany) for 30 minutes. The solutions were filtered through a folding filter into new Teflon tubes before 4 mL of 0.9% NaCl was added. The Teflon tubes were then centrifuged at 2000g (g-force) for 10 minutes by a Hereasus Multifuge 1 S_R (Thermo Scientific, MA, USA). Two separate phases were formed after centrifugation. The upper phase consisted of water and methanol, while the lower one of DCM and lipids. A glass pipette was used to

pipette the bottom layer into pre-weighed glass tubes. The lipid solution was then evaporated to dryness using a nitrogen evaporator (Sample Concentrator SBHCONC/1, Stuart Equipment, Staffordshire, UK) at 30°C, $100\ mbar$. The glass tubes were weighed again, and the fat content was calculated by the following formula: 2.1.

$$\text{Fat (\%)} = \frac{\text{Glass tube}_{\text{with content}} - \text{Glass tube}_{\text{empty}}}{\text{Weighted amount of sample}} \times 100 \tag{2.1}$$

2.3 | Fatty acids composition and gas chromatography

The fatty acids composition of the lipids was determined by a method modified from Stoffel, Chu, and Ahrens (1959). The lipid extract was re-dissolved to $10\ mg\ mL^{-1}$ with DCM:MeOH before methylation. Each sample ($100\ \mu L$) was mixed with $0.9\ mL$ DCM and $2\ mL$ 2% H_2SO_4 and placed in a heating block (Drybath Stdrd, Thermo Scientific, MA, USA) at about 100°C for one hour.

Then $3.5\ mL$ of heptane and $3.5\ mL$ of 5% NaCl were added, blended, and the top phase, containing lipids and heptane, was pipetted in new tubes, dried under N_2 gas (Aga, Oslo, Norway) and dissolved in $100\ \mu L$ of heptane. The samples were analysed using gas chromatography. The instrument used was an Agilent 6890N equipped with a flame ionization detector (FID)(Agilent Technologies, Santa Clara, CA, USA) and a Varian CP7419 capillary column ($50\ m \times 250\ \mu m \times 0.25\ \mu m$ nominal) (Varian Inc., Middelburg, Netherlands). Helium was used as the carrier gas. The fatty acids were identified by retention time, and by comparing with the fatty acids standards PUFA no. 1, PUFA no. 2 and PUFA no. 3 from Supelco (Supelco Analytical, Bellefonte, PA, USA).

The quantity of fatty acids were determined by calculation from the area of the fatty acids following formula 2.2.

$$\frac{\text{Amount of fatty acid}}{100\ g \text{ of sample}} = \frac{\text{area peak FA}}{\text{area peak IS}} \times \frac{\text{added IS}(g)}{\text{weight sample}(g)} \tag{2.2}$$

2.4 | WATER AND ASH CONTENT

Water content ($n = 3$) was performed by using a modified version of AOAC method 950.46b (Association of Official Analysis Chemists International 2005). Approximately $10\ g$ sample was dried at $105°$C until constant weight. Analyses were performed in triplicates. Water content was determined gravimetrically and calculated follwing formula 2.3.

Ash content was performed using a modified version of AOAC 938.08 (Association of Official Analysis Chemists International 2005). The dried samples were further combusted at $540°$C for $16\ h$ in a muffle furnace (Nabertherm, GmbH, Program Controller S27, Lilienthal, Germany). Ash content was determined gravimetrically and calculated following formula 2.4.

$$\text{Water content (\%)} = \frac{\text{Weight}_{\text{before drying}} - \text{Weight}_{\text{after drying}}}{\text{Weight}_{\text{before drying}}} \times 100 \quad (2.3)$$

$$\text{Ash content (\%)} = \frac{\text{Weight}_{\text{after drying and combustion}}}{\text{Weight}_{\text{before drying and combustion}}} \times 100 \quad (2.4)$$

2.5 | AMINO ACID ANALYSIS

In accordance with Moore and Stein (1963), the analysis of total amino acids was performed after acid hydrolysis. However, as acid hydrolysis breaks down tryptophan, this amino acid is not detected in the analysis. Before analysis, the protein structure must be broken down. The peptide bonds were broken by acid hydrolysis. To enable quantitative analysis of amino acids in the samples, an internal standard was added before hydrolysis. The internal standard was N-leucin (Sigma Aldrich, MO, USA) with a known concentration of $20\ mM$.

Approximately $40\ mg$ of freeze-dried sample was mixed with $0.7\ mL$ distilled H_2O and $0.5\ mL$ internal standard (N-leu $20\ mM$). $1.2\ mL$ of concentrated hydrochloric acid (37%) was added and the samples were flushed with N_2 gas (Aga, Oslo, Norway) for 10-15 seconds to prevent oxidation. The samples were placed in a heating cabinet (Termaks-Labolytic AS, Trondheim, Norway) at $110°$C for 22-24 h. The samples were cooled down and $100\ \mu L$ of sample was transferred to assay tubes and evaporated into dryness with N_2 gas (Aga, Oslo, Norway). The samples were dissolved in $1\ mL$ of lithium citrate buffer, pH 2.2 (Biochrom Co. Cambridge, UK).

Chromatographic separation on an ion exchange column was used to analyse all amino acid samples. As described in Spackman, Stein, and Moore (1958), lithium-citrate buffer with different pH and ionic strength and a pre-defined temperature programme was used.

The samples were analysed using a Biochrom 30 Amino Acid Analyser (Biochrom C, Cambridge, UK) with lithium citrate equilibrated column and post-column derivatization with ninhydrin. The signals were analysed with Chromeleon Software (Dionex, Sunnyvale, CA, USA) and the identification of the amino acids were done by comparison with an A9906 physiological amino acid standard (Sigma Chemicals Co, ST. Louis, MO, USA). According to guidance by FAO (2003), sums of individual amino acid residues (the molecular weight of each amino acid minus the molecular weight of water) are the protein content of the samples.

2.6 | Ferric reducing antioxidant power-assay

The ferric reducing antioxidant power (FRAP)-assay was performed by following the FRAP assay described Benzie and Strain (1996), with some modifications. The FRAP-solution was prepared by mixing $50\ mL$ acetate buffer ($1.505\ g$ $C_2H_3NaO_2$, $8\ mL$ $C_2H_4O_2$ and $500\ mL$ distilled H_2O), pH 3.6, $5\ mL$ 2,4,6-Tri(2-pyridyl)-2-triazine (TPTZ) ($10\ mM$ in $40\ M$ HCl) and $5\ mL$ $FeCl_3 \cdot 6H_2O$ ($19\ mM$) and heated to 37°C before use.

Trolox, the water-soluble analog of Vitamin E, was used as a standard. It was dissolved in methanol to $1\ mM$ before dilution with water to a concentration ranging from 15.625 to $1000\ mM$. Samples were prepared by mixing approximately $100\ mg$ of dry matter with $10\ mL$ distilled H_2O in an automatic homogenizer (Multi Reax, Heidolph Instruments, Schwabach, Germany) for 30 minutes. Then about $500\ \mu L$ sample was placed in Eppendorf tubes and centrifuged for 2 minutes at $13000\ rpm$ in a 5424 R centrifuge (Eppendorf, Hamburg, Germany) to separate the solids from the liquid. Samples were diluted to a suitable concentration.

In the microplate $10\ \mu L$ of sample or standard solution, $30\ \mu L$ of distilled H_2O and $300\ \mu L$ of FRAP-solution were added. This was incubated for 30 minutes at 37°C before reading at $593\ nm$ in a microplate reader (SpectraMax i3, Molecular Devices, USA). The results were expressed in μmol Trolox equivalents per $100\ g$ (TE/100 g) of wet weight.

2.7 | Oxygen radical absorbance capacity-assay

The oxygen radical absorbance capacity (ORAC)-assay was performed as described by Dávalos, Gómez-Cordovés, and Bartolomé (2004). In a black microplate, $25\ \mu L$ sample or Trolox (standards) and $125\ \mu L$ of $50\ mM$ fluorescein were added and incubated for 15 minutes at 37°C. Thereafter, $50\ \mu L$ of $38\ mM$ 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) was added before the fluorescence was measured kinetically at 485 and $520\ nm$ in a microplate reader (SpectraMax i3, Molecular Devices, USA). The microplate was automatically shaken for 5 seconds before the first reading and 3 seconds prior to each reading every minute for 35

minutes when the fluorescence was completely lost.

Trolox dissolved in FB to concentrations of 5-$50\ \mu M$ was used as a standard. The samples were prepared similar as to those for the FRAP-assay. However, the samples for the ORAC assay were diluted in phosphate buffer (pH 7.4) before being mixed in an automatic homogenizer (Multi Reax, Heidolph Instruments, Schwabach, Germany) for 30 minutes. About $500\ \mu L$ sample was pipetted in Eppendorf tubes and centrifuged at $13000\ rpm$ in a 5425 R centrifuge (Eppendorf, Hamburg, Germany) for 2 minutes and diluted to a suitable concentration. A regression equation between the Trolox concentration and the net area under the curve (AUC) was used to determine the ORAC-values. To express final ORAC-value, μmol of TE was used.

2.8 | Statistical analysis

Statistical analyses were conducted in R R and Excel. The analyses are presented as the arithmetic mean with standard deviation. A t-test was used to test for statistically significant differences between species. Significance level was set to 5% ($p \leq 0.05$).

3 | RESULTS

3.1 | PROXIMATE COMPOSITION

There were significant differences among *P. tremulus* and *C. frondosa* in their proximate composition; more specifically, there were differences in the fat, water, ash and protein content (Table 3.1). The fat content differed between *P. tremulus* and *C. frondosa* ($p < 0.05$), which contained 0.4% and 2.0%, respectively. Further, water content significantly differed ($p < 0.05$) with *C. frondosa* consisting of an average of 83.2% water, while *P. tremulus* consisted of an average of 89.0% water. The protein content was significantly different ($p < 0.05$) for the species and was 3.2% and 6.6% for *P. tremulus* and *C. frondosa*, respectively. Lastly, ash content was 3.2% for *C. frondosa* and 4.5% for *P.tremulus* ($p < 0.05$).

Table 3.1: Proximate composition; water, lipid, ash, protein (% of wet weight) of whole *P. tremulus* (Mean ± SD, $n = 10$) and *C. frondosa* (Mean ± SD, $n = 3$)

Component (% of wet weight)	**Species**	
	P. tremulus	*C. frondosa*
Fat	0.4 ± 0.1^a	2.0 ± 0.2^b
Water	89.0 ± 0.8^a	83.2 ± 0.7^b
Ash	4.5 ± 0.7^a	3.2 ± 0.07^b
Protein	3.2 ± 4.2^a	6.6 ± 0.7^b

Different letter in the same row means significant difference $p < 0.05$

3.2 | AMINO ACID COMPOSITION

Seventeen different amino acids (AAs) were identified, seven of which were essential amino acids (EAAs) and ten were nonessential amino acids. The amount of the individual amino acids (mg AA/g) in *P. tremulus* and *C. frondosa* are presented as dry weight (DW) in Table 3.2.

There was a significant difference ($p < 0.05$) in total amino acid (TAA) content between the species. The TAA content of *P. tremulus* was $315.7\ mg/g$ DW while it was $423.2\ mg/g$ DW for *C. frondosa*. A significant difference was also found for the ratio of EAA to TAA which

was 0.27 for *P. tremulus* and 0.28 *C. frondosa*. The only three amino acids that did not differ between the species were glycine, methionine and proline.

The EAA content of the species varied significantly, with 95.9 mg/g in *P. tremulus* and 117.9 mg/g in *C. frondosa*. The most abundant EAA was leucine for both species, 18.8 mg/g in *P. tremulus* and 23.9 mg/g in *C. frondosa*.

Glutamic acid dominated in both species and was significantly different ($p < 0.05$) between species. The content was 48.2 mg/g in *P. tremulus* and 59.6 mg/g in *C. frondosa*. Also, the second most abundant amino acid, glycine, was similar for both species with 30.6 mg/g in *P. tremulus* and 34.8 mg/g in *C. frondosa*. Followed by aspartic acid (24.8 mg/g) and arginine (21.6 mg/g) for *P. tremulus* and aspartic acid (27.7 mg/g) and proline (23.5 mg/g) for *C. frondosa*.

Table 3.2: Amino acid profile (mg per g of dry sample) for whole *P. tremulus* (Mean ± SD, $n = 10$) and *C. frondosa* (Mean ± SD, $n = 3$). $n.d$ = not detected.

Amino acid	**Species**	
	P. tremulus	*C. frondosa*
Histidine	4.0 ± 0.5^a	6.1 ± 0.4^b
Isoleucine	10.1 ± 0.5^a	11.7 ± 0.2^b
Leucine	18.8 ± 1.1^a	23.9 ± 0.6^b
Lysine	15.1 ± 1.3^a	22.0 ± 0.8^b
Methionine	6.3 ± 0.6^a	6.7 ± 0.5^a
Phenylalanine	12.1 ± 1.0^a	13.9 ± 0.6^b
Threonine	17.0 ± 1.6^a	19.1 ± 0.3^b
Tryptophan	$n.d$	$n.d$
Valine	12.7 ± 0.8^a	14.4 ± 0.3^b
EAA	95.9 ± 6.0^a	117.9 ± 3.5^b
Arginine	21.6 ± 4.0^a	9.8 ± 0.3^b
Alanine	16.8 ± 2.1^a	22.8 ± 0.4^b
Aspartic acid	24.8 ± 2.7^a	27.7 ± 0.4^b
Cysteine	3.1 ± 0.7^a	4.6 ± 0.2^b
Glutamic acid	48.2 ± 4.9^a	59.6 ± 1.4^b
Glycine	30.6 ± 8.0^a	34.8 ± 0.6^a
Proline	21.2 ± 5.4^a	23.5 ± 0.7^a
Serine	15.5 ± 1.6^a	20.6 ± 0.1^b
Tyrosine	9.6 ± 1.4^a	12.8 ± 0.4^b
TAA	351.7 ± 41.9^a	423.2 ± 9.0^b
EAA/TAA	0.27 ± 0.14	0.28 ± 0.39

Different letter in the same row means significant difference $p < 0.05$
EAA = Essential amino acids, TAA = Total amino acids

3.3 | Fatty acid composition

The fatty acid composition (% of total fatty acids) and the total amount of fatty acids per 100 g of *C. frondosa* and *P. tremulus* are presented in Table 3.3. Twenty-seven different fatty acids (FA) were identified in the assay in addition to several unidentified FAs, which are included in the total value. Seventeen FAs (excluding C14:1, C16:2 n-4, C16:3 n-4, C18:2 n-6, C18:3 n-3, C18:3 n-6, C20:2 n-6, C20:3 n-3, C20:3 n-6, and C22:4 n-6) were found to differ significantly ($p < 0.5$) between the species according to area percentage. Whereas for amount, g per 100 g of sample, docosahexaenoic acid (DHA, C22:6 n-3) were the only FAs that were not significantly different between the species. There was also significant difference ($p < 0.05$) in the total content of fatty acids g per 100 g, found in the species.

Table 3.3 shows the area percentage of the fatty acid composition (% of the total fatty acids) of *P. tremulus* and *C. frondosa*. *P. tremulus* contained 18.2%, 29.6% and 37.0% of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), respectively. While the unidentified FAs made up 1.5%. The PUFAs contained 25.2% n-3 fatty acids, which consisted of 12.6% eicosapentaenoic acid (EPA, C20:5 n-3), 1.2% DPA and 7.8% DHA. The n-6 fatty acids reached a total of 11.8% of which 1.2% was linoleic acid (LA, C18:2 n-6) but aracidonic acid (ARA, 20:4 n-6) was the most abundant (8.1%). *C. frondosa* contained 11.1% of SFA, 30.5% of MUFA and 41.8% of PUFA. While the unidentified FAs made up 1.9%. The PUFAs contained 36.7% n-3 fatty acids, with EPA, DPA and DHA accounting for 30.5%, 0.5% and 1.9%, respectively. While the n-6 fatty acids were 3.0% and LA was about 0.5% and ARA was most abundant for *C. frondosa* as well (1.5%).

Table 3.3 also shows the FA content ($g/100$ g) for each species. Per 100 g, *P. tremulus* contained 0.06 g of SFA 0.10 g of MUFA and 0.13 g of PUFA. The PUFAs contained 0.087 g of the long-chain omega-3 fatty acids (LC n-3). *C. frondosa* had a slightly higher content per 100 g than *P. tremulus* with 0.18 g, 0.50 g and 0.68 g of SFA, MUFA and PUFA, respectively. The PUFAs contained 0.59 g LC n-3.

Table 3.3: Area percentage of the fatty acid composition (% of total fatty acids) and the fatty acid composition (g per 100 g) of *P. tremulus* (Mean ± SD, $n = 10$) and *C. frondosa* (Mean ± SD, $n = 3$). $n.d$ = not detected

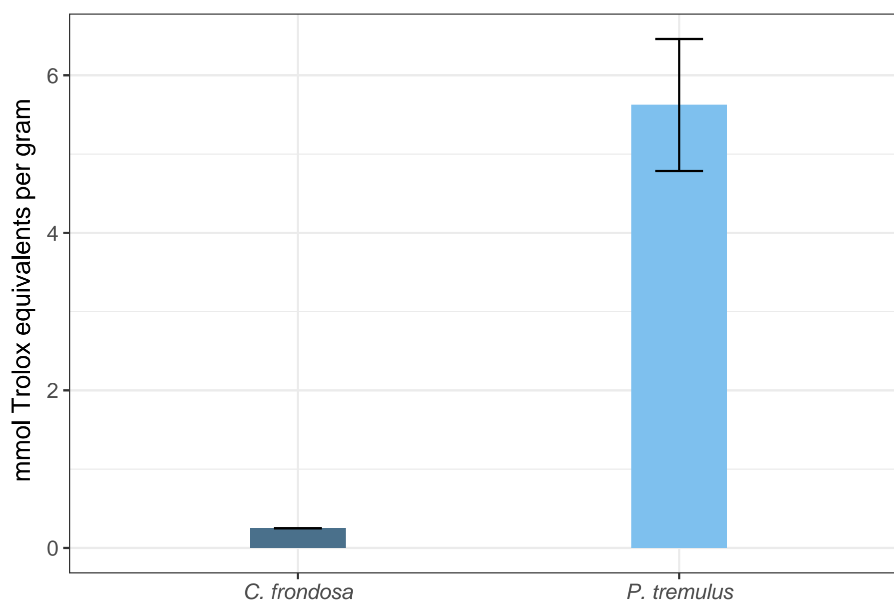
Fatty acid	**Species**			
	P. tremulus		*C. frondosa*	
	Composition	Amount	Composition	Amount
Unidentified	1.5 ± 0.38^a	0.005 ± 0.001^a	1.9 ± 0.39^a	0.031 ± 0.01^b
C14:0	4.1 ± 0.52^a	0.014 ± 0.01^a	3.1 ± 0.15^b	0.050 ± 0.01^b
C16:0	6.4 ± 0.91^a	0.022 ± 0.01^a	2.8 ± 0.18^b	0.045 ± 0.01^b
C18:0	6.3 ± 1.17^a	0.021 ± 0.005^a	4.5 ± 0.26^b	0.073 ± 0.01^b
C20:0	1.4 ± 0.23^a	0.005 ± 0.01^a	0.7 ± 0.02^b	0.012 ± 0.002^b
Total SFA	18.2 ± 2.38	0.06 ± 0.01	11.1 ± 1.56	0.18 ± 0.03
C14:1	4.7 ± 0.75	0.016 ± 0.005	$n.d$	$n.d$
C16:1 n-7	7.9 ± 1.13^a	0.028 ± 0.01^a	17.5 ± 0.56^b	0.285 ± 0.05^b
C18:1 n-7	6.6 ± 0.45^a	0.023 ± 0.01^a	3.2 ± 0.02^b	0.052 ± 0.001^b
C18:1 n-9	2.4 ± 0.52^a	0.009 ± 0.005^a	5.0 ± 0.39^b	0.082 ± 0.02^b
C20:1 n-9	1.5 ± 0.23^a	0.005 ± 0.001^a	0.8 ± 0.03^b	0.013 ± 0.002^b
C22:1 n-9	1.5 ± 0.09^a	0.005 ± 0.002^a	1.1 ± 0.03^b	0.018 ± 0.003^b
C22:1 n-11	1.9 ± 0.40^a	0.006 ± 0.002^a	1.3 ± 0.05^b	0.021 ± 0.003^b
C24:1 n-9	3.1 ± 0.61^a	0.010 ± 0.003^a	1.6 ± 0.07^b	0.026 ± 0.005^b
Total MUFA	29.6 ± 2.44	0.10 ± 0.01	30.5 ± 5.99	0.50 ± 0.10
C16:2 n-4	$n.d$	$n.d$	1.2 ± 0.02	0.019 ± 0.003
C16:3 n-4	$n.d$	$n.d$	0.9 ± 0.08	0.015 ± 0.003
C18:2 n-6	1.2 ± 0.13	0.004 ± 0.002	0.5	0.008
C18:3 n-3	1.2 ± 0.46	0.005 ± 0.003	$n.d$	$n.d$
C18:3 n-6	0.3	0.001	$n.d$	$n.d$
C18:4 n-3	1.6 ± 0.50^a	0.005 ± 0.002^a	3.3 ± 0.36^b	0.053 ± 0.01^b
C20:2 n-6	1.5 ± 0.11	0.005 ± 0.001	$n.d$	$n.d$
C20:3 n-3	0.9 ± 0.16	0.003 ± 0.001	0.5	0.009
C20:3 n-6	0.6	0.003	$n.d$	$n.d$
C20:4 n-6	8.1 ± 1.06^a	0.028 ± 0.01^a	1.5 ± 0.24^b	0.024 ± 0.01^b
C20:5 n-3 EPA	12.6 ± 1.05^a	0.043 ± 0.001^a	30.5 ± 0.63^b	0.494 ± 0.06^b
C22:4 n-6	$n.d$	$n.d$	1.1 ± 0.04	0.017 ± 0.002
C22:5 n-3 DPA	1.2 ± 0.18^a	0.004 ± 0.002^a	0.5 ± 0.01^b	0.008 ± 0.001^b
C22:6 n-3 DHA	7.8 ± 0.75^a	0.027 ± 0.01^a	1.9 ± 0.11^b	0.031 ± 0.005^a
Total PUFA	37.0 ± 3.90	0.13 ± 0.01	41.8 ± 7.9	0.68 ± 0.13
n-3	25.2 ± 4.89	0.087 ± 0.017	36.7 ± 12.01	0.60 ± 0.19
n-6	11.8 ± 0.39	0.041 ± 0.003	3.04 ± 0.1	0.05 ± 0.001
n-6/n-3	0.5 ± 0.085	0.47 ± 0.18	0.1 ± 0.01	0.08 ± 0.004

Different letter in the same row means significant difference $p < 0.05$

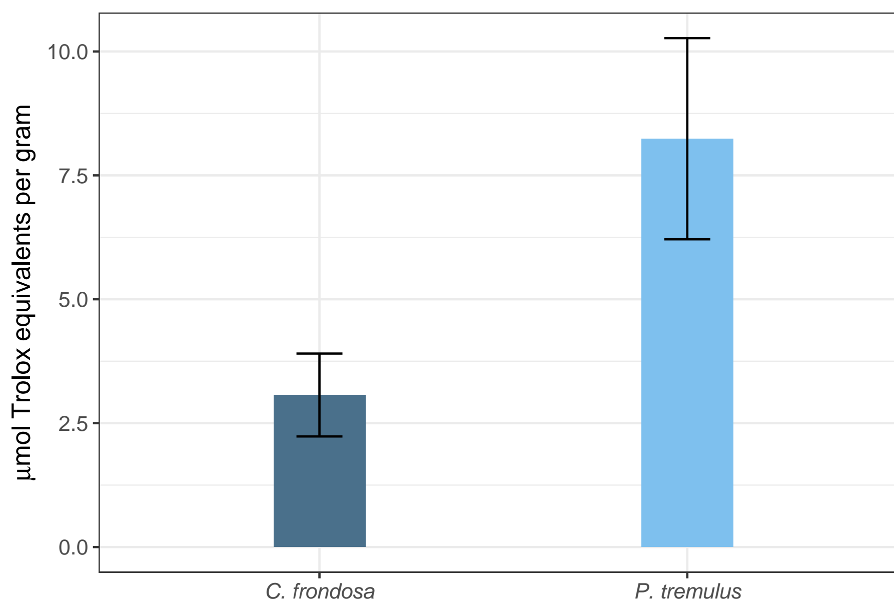
3.4 | ANTIOXIDANT CAPACITY

The results from FRAP-assay are presented in Figure 3.1a. The species were significantly different from each other ($p < 0.05$) where *P. tremulus* had an average value of $5.6\ mM\ TE/g$ and *C. frondosa* of $0.25\ mM\ TE/g$.

Figure 3.1b presents the antioxidant activity/scavenging ability of *P. tremulus* and *C. frondosa* against AAPH. The species were significantly different ($p < 0,05$) with an average TE-value of $3.1\ \mu mol\ TE/g$ for *C. frondosa* and $8.2\ \mu mol\ TE/g$ for *P. tremulus*.



(a) Antioxidant activity measured by ferric reducing antioxidant power (FRAP) assay for whole *Cucumaria frondosa* (dark blue) and *Parastichopus tremulus* (light blue). Results are presented as $mmol$ of Trolox equivalents per gram of wet sample. Mean± S.D, $n = 10$ for *P. tremulus* and $n = 3$ for *C. frondosa*.



(b) Antioxidant activity measured by oxygen radical absorbance capacity (ORAC) assay for whole *Cucumaria frondosa* (dark blue) and *Parastichopus tremulus* (light blue). Results are presented as μmol of Trolox equivalents per gram of wet sample. Mean± S.D, $n = 10$ for *P. tremulus* and $n = 3$ for *C. frondosa*.

Figure 3.1

4 | DISCUSSION

4.1 | PROXIMATE COMPOSITION

This study shows significant differences in proximate composition between *C. frondosa* and *P. tremulus*. Specifically, *P. tremulus* had a lower fat and protein content and a higher water and ash content than *C. frondosa*.

Overall, the proximate composition of sea cucumber presented in this study was similar to those found in previous research. Proximate composition is presented and discussed as the percentage of wet weight (WW). The content of fat reported by Kjerstad, Bjørkevoll, and Christophersen (2020) for *P. tremulus* ranged from 0.4% in June to 0.9% in September. The fat content of *P. tremulus* in this study was on average 0.4% which is in line with the values found by Kjerstad, Bjørkevoll, and Christophersen (2020) and Kjerstad and Rønneberg (2005). The protein content was reported by Kjerstad, Bjørkevoll, and Christophersen (2020) to range from 3.3% in June to 4.3% and 5.3% in March and September, respectively. While Kjerstad and Rønneberg (2005) reported a protein content of 3.4%, the protein content of *P. tremulus* found in this study was measured to be 3.2% for the same season (June) as in Kjerstad, Bjørkevoll, and Christophersen (2020). The ash content measured for *P. tremulus* (4.5%) in this study was higher than values (3.5%) for the same period by Kjerstad, Bjørkevoll, and Christophersen (2020) and lower than values (6.2%) reported by Kjerstad and Rønneberg (2005). However, the water content found for *P. tremulus* in this study was lower (89.0%) than what Kjerstad, Bjørkevoll, and Christophersen (2020) reported for the same period (91.0%) and higher than values (88.9%) reported by Kjerstad and Rønneberg (2005). The proximate composition in *P. tremulus* was also comparable to that of both *A.japonicus* (Dong et al. 2006) and *Stichopus herrmanni* (Wen, Hu, and Fan 2010).

The fat content measured in *C. frondosa* is somewhat higher (2.0%) than previously measured (0.5-1.27%) by Zhong, Khan, and Shahidi (2007). The protein content found for *C. frondosa* in this study was on average 6.6%, which is within the range of values found by Zhong, Khan, and Shahidi (2007). The ash content of *C. frondosa* was measured to be 3.2% in this study, which is slightly higher than the content reported by Zhong, Khan, and Shahidi (2007)

where the ash content ranged from 0.67% to 3.03%. While the water content of *C. frondosa* in this study (83.2%) is lower than previously measured (83.3%-90.1%) by Zhong, Khan, and Shahidi (2007). The proximate composition of *C. frondosa* was similar to that of *Thelenota ananas* (Wen, Hu, and Fan 2010).

The proximate composition has a major impact on the quality of the sea cucumbers and varies due to geographical location, climatic conditions, type, season, weight and environmental factors (Salarzadeh et al. 2012; Dj Ratoe Oedjoe 2017). It is plausible that proximate composition may change prior to and after spawning due to reallocation of nutrients to gonads. This has previously been observed for several species such as the *Atlantic salmon* (Shearer et al. 1994), several species of *Gastropoda* (Vasconcelos, Gaspar, and Castro 2009) and in particular in the same and comparable species of sea cucumber *C. frondosa* (Hamel and Mercier 1996a; Hamel and Mercier 1996b) and *A. japonicus* (Gao, Xu, and Yang 2011).

Hamel and Mercier (1995) reported that *C. frondosa* from arctic waters had a spawning season in July. This is supported by the fact that fishing for *C. frondosa* in Icelandic waters is stopped for about a month during summer due to spawning and poor quality (Kjerstad, Bjørkevoll, and Christophersen 2020). Studies from Kjerstad, Bjørkevoll, and Christophersen (2020) indicate that this spawning season also applies to *P. tremulus*, although their publication does not explain why *P. tremulus* has a lower content of protein and fat. Nevertheless, their study shows that *P. tremulus* has the lowest quality in June measured as 0.4%, 3.3%, 3.5% and 91.0% for fat, protein, ash and water content, respectively. However, their conclusion is that more data should be obtained to better understand the variations in proximate composition and the relationship between spawning and proximate composition.

Kjerstad, Bjørkevoll, and Christophersen (2020) reported that when the content of nutritional components such as fat and protein declines, the water content increases. Which may also explain why the water content of *P. tremulus* is greater than that of *C. frondosa*, where both fat and protein content are higher. A previous study by Tan et al. (2018) found that several freeze-thaw cycles can have a significant impact on quality properties in sea cucumber such as thaw loss, texture, protein content, water durability, colour parameters and even expand the interstice between the fibre networks in the microstructure. With this in mind, differences in preparation of samples of *P. tremulus*, may also have been an influential reason as to why the proximate composition of *P. tremulus* were different from *C. frondosa* in this study.

C. frondosa was collected in the vicinity of Tromsø and transported live almost directly to the laboratory at the Norwegian College of Fishery Science (NCFS, in Norwegian; NFH). Due to necessity of transportation, the *P. Tremulus* samples had to be frozen, stored, transported and thawed before equal preparation in the laboratory. This may have impacted the difference in water content and in turn the proximate composition of *P. tremulus* and *C. frondosa*. The different species went through the same treatment after arrival, and were ground, frozen and

freeze-dried.

4.2 | Amino acid composition

The total protein content of the sea cucumbers was determined to be about 3.2% WW (of wet weight) and 6.6% WW for *P. tremulus* and *C. frondosa*, respectively. It is approximately $35.5\ g$ protein per kg for *P. tremulus* and $65.5\ g$ protein per kg for *C. frondosa*. Essential amino acids make up about $9.5\ g$ per kg for *P. tremulus* and $11.7\ g$ per kg for *C. frondosa*.

This study showed that both total amino acid (TAA) content and essential amino acid (EAA) content differed significantly between the *P. tremulus* and *C. frondosa*. Significant differences were found for all amino acids except for glycine, methionine and proline.

There are recommendations for intake of various nutrients, including amino acids. One method of assessing protein quality is to determine its ability to cover the requirements of essential amino acids (EAAs). Figure 4.1 shows the contribution of EAAs from the intake of $100\ g$ of sea cucumber based on the results of this study. The results of this study were based on the recommendations of the Institute of Medicine (2005). Average weight is set at $70\ kg$ per person.

Figure 4.1 shows that consumption of $100\ g$ of *C. frondosa* and *P. tremulus* does not cover daily recommended intake for all the essential amino acids. Both species meet the need for threonine, while *C. frondosa* also covers daily recommended intake for phenylalanine + tyrosine. *P. tremulus*, on the other hand, almost covers the daily recommended intake for phenylalanine + tyrosine. Given a consumption of $150\ g$ of *C. frondosa* and P. *tremulus* respectively, daily recommended intake for all essential amino acids, but histidine, would have been covered. Since several of them are just below the recommended daily intake at $100\ g$.

The results showed that the sea cucumbers contained all EAA except tryptophan which is destroyed in acid hydrolysis and thus not detected or reported. Bechtel et al. (2013) reported that EAA often are restricted to methionine, threonine, lysine and tryptophan. The limiting EAAs in this study were histidine and lysine, which were also reported by Wen, Hu, and Fan (2010) for eight different sea cucumbers, including methionine. While Bechtel et al. (2013) reported that histidine and methionine were the limited EAAs for *Parastichopus californicus*.

In general, the EAA contents were about $1/4$ of total amino acid (TAA) content and the ratio between EAA/TAA was $0.27\ mg/g$ and $0.28\ mg/g$ for *P. Tremulus* and *C. frondosa*, respectively. Wen, Hu, and Fan (2010) reported an EAA/TAA ratio varied from 0.38 to 0.60 for eight different sea cucumbers, with *Holothuria fuscogilva* having the lowest value and *T. ananas* having the highest. Compared to these species investigated by Wen, Hu, and Fan (2010),

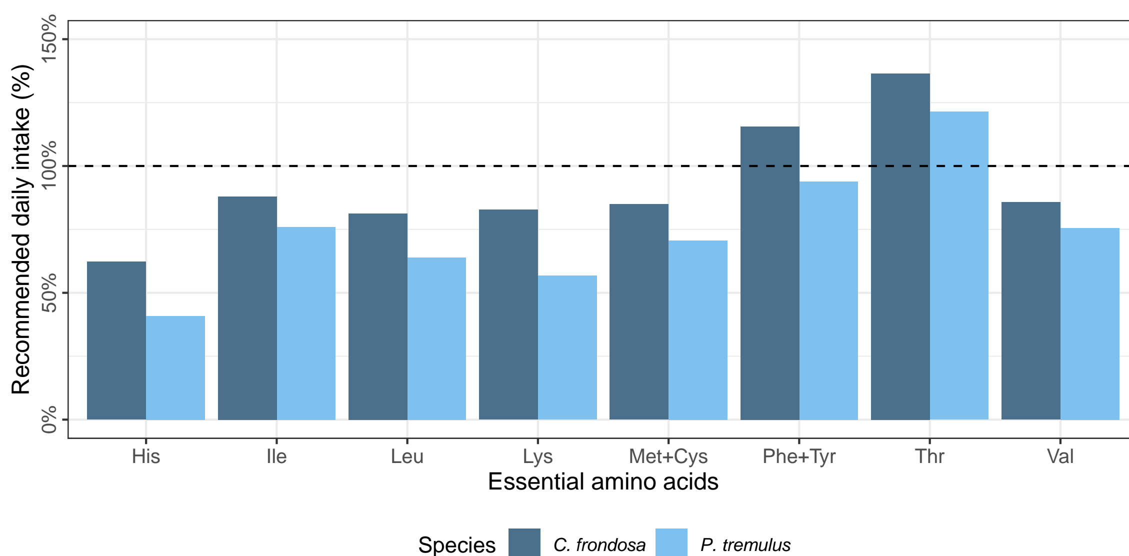


Figure 4.1: Estimated intake of various essential amino acids from 100 g of *Cucumaria frondosa* (dark blue) and *Parastichopus tremulus* (light blue), respectively. The estimates were made for a 70 kg person based on Institute of Medicine (2005) recommendations (dotted line). (The recommended daily intake in mg per 70 kg of the various amino acids is; Histidine (His) = 980 mg/day, Isoleucine (Ile) = 1330 mg/day, Leucine (Leu) = 2940 mg/day, Lysine (Lys) = 2660 mg/day, Methionine+Cystine (Met+Cys) = 1330 mg/day, Phenylalanine+Tyrosine (Phe+Tyr) = 2310 mg/day, Threonine (Thr) = 1400 mg/day and Valine (Val) = 1680 mg/day for a 70 kg person. Both Met+Cys and Phe+Tyr were merged as the article used to calculate the recommended daily intake operated with this data merged).

the EAA/TAA ratio of *P. tremulus* and *C. frondosa* species in this study is low. The overall EAA value of *P. tremulus* and *C. frondosa* is likely lower due to tryptophan being destroyed during conventional hydrolysis procedures.

Wen, Hu, and Fan (2010) make no mention of the eight sea cucumbers being harvested at a time close to spawning. This may skew the comparison considerably, and thus, comparisons may be hampered. For example, *P. tremulus* may not be comparable with *C. frondosa* or the other eight species of sea cucumber as *P. tremulus* in this study was harvested at a time of year when the nutritional content may have been sub-optimal due to spawning. This may cause the composition of protein and contents of amino acids to differ from the content reported from a different season.

4.3 | Fatty acid composition

Fatty acid composition (% of total fatty acids) and the total amount of fatty acids per $100\ g$ are shown in Table 3.3. The fatty acid (FA) composition showed that more than $1/3$ of the fat in *P. tremulus* consisted of PUFAs (37.0%). The majority of these FAs were n-3 fatty acids, with EPA accounting for approximately 12.6%, DPA 1.2% and DHA accounting for 7.8%. This combined with a low n-6 content (11.8%) gives *P. tremulus* a low n-6/n-3 ratio of 0.5. The most abundant FA in *P. tremulus* was EPA with 12.5%, while the second most abundant FA was arachidonic acid (ARA) (8.1%).

C. frondosa has a relatively similar FA composition, saturated, monounsaturated and polyunsaturated, compared to *P. tremulus*. *C. frondosa* contained most of both MUFAs (30.5%) and PUFAs (41.8%) of the two species. EPA accounted for as much as 30.5% in the PUFAs, while palmitoleic acid (16:1n-7) was the second most abundant FA and the most abundant MUFA. The n-6/n-3 ratio in *C. frondosa* was even lower with a value of 0.1.

As for $g/100\ g$, the trend is quite similar to that of % of total fatty acids. Both species have the highest content of PUFA with $0.13\ g$ for *P. tremulus* and $0.68\ g$ for *C. frondosa*. Followed by MUFA and PUFA for both species, with $0.10\ g$ and $0.6\ g$ for *P. tremulus* respectively. Whereas *C. frondosa* had a higher content with $0.50\ g$ of MUFA and $0.18\ g$ of SFA.

P. tremulus and *C. frondosa* generally had a higher content of MUFAs and PUFAs when compared to values reported by Wen, Hu, and Fan (2010) for eight different sea cucumbers. The SFA content was lower than reported for *A. japonicus* (Jiang et al. 2013; Gao et al. 2016). The content of unsaturated FAs was much higher than of saturated FAs, and PUFAs accounted for over 40% of the fatty acids. The proportions of PUFAs was similar to that of tuna and higher than in salmon (Strobel, Jahreis, and Kuhnt 2012), while both *P. tremulus* and *C. frondosa* had a lower SFA than Carp (Tkaczewska, Migdał, and Kulawik 2014).

Mozaffarian and Rimm (2006) recommend a daily intake of between 250-500 mg of EPA+DPA per day to lower the risk of coronary heart disease (CHD) death by 25%. I have chosen to use $250\ mg$ in this study, as Mozaffarian and Rimm (2006) state that the efficacy above $250\ mg$ per day provides minimal additional reduction of CHD mortality. By consuming $100\ g$ of *C. frondosa*, caught in January, you gain more than 100% (EPA+DPA+DHA $533mg/100g$) than the minimum recommended daily intake of EPA and DPA (Figure 4.2). While consumption of $100\ g$ of *P. tremulus*, caught in June, did not and by far, fulfil the recommended daily intake of EPA and DHA(EPA+DPA+DHA $74mg/100g$) (Figure 4.2).

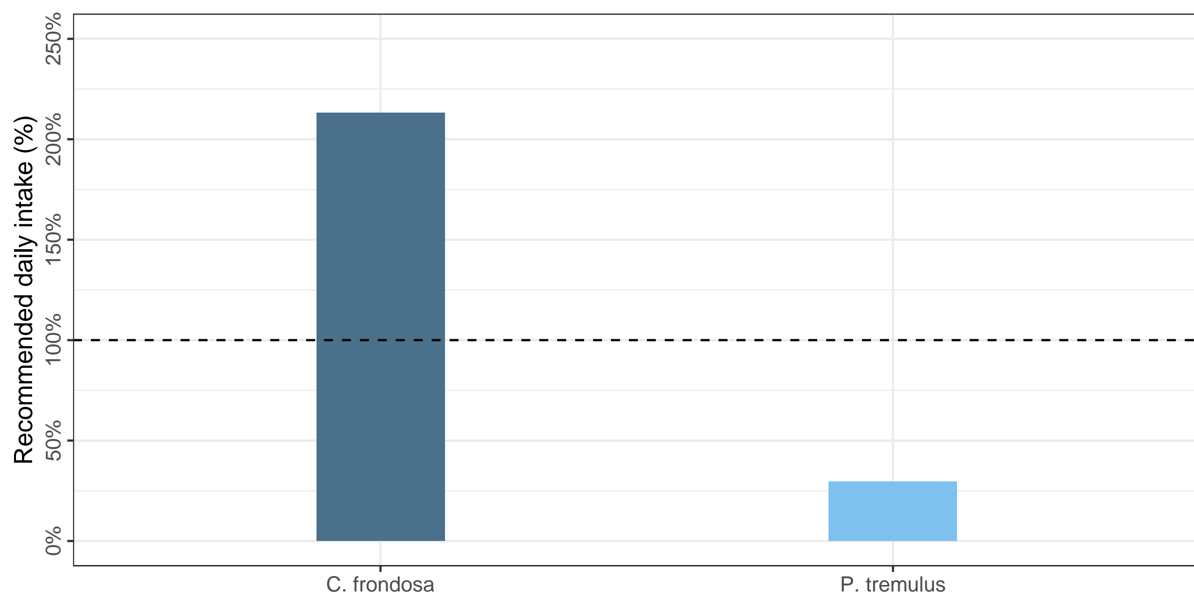


Figure 4.2: The relative ability to cover daily requirements of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) by $100\ g$ sea cucumber. Based on the recommendation from Mozaffarian and Rimm (2006) of a minimum of $250\ mg$ EPA and DHA per day. $100\% =$ dotted line. Dark blue = *Cucumaria frondosa* and light blue = *Parastichopus tremulus*.

4.4 | Antioxidant capacity

In this study, we used two of the most common methods for measuring antioxidant capacity (AOC) in tissues, which are ferric reducing antioxidant power (FRAP) assay and oxygen radical absorbance capacity (ORAC) assay. In addition, several papers use ABTS [2,2’- azinobis-(3-ethylbenzothiazoline-6-sulfonate)]- assay and DPPH [(1,1-Diphenyl-2- picrylhydrazyl)]-assay (Pérez-Jiménez et al. 2008). Comparison of antioxidative capacity among these methods is difficult as they often use different standards (e.g., Trolox equivalents). Moreover, they measure different mechanisms and are conducted under different conditions such as pH and temperature. In this study, the results are presented as wet weight and might be difficult to compare to values presented as dry weight DW. By using FRAP and ORAC the results of this study, therefore, provides a better insight into the AOC of *P. tremulus* and *C. frondosa* than only using one method.

The results of this study show significant differences in AOC of *P. tremulus* and *C. frondosa*. In essence, *C. frondosa* had lower antioxidative efficiency than *P. tremulus*, as demonstrated by the FRAP- and ORAC-assays. Based on the ORAC-assay, *P. tremulus* had more than twice the antioxidative capacity than *C. frondosa*. At the same time, FRAP-assay found *P. tremulus* to be six times more efficient than *C. frondosa*. In a study by Zhong, Khan, and Shahidi (2007) *C. frondosa* had a content of 2.09-2.60 $mmol$ of TE/g dry weight DW, which is substantially larger than found for *C. frondosa* in this study (3.1 $\mu mols$ of TE/g wet weight). Keep in mind that this comparison of wet weight and dry weight DW might be misleading. Additionally, Zhong, Khan, and Shahidi (2007) had a different method of preparing the samples, using methanol as an extraction solvent, and repeated extractions, which may lead to higher concentrations of antioxidants.

The use of ORAC and FRAP in this study showed relatively low values of antioxidant capacity. This is partly a result of not optimising the samples for measurement of antioxidant capacity, as studies show that hydrolysis (Neklyudov, Ivankin, and Berdutina 2000; Chalamaiah et al. 2012), enzymatic proteolysis or aspects of denaturation (Aluko 2015) expose otherwise inaccessible active amino acids and are shown to increase the AOC.

Several amino acids are shown to possess larger antioxidative properties, due to the presence of reactive groups (i.e., hydroxyl, carboxyl and aromatic units) and in terms of whole proteins, active amino acid residues may be shielded by the conformation of the protein (Aluko 2015). Thus, if the protein structure, degree of denaturation found in *P. tremulus* differs from that of *C. frondosa*, this might explain the higher antioxidative capacity in spite of the lower protein content of *P. tremulus*. Additionally, variation may be explained by trace elements (*e.g.,* selenium), organic antioxidants (*e.g.,* carotenoids) or differing presence or absence of amino acids between the two species (Table 3.2).

Seasonal effects may have a profound influence on antioxidant capacity. Pertaining to this study, light conditions at the time of harvest may have affected the antioxidant capacity of the two sea cucumber species. In a study by Chua et al. (2015), light intensity and temperature were found to be negatively correlated with antioxidant capacity in the plant *Premna serratifolia*. In short, seasonal light conditions may not explain the higher antioxidant capacity of *P. tremulus* in this study. A reasonable explanation, however, is the difference in depth at which the *P. tremulus* and *C. frondosa* occur. Given the attenuation of light at 15 meters (*C. frondosa*) versus at 200 meters (*P. tremulus*) it is likely that *P. tremulus* is more shielded from light and, thus, expend less of their antioxidant capacity due to free radicals caused by exposure to sunlight. Furthermore, considering seasonal variation in vertical distributions of water temperature, it is likely that the temperature at 200 meters depth is on average lower than at 15 meters depth.

4.5 | Limitations

The findings of this study are subject to limitations through study design. Comparisons of biochemical composition are only representative for the difference between *P. tremulus* harvested in June and *C. frondosa* harvested in January. Thus, this study pertains only to this specific comparison, and as discussed seasonal variations may cause differences in chemical composition. Additional deviance in chemical composition may result from observational errors, more specifically systematic errors. However, all analyses were performed in triplicates and only minor deviances around the mean were observed within batches.

On a positive note, there are several applications for the results found in this study. The results may serve for comparisons in future studies and provide insight into the biochemical composition of *P. tremulus* harvested in June, and *C. frondosa* harvested in January. Pertaining to commercial harvest it seems crucial to account for seasonal variation in biochemical composition to ensure that optimal quality of sea cucumber is achieved.

5 | Conclusion

In conclusion, *P. tremulus* and *C. frondosa* appear to have a beneficial biochemical composition for human consumption. Even if it is difficult to conclude due to the research design, there seems to be a difference in the biochemical composition between the two species. Therefore, we recommend that future studies account for time of harvest and geographical variation for improved validity of comparisons.

References

AkvaplanNIVA (2019). *Kunnskapsgrunnlag for nye arter i oppdrett. Utredning for Norges forskningsråd, Område for ressursnæringer og miljø*. URL: https://www.akvaplan.niva.no/prosjekter-nettverk/nye-arter/.

Aluko, R. E. (2015). "Amino acids, peptides, and proteins as antioxidants for food preservation". In: *Handbook of Antioxidants for Food Preservation*. Elsevier Ltd, pp. 105–140. DOI: 10.1016/B978-1-78242-089-7.00005-1.

Arterburn, L.M., E.B. Hall, and H. Oken (2006). "Distribution, interconversion, and dose response of n− 3 fatty acids in humans". In: *The American Journal of Clinical Nutrition* 83.6, pp. 1467–1476. DOI: 10.1093/ajcn/83.6.1467S.

Association of Official Analysis Chemists International (2005). *Official Methods of Analysis of AOAC International*. ISBN: 0935584544.

Bechtel, P.J. et al. (2013). "Chemical composition of the giant red sea cucumber, *Parastichopus californicus*, commercially harvested in Alaska". In: *Food Science & Nutrition* 1.1, pp. 63–73. DOI: 10.1002/fsn3.12.

Benzie, I.F.F. and J.J. Strain (1996). "The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay". In: *Analytical Biochemistry* 239.1, pp. 70–76. DOI: 10.1006/abio.1996.0292.

Chalamaiah, M. et al. (2012). "Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review". In: *Food Chemistry*. Vol. 135. 4. Elsevier, pp. 3020–3038. DOI: 10.1016/j.foodchem.2012.06.100.

Chen, J. (2004). "Present status and prospects of sea cucumber industry in China". In: *FAO Fisheries and Aquaculture Technical Paper* 463, pp. 25–38. URL: http://www.fao.org/3/a-y5501e.pdf.

Chua, I. Y.P. et al. (2015). "Influence of light intensity and temperature on antioxidant activity in *Premna serratifolia* L." In: *Journal of Soil Science and Plant Nutrition* 15.3, pp. 605–614. DOI: 10.4067/S0718-95162015005000027.

Conand, C. (2004). "Present status of world sea cucumber resources and utilization: An international overview". In: *FAO Fisheries and Aquaculture Technical Paper* No. 463, pp. 13–23. URL: https://www.researchgate.net/publication/285166239_Present_status_of_world_sea_cucumber_resources_and_utilization_An_international_overview.

Dávalos, A., C. Gómez-Cordovés, and B. Bartolomé (2004). "Extending Applicability of the Oxygen Radical Absorbance Capacity (ORAC-Fluorescein) Assay". In: *Journal of Agricultural and Food Chemistry* 52.1, pp. 48–54. DOI: 10.1021/jf0305231.

Dj Ratoe Oedjoe, M. (2017). "Composition of Nutritional Content of Sea Cucumbers (*Holothuroidea*) in Mania Waters, Sabu Raijua Regency, East Nusa Tenggara". In: *Journal of Aquaculture Research & Development* 08.07. DOI: 10.4172/2155-9546.1000502.

Dong, Y. et al. (2006). "Effects of diel temperature fluctuations on growth, oxygen consumption and proximate body composition in the sea cucumber *Apostichopus japonicus* Selenka". In: *Aquaculture* 255.1-4, pp. 514–521. DOI: 10.1016/j.aquaculture.2005.12.013.

FAO (2003). *Food energy - methods of analysis and conversion factors*. Ed. by FAO. Vol. 77. Rome (Italy): FAO Food and Agriculture organization of the United Nations. ISBN: 92-5-105014-7.

Folch, J., M. Lees, and G.H. Sloane (1957). *A simple method for the isolation and purification of total lipids from animal tissues*, pp. 497–509. URL: https://www.ncbi.nlm.nih.gov/pubmed/13428781.

Fredalina, B.D. et al. (1999). "Fatty acid compositions in local sea cucumber, *Stichopus chloronotus*, for wound healing". In: *General Pharmacology* 33 (4), pp. 337–340. DOI: 10.1016/s0306-3623(98)00253-5.

Gao, F., Q. Xu, and H. Yang (2011). "Seasonal biochemical changes in composition of body wall tissues of sea cucumber *Apostichopus japonicus*". In: *Article in Chinese Journal of Oceanology and Limnology* 29 (2), pp. 252–260. DOI: 10.1007/s00343-011-0041-7.

Gao, Y. et al. (2016). "Proximate composition and nutritional quality of deep sea growth sea cucumbers (*Stichopus japonicus*) from different origins". In: *Journal of the Science of Food and Agriculture* 96 (7), pp. 2378–2383. DOI: 10.1002/jsfa.7353.

Hamel, J. F. and A. Mercier (1996a). "Evidence of chemical communication during the gametogenesis of holothuroids". In: *Ecology* 77.5, pp. 1600–1616. ISSN: 00129658. DOI: 10.2307/2265555.

Hamel, J.F. and A. Mercier (1995). "Spawning of the sea cucumber *Cucumaria frondosa* in the St Lawrence Estuary, eastern Canada". In: *SPC Beche-de-mer Information Bulletin*, pp. 12–18. URL: https://www.researchgate.net/publication/239582652_Spawning_of_the_sea_cucumber_Cucumaria_frondosa_in_the_St_Lawrence_Estuary_eastern_Canada.

Hamel, J.F. and A. Mercier (1996b). "Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (*Echinodermata*: *Holothuroidea*)". In: *Canadian Journal of Fisheries and Aquatic Sciences* 53, pp. 253–271. DOI: 10.1139/cjfas-53-2-253.

Han, Q., J. K. Keesing, and D. Liu (2016). "A Review of Sea Cucumber Aquaculture, Ranching, and Stock Enhancement in China". In: *Reviews in Fisheries Science and Aquaculture* 24 (4), pp. 326–341. DOI: 10.1080/23308249.2016.1193472.

Institute of Medicine (2005). *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. National Academies Press, pp. 1–1331. DOI: 10.17226/10490.

Jiang, S. et al. (2013). "Comparative study on nutrient composition and growth of green and red sea cucumber, *Apostichopus japonicus* (Selenka, 1867), under the same culture conditions". In: *Aquaculture Research* 44 (2), pp. 317–320. DOI: 10.1111/j.1365-2109.2011.03033.x.

Kjerstad, M., I. Bjørkevoll, and G. Christophersen (2020). *Sesongmessig variasjon i kvalitet hos rødpølse [Seasonal variation in quality of Norwegian red sea cucumber]*. Rapport nr. MA 20-02. Personally acquired from Kjerstad, M., pp. 1–51.

Kjerstad, M. and J. E. Rønneberg (2005). *Utnytting av rødpølse i fjordane i Møre og Romsdal [Utilisation of Norwegian red sea cucumber in the fjords of Møre and Romsdal]*. Rapport Nr. Å0501. Closed report from Møreforskning; Personally acquired from Kjerstad, M., pp. 1–38.

Kjerstad, M. et al. (2015). "Preliminary study on the utilisation of Norwegian red sea cucumber, *Parastichopus tremulus* (Gunnerus, 1767) (*Holothuroidea*, *Echinodermata*), from Norwegian waters: resource, biology and market". In: URL: www.marbef.org.

Lobo, V. et al. (2010). "Free radicals, antioxidants and functional foods: Impact on human health". In: *Pharmacognosy Reviews* 4 (8), pp. 118–126. DOI: 10.4103/0973-7847.70902.

Madsen, F.J. and B. Hansen (1994). *Echinodermata, Holothurioidea*. Vol. 9. Scandinavian University Press, p. 147. URL: https://www.forskningsdatabasen.dk/en/catalog/2398160525.

Moore, S. and W.H. Stein (1963). "Chromatographic determination of amino acids by the use of automatic recording equipment". In: *Methods in enzymology: Liposomes*. Vol. 6. Elsevier, pp. 819–831. DOI: 10.1016/0076-6879(63)06257-1.

Mozaffarian, D. and E.B. Rimm (2006). "Fish Intake, Contaminants, and Human Health Evaluating the Risks and the Benefits". In: *Clinical Review Clinician´s Corner* 296 (15), pp. 1885–1900. DOI: 10.1001/jama.296.15.1885.

Nærings og Fiskeridepartementet (2015). "Masterplan for marin forskning". URL: https://www.regjeringen.no/no/dokumenter/masterplan-for-marin-forskning/id2437919/.

Neklyudov, A.D., A.N. Ivankin, and A.V. Berdutina (2000). "Properties and Uses of Protein Hydrolysates (Review)". In: *Prikladnaya Bioldaimiya i Mikrobiologiya* 36 (5), pp. 525–534.

Norwegian Seafood Council (2020). *Sjømateksport for 107,3 milliarder kroner i 2019*. URL: https://seafood.no/aktuelt/nyheter/sjomateksport-for-1073-milliarder-kroner-i-2019/ (visited on 04/27/2020).

Olsen, R.L. (2017). *Lipidkjemi med vekt på fisk [Lipid chemistry with an emphasis on fishes]*. In compendium. Kompendium utgave 4 [Compendium no. 4].

Pérez-Jiménez, J. et al. (2008). "Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results". In: *Food Research International* 41 (3), pp. 274–285. DOI: 10.1016/j.foodres.2007.12.004.

Purcell, S.W. et al. (2010). “Managing sea cucumber fisheries with an ecosystem approach”. In: *FAO Fisheries and Aquaculture Technical Paper* 520, pp. 1–157. URL: http://www.fao.org/3/i1384e/i1384e00.htm.

Rustan, A.C. and C.A. Drevon (2005). “Fatty Acids: Structures and Properties”. In: *Encyclopedia of Life Sciences*, pp. 1–7. DOI: 10.1038/npg.els.0003894.

Salarzadeh, A.R. et al. (2012). “Proximate composition of two sea cucumber species *Holothuria pavra* and *Holothuria arenicola* in Persian Gulf”. In: *Annals of Biological Research* 3 (3), pp. 1305–1311. URL: https://pdfs.semanticscholar.org/7e02/afa840e823f53a31a9bb80fe3cf21b6753c4.pdf.

Shearer, K.D. et al. (1994). “Whole body elemental and proximate composition of Atlantic salmon (*Salmo salar*) during the life cycle”. In: *Journal of Fish Biology* 44.5, pp. 785–797. DOI: 10.1111/j.1095-8649.1994.tb01255.x.

Spackman, D.H., W.H. Stein, and S. Moore (1958). “Automatic Recording Apparatus for Use in the Chromatography of Amino Acids”. In: *Analytical Chemistry* 30 (7), pp. 1190–1206. DOI: 10.1021/ac60139a006.

Stoffel, W., F. Chu, and E.H. Ahrens (1959). “Analysis of Long-Chain Fatty Acids by Gas-Liquid Chromatography: Micromethod for Preparation of Methyl Esters”. In: *Analytical Chemistry* 31 (2), pp. 307–308. DOI: 10.1021/ac60146a047.

Strobel, C., G. Jahreis, and K. Kuhnt (2012). “Survey of n-3 and n-6 polyunsaturated fatty acids in fish and fish products”. In: *Lipids in Health and Disease* 11 (144), pp. 1–10. DOI: 10.1186/1476-511X-11-144.

Tan, M. et al. (2018). “Effect of multiple freeze-thaw cycles on the quality of instant sea cucumber: Emphatically on water status of by LF-NMR and MRI”. In: *Food Research International* 109, pp. 65–71. DOI: 10.1016/j.foodres.2018.04.029.

Tkaczewska, J., W. Migdał, and P. Kulawik (2014). “The quality of carp (*Cyprinus carpio* L.) cultured in various Polish regions”. In: *Journal of the Science of Food and Agriculture* 94 (14), pp. 3061–3067. DOI: 10.1002/jsfa.6661.

Toral-Granda, V. et al. (2008). “Sea cucumbers : a global review of fisheries and trade”. In: *FAO Fisheries and Aquaculture Technical Paper* No. 516, p. 331. URL: http://www.fao.org/3/i0375e/i0375e00.htm.

Uthicke, S. et al. (2004). “Overfishing of holothurians: lessons from the Great Barrier Reef”. In: *FAO Fisheries and Aquaculture Technical Paper* No. 463, pp. 163–173. URL: http://agris.fao.org/agris-search/search.do?recordID=XF2016031090.

Vasconcelos, P., M.B. Gaspar, and M. Castro (2009). “Influence of growth and reproductive cycle on the meat yield and proximate composition of *Hexaplex trunculus* (*Gastropoda*: *Muricidae*)”. In: *Journal of the Marine Biological Association of the United Kingdom* 89 (6), pp. 1223–1231. DOI: 10.1017/S0025315409003026.

Wen, J., C. Hu, and S. Fan (2010). “Chemical composition and nutritional quality of sea cucumbers”. In: *Journal of the Science of Food and Agriculture* 90 (14), pp. 2469–2474. DOI: 10.1002/jsfa.4108.

Wu, G. (2009). “Amino Acids”. In: 37.1, pp. 1–17. DOI: 10.1007/s00726-009-0269-0.

Zhong, Y., M.A. Khan, and F. Shahidi (2007). “Compositional characteristics and antioxidant properties of fresh and processed sea cucumber (*Cucumaria frondosa*)”. In: *Journal of Agricultural and Food Chemistry* 55.4, pp. 1188–1192. DOI: 10.1021/jf063085h.