NORWEGIAN COLLEGE OF FISHERIES SCIENCE

Proximate composition and ACE-inhibitory peptides from two species of bivalve mollusks.

(Mytilus edulis and Pecten maximus)

Chris Christensen

Master's Degree Thesis in Fisheries Science
Field of Study - Fisheries and aquaculture (60 credits)

May 2012

Acknowledgements

The work behind this thesis was carried out at the Norwegian College of Fishery Science, University of Tromsø. I would like to thank my supervisor Karl-Erik Eilertsen for the encouragement and help through my work. Writing this thesis has undoubtedly been the most challenging and interesting part of my education. I would not have been able to do this without your help.

Special thanks to Hanne K. Mæhre for all the help in the lab, help with the writing process and for having the impressive patience to deal with a whole year of silly questions from me. And thanks to Ida-Johanne Jensen for help in the lab, and making sure I did not get lost in Vietnam.

Thanks to Karina for taking care of me during especially stressful times, and supporting me in my quest to travel the world and ascend mountains, cliffs, rocks, trees and other objects. I would also like to thank the girls; Natasha, Makoto for being my supportive friends, and Taiana and for being a small and aggressive source of inspiration. Lastly I would thank my family for their help and regular Sunday dinners.

Chris Christensen
Tromsø , May 2012

Sammendrag/Norwegian summary

Å kjenne til sammensetningen av makronæringsstoffer som fett og proteiner er nødvendig for å forstå eventuelle helsemessige effekter av ulike matvarer. Forskning på marine matvarer har i stor grad vært rettet mot lipider og helserelaterte effekter av disse. Anbefalt daglig inntak av eicosapentaenoic acid (EPA) og docosahexaenoic acid (DHA) er 0,2 til 0,5g/dag, noe man kan oppnå med to porsjoner fet fisk i uken. Men også sammensetningen av proteiner og forholdet mellom de forskjellige aminosyrene har betydning for de helsemessige effektene av næringsmidler. Kjennskap til aminosyresammensetningen viser seg derfor å være et viktig aspekt for å kunne forklare de helsemessige effektene forbundet med enkelte næringsmidler. Ved enzymatisk hydrolyse i fordøyelsen kan man få dannet peptider med biologiske aktiviteter, for eksempel blodtrykkssenkende peptider og peptider med antioksidativ kapasitet.

Hovedmålet med denne oppgaven har vært å fastslå sammensetningen av lipider og aminosyrer, i tillegg til vann og askeinnhold i blåskjell og kamskjell. Evnen til å hemme angiotensin converterende enzym (ACE) ble også undersøkt. I tillegg ble det undersøkt hvilken effekt to ulike typer tilberedning hadde på de ulike faktorene.

Resultatene viste en tydelig forskjell i mengde aminosyrer og lipider, og ulik sammensetning av aminosyrer mellom de to artene. Lipidsammensetningen var ulik for de to artene, men begge hadde en typisk marin lipidprofil med høy mengde EPA og DHA. Koking viste seg å ha større effekt på sammensetningen enn hurtig steking. ACE-hemming var kun påvirket av koking, men så ikke ut til å påvirkes av tilberedning når man justerte for proteininnhold.

Konklusjonen er at både kamskjell og blåskjell er gode kilder til EPA og DHA, gode proteinkilder med stor mengde essensielle aminosyrer. Kamskjell og blåskjell har ACE-hemmende effekt og effekten ser ut til å ha sammenheng med proteinmengden.

Summary

Knowing the composition of macronutrients such as fats and proteins are necessary for understanding the potential health effects of various foods. Research on marine foods have largely been directed towards lipid and health effects of these. The recommended daily intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is 0.2 to 0.5 g / day, which that can be achieved with about two servings of fatty fish a week. But also the composition of proteins and the relationship between the different amino acids are important for the health effects of foods. Knowledge of amino acid composition appears therefore to be an important aspect in order to explain the health effects associated with certain foods. During the enzymatic hydrolysis in the digestion process peptides with biological activities can be formed, such as antihypertensive peptides and peptides with anti-oxidative capacity.

The main objective of this study was to determine the composition of lipids and amino acids, in addition to water and ash content in mussels and scallops. The ability to inhibit angiotensin-converter enzyme (ACE) was also investigated. In addition, the effects of household preparation on the various factors was investigated.

The results showed a clear difference in the amount of amino acids and lipids, and the different composition of amino acids between the two species. Lipid composition was different for the two species, but both had a typical marine lipid profile with high amounts of EPA and DHA. Boiling appeared to have greater effect on the composition than panfrying. ACE inhibition was only affected by boiling, but when adjusted for protein content this effect was not significant.

The conclusion is that both the scallops and mussels are good sources of EPA and DHA, good sources of protein with a large proportion of essential amino acids. Scallops and mussels have ACE-inhibitory effect and the effect appears to be related to the amount of protein.

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Introduction

Cardiovascular disease (CVD) is the leading cause of death in the world. According to the WHO ischaemic heart disease and stroke constitute 12 and 10 percent, respectively, of worldwide deaths every year (WHO 2011). Elevated blood pressure (BP), or hypertension, is seen as one of the most important risk factors for developing CVD, and a reduction in BP in a population is assumed to have a significant impact on development and mortality of CVD (Rédei 2008). Despite great therapeutic advances the prevalence of hypertension is increasing (Chobanian 2009).

While blood pressure lowering drugs are generally accepted as safe and effective they are not without side effects. For patients with mild hypertension, alternative methods of reducing BP such as diet, activity and naturally occurring compounds are therefore preferred. It is therefore of great interest to further explore food items and bioactive compounds that can have a beneficial effect on human health.

The interest for marine bioprospecting is rapidly increasing. The marine environment is not well explored in this regard, compared to terrestrial species. Marine animals have unique challenges in their environment and contain a variety of diverse molecules that are used to deal with the environoment they are exposed to (Chin, Balunas *et al.* 2006).

Bivalves are a large group of mollusks, currently numbering around 9200 different species, the majority of which are filter feeders. A high amount of n-3 PUFA, taurine and the possibility of novel bioactive molecules make bivalve mollusks an interesting group of animals for marine bioprospecting.

The purpose of this study was to look at proximate composition of two different species of bivalve mollusks, blue mussels (*Mytilus edulis)* and scallops (*Pecten maximus*). Their ability to inhibit angiotensin converting enzyme (ACE) was also tested. Finally the effects of normal household preparation techniques on these factors were examined.

Blood pressure and cardiovascular disease

Cardiovascular disease

CVD is the biggest contribution to total healthcare expenditures in USA, direct and indirect cost for CVD and related conditions was around 297 billion in 2008. Between 2012 and 2030 these cost are projected to triple. A reduction in CVD will therefore have a great economic impact (Roger, Go *et al.* 2012).

Some of the risk factors associated with CVD such as increased age, gender and genetic predispositions are not possible to change. The most common modifiable risk factors affecting arteries are cigarette smoking, hypertension, and hypercholesterolemia (Wong, Wilson *et al.* 1991).

Atherosclerosis is a disease affecting elastic arteries. Coronary, carotid, cerebral, renal arteries and the aorta are often affected. Atherosclerotic plaques consist of fat, cholesterol, various inflammatory cells and other substances. These plaques can cause problems with blood flow in the affected area, or they can break loose and cause stenosis or thrombosis in other organs in the body. Although the obvious consequence of plaques seems to be the mechanical effect on blood flow other risk factors are associated with them. A high degree of inflammatory cells in plaques seem to increase the risk of fatal events. Certain drugs like statins are also able to reduce clinical events associated with established atherosclerosis without reducing the size of plaques (Cohen and Rudd 2006a)

The primary event in the formation of atherosclerotic plaques is probably endothelial dysfunction, damage to endothelial cells can lead to lipid accumulation. Endothelial cells synthesize nitrogen oxide (NO) which works as a powerful inhibitor of platelet aggregation and has anti-inflammatory properties. If the endothelium breaks down it become more adhesive to inflammatory cells and platelets. Endothelial function is improved by certain drugs, including statins and ACE-inhibitors (Cohen and Rudd 2006c).

The link between hypertension and CVD

Hypertension is often seen in combination with other cardiovascular risk factors such as diabetes mellitus, hyperinsulinemia, dyslipidemia, inflammation and endothelial dysfunction and obesity(Cachofeiro, Miana *et al.* 2009; Cassis and Police 2010; Zhang, Thompson *et al.* 2010). The risk of coronary artery disease more than doubles among patients with hypertension and dyslipidemia, when compared to those with either condition alone. A history of smoking and use of alcohol also contribute to cardiovascular morbidity (Criqui, Mebane *et al.* 1982; Johnson, Pietz *et al.* 2004). The connection between glucose metabolism and hypertension is well known in animal models. The best known example is fructose induced hypertension in rat (Hwang, Ho *et al.* 1987; Tran, Yuen *et al.* 2009). Human studies have shown that improvement of insulin sensitivity has a positive effect on blood pressure (Manrique, Lastra *et al.* 2009).

Renin-angiotensin-aldosterone-system

The Renin-angiotensin-aldosterone-system (RAAS) plays an important role in the cardiovascular system, controlling extracellular fluid volume, sodium excretion, and other vascular effects. RAAS over activity is associated with hypertension, atherosclerosis and other CVD (Widmaier 2007).

Renin converts the inactive angiotensinogen to angiotensin I (AngI). The zinc metallo-peptidase Angiotensin-I-converting-enzyme (ACE) will form angiotensin II (AngII) by removing the dipeptide His-Leu from AngI. AngII is a powerful vasoconstrictor, and will promote sodium retention by increasing tubular reabsorption of sodium. RAAS systems are found in various tissues in the body. Kidneys, heart, brain and vessels contain angiotensin that are either expressed locally or imported from the circulation (Bader 2010). In the kidneys, AngII is formed from systemically delivered angiotensinogen and AngI, as well as from locally produced angiotensinogen. Transgenic mice who overexpress AngII in the kidneys develop hypertension and renal injury without increasing circulating AngII. This effect means ACE-inhibitors can lead to decreased BP by reducing local AngII production without affection plasma ACE (Bader 2010).

Angiotensin II is, in addition to sodium retention and vasoconstriction, a promotor of inflammatory substances and oxidative stress in kidneys and vascular tissues. There is also evidence showing that increasing oxidative stress in animals can cause up-regulation of AngII and hypertension (Vaziri and Rodriguez-Iturbe 2006). In normal physiological situations (non-pathological) production of AngII in the RAAS does not lead to oxidative stress or inflammation. Increased levels of AngII in a high sodium diet led to oxidative stress in rats (Kitiyakara, Chabrashvili *et al.* 2003). In humans oxidative stress and inflammation resulting from hypertension is confined to the kidneys and cardiovascular tissues (Vaziri and Rodriguez-Iturbe 2006).

Kinin-kallikrein system

ACE affects the kinin-kallikrein system by degrading kinin, which is a vasodilating substance. ACE-inhibitors can therefore reduce hypertension by increasing kinin concentration. Kinin has known cardio- and renal protective actions and might be responsible for some of the beneficial effects seen with use of ACE-inhibitors (Bader 2010).

Hypertension

Its estimated that blood-pressure related disease causes the death of about 8 million people a year, the majority of cases occur in low-income and middle-income countries (Abegunde, Mathers *et al.* 2007). Despite a high amount of blood-pressure related diseases compared to high-income countries, low and middle-income countries have access to less than 10% of global treatment resources (Lawes, Hoorn *et al.* 2008; MacMahon, Alderman *et al.* 2008).

Blood-pressure (BP) involves two measurements, systolic and diastolic. BP in the range 90-119 mm/hg systolic and 60-79 mm/hg diastolic is considered as normal. Anything above 140/90 is defined as hypertension. Blood-pressure between these stages is referred to as pre-hypertension. In the human body BP is regulated by cardiac output (CO) and peripheral resistance (PR). CO is adjusted by heart rate and stroke volume, PR is adjusted by the changes in smooth muscle in arteries and arterioles (Widmaier 2007).

Hypertension can be divided into primary hypertension, and secondary hypertension. Primary is the most common in adults. It has no identifiable distinct cause and can develop over a course of several years. Secondary hypertension is a result of some underlying condition or can be caused of certain medications. It appears suddenly and can result in higher BP than primary hypertension (Cohen and Rudd 2006c). Although there is no distinct cause for primary hypertension there are several factors associated with chronic elevation in BP, such as smoking, obesity, sedentary lifestyle, sodium sensitivity and high level of alcohol consumption among others (Forman, Stampfer *et al.* 2009).

Hypertension can be seen as a distinct disease or as a major risk factor for cardiovascular disease, it contributes directly to artery disease, stroke, congestive heart failure, renal failure, and peripheral arterial disease. Successful reduction of BP and other cardiovascular risk factors can dramatically reduce the incidence of coronary mortality, especially for those with multiple risk factors and the elderly (Stamler, Stamler *et al.* 1989; Mac Mahon and Rodgers 1993).

BP is strongly related to CVD during middle and older age. Of the known cardiovascular risk factors, hypertension is the most common. The prevalence increases with age, for adults over

65 BP-lowering drugs are among the most common prescription drugs (Sarah, Robert *et al.* 2002; Cohen and Rudd 2006c).

If left untreated hypertension can lead to damage on various organs in the body. Common organs that are affected by hypertension are: heart (diastolic dysfunction, left ventricular hypertrophy, endothelial scarring), large and medium sized arteries (accelerated atherosclerosis, aneurism formation), brain (ischemia, hemorrhagic and thrombotic infarction), and the kidneys (Cohen and Rudd 2006c).

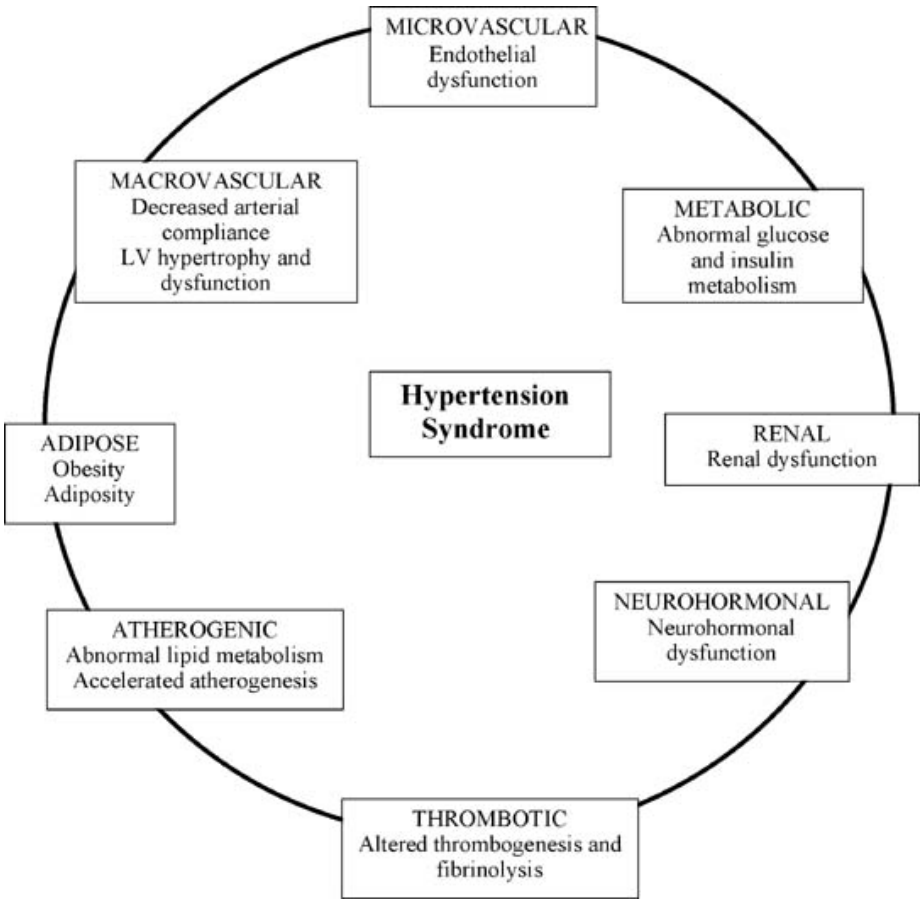

Figure 1 – Hypertension syndrome(Cohen and Rudd 2006c).

Management of hypertension

Management of hypertension can be done in several ways. The different methods can be classified as non-pharmacological and pharmacological. Non-pharmacological involves diet, lifestyle changes and exercise. While non-pharmacological methods may prevent hypertension, combining non-pharmacological and pharmacological has the best effect at reducing BP (Cohen and Rudd 2006c).

Hypertension is a condition that can be influenced by diet and lifestyle, and a modest reduction of BP can greatly reduce the risk of CVD. In a larger population a very small reduction in average BP will greatly affect number of deaths per year due to CVD. Therefore any food item that can lover BP should be considered a possible component of a diet that has the aim of reducing CVD in a population (Boelsma and Kloek 2009).

Pharmacological therapy

Pharmacological treatment is useful if non-pharmacological methods alone are not sufficient at reducing BP, or if patients are showing signs of CVD, target organ damage or stage 2 hypertension. Drugs used for controlling BP can be divided into following groups: Diuretics, antiadrenergics, ACE inhibitors, angiotensin receptor blockers, calcium antagonists and direct vasodilators (Cohen and Rudd 2006c).

The effect of ACE inhibitors as treatment for hypertension is well known, and is regarded as a safe and efficient way of reducing BP (Frank 1989). There are however side effects associated with drugs, and they might not be an optimal solution for people with pre-hypertension (Izzo 2007). The adverse effects of ACE inhibitors include first-dose hypotension, renal dysfunction, hyperkalemia, and persistent dry cough. Because of effects on the fetus it is not recommended to use during pregnancy (Alderman 1996). Due to side effects associated with drugs there is a great interest in alternative therapeutic approaches.

Non- Pharmacological therapy

Diet plays an important role in the prevention and management of CVD. In the case of hypertension it is recommended to reduce sodium intake, increase intake of fruits, vegetables and low-fat dairy. If needed, weight reduction and exercise is also recommended (Cohen and Rudd 2006b).

There have been three large studies focused on macronutrient intake on blood pressure and blood lipids. The Dietary Approaches to Stop Hypertension (DASH), DASH – Sodium, and Optimal Macro-Nutrient Intake to Prevent Heart Disease (Omniheart diet). All of these studies tested the effect of different macronutrient ratios on CVD markers while maintaining a stable bodyweight (Miller, Erlinger *et al.* 2006). The diets were very successful at reducing BP. Hypertensive subjects in the DASH trial had a reduction of 11.4 mm Hg and 5.5 mm Hg systolic and diastolic BP respectively (Appel, Moore *et al.* 1997).

Naturally occurring ACE-inhibitory peptides is one alternative method for treating hypertension. The safety of these molecules makes it an interesting alternative to existing therapies.

The focus in this paper will be on diet, especially the effect of seafood on hypertension and the role of natural occurring ACE-inhibitors in management of hypertension.

Bioactive proteins and health

Certain proteins and peptides can have biological effects that are not due to the individual constituting AA. Bioactive proteins and peptides can be divided into different groups depending on their functionality. Opioids, mineral – binding peptides, anti – oxidative peptides, antimicrobial peptides, immune – and cyto – modulatory peptides as well as enzyme inhibiting peptides (Walther and Sieber 2011). The type of peptide that is formed depends on two factors; type of AA sequence in the protein and the enzyme(s) used to generate the peptides. Presence of certain AA are associated with certain biological activities. Binding to ACE is for instance strongly influenced by the presence of tyrosine, phenylalanine,

tryptophan, proline, lysine, isoleucine, valine, leucine, and arginine (Harnedy and FitzGerald 2011).

Absorption of bioactive proteins over the GI tract is limited due to the size of the proteins, current knowledge indicates that mainly dipeptides and tripeptides can cross the intestional wall and enter the blood. Larger bioactive peptides and proteins can have activity in the GI tract itself by enhancing nutrient absorption, inhibiting enzymes and modulating the immune system (Federal commission for nutrition 2011). Peptides with proline and hydroxyproline are generally more resistant to digestive enzymes, and may therefore have a better chance of reaching the target site intact (Sarmadi and Ismail 2010).

ACE-inhibitory peptides

Several studies on hypertensive rats and humans have shown ACE-inhibitory (ACEI) peptides to be effective at reducing blood pressure. The peptides in these studies had little or no effect on normotensive subjects (Erdmann, Cheung *et al.* 2008). The first commercial product containing ACEI-peptides was a milk based drink containing lacto-tripeptides. It was first sold in Japan in 1991. Some of the best documented ACE-inhibitory peptides found in fermented milk are isoleucine-proline-proline (IPP, IC_{50} = 5µmol/L), and valine-proline-proline (VPP, IC_{50} = 9µmol/L) (Boelsma and Kloek 2009).

Lactopeptides are considered safe. Even in very large doses they had no adverse effect on rats. Milk proteins are a normal part of a human diet in the Western world, and humans are regularly exposed to their hydrolyzed products either by digestion or consumption of fermented products (Boelsma and Kloek 2009).

Marine organisms are an interesting source of biologically active compounds and are still relatively unexplored in this regard compared to other protein-rich foods. Potent ACE-inhibitors have been found in marine sources, and it’s of great interest to isolate other similar compounds (Wijesekara and Kim 2010). *In vitro* potency of marine peptides are comparable to the two lacto-tripeptides IPP and VPP present in commercially sold beverages (Howell and Kasase 2010).

Taurine and CVD risk factors

In addition to the essential AA, methionine, sulfur-containing AA (methionine, cysteine, homocysteine and taurine) are important. Sulfur AA are used in many physiological pathways, glutathione production, protein synthesis and methylation (Brosnan and Brosnan 2006).

Taurine (2-aminoethanesulfonicacid) is an amino acid that does not participate in the formation of peptide bonds, and is therefore only found as a free amino acid. Taurine is counted as a conditionally essential AA. The main source of taurine is through diet, but small amounts can be synthesized from methionine and cysteine (Mark 2005).

Taurine is necessary for several biological functions such as bile acid conjugation, cell membrane stabilization, development of the central nervous system and retina, osmoregulation, modulation of cellular calcium and the immune system. Taurine can also have some effect on regulation of blood pressure, antioxidative capacity, anti-inflammatory effects and cholesterol lowering effects. It might have an effect on CHD, but the data on humans is limited (Harnedy and FitzGerald 2011).

Taurine and blood pressure

Epidemiological studies have shown an inverse relationship between urinary taurine excretion and CVD risk and mortality in humans (Moriguchi, Moriguchi *et al.* 2004; Yamori, Liu *et al.* 2009). These findings are supported by studies on spontaneously hypertensive rats (SHR) (Nandhini and Anuradha 2004; Hu, Xu *et al.* 2009; Yamori, Taguchi *et al.* 2010), and studies on fructose induced hypertension in rat models (Rahman, Park *et al.* 2011).

n-3 FA and blood pressure

Several studies have shown an association between n-3 PUFA and a reduction in blood pressure. However the effects seen are modest, and doses used to achieve this tend to be high, ≥3g per day. The observed effects are greater in older individuals, ≥45 years. No effect has been observed on normotensive subjects. Proposed mechanisms for reduction in BP are reduced production of thromboxane A2, increased nitric oxide (NO) production, improved vascular compliance and an effect on autonomic nerve function (Saravanan, Davidson *et al.* 2010). A recent review confirmed this by finding small but statistically significant BP-lowering effects of fish oil supplements (Campbell, Dickinson *et al.* 2012). Marine n-3 can also be used in combination therapy against hypertension. About one gram of n-3 PUFA used in combination with diuretics and beta-blockers by hypertensive patients gave a reduction of 3 mmHg DSP and 2 mmHg SBP, showing that n-3 PUFA can be a useful addition to pharmacological therapy, even if the effect is only a modest reduction. There was also a 21% reduction of plasma triglycerol and an 15% increase in HDL cholesterol in the group receiving n-3 PUFA (Lungershausen, Abbey *et al.* 1994).

Materials and Methods

Design of study

Two species of bivalve mollusks, blue mussels (*Mytilus edulis)* and scallops (*Pecten maximus*) were investigated in this study.Blue mussels and scallops were purchased from Dragøy AS (Tromsø, Norway)

To examine the effect of preparation scallops and mussels were divided into four subgroups; raw mussels, prepared mussels, raw scallops and prepared scallops. Each subgroup was divided into 5 batches. Weight and yield of the various subgroups are described in table 1.

Blue mussels were steamed until all the mussels were open (2:15 – 2:45 minutes) and scallops were fried at high temperature, without butter or oil, for about one minute on each side. The preparation methods are the most commonly used for the two species, steaming for blue mussels and panfrying for scallops, respectively. Both raw and prepared samples were subsequently homogenized and frozen. Due to a large and variable water content the samples were freeze dried. Both species were freeze dried (VirTis Genesis 35L) until constant weight. The samples were homogenized to a fine powder using a grinder (MF 10 basic, IKA werke) and stored in the dark at room room temperature. The samples were subjected to a simulated gastrointestinal digestion prior to analysis of ACE inhibiting effects.

Water and ash content

The water content was determined according to AOAC method 950.46b (Horwitz 2004). 2 grams of freeze dried sample was dried at 105° C until constant weight.

Ash content was determined according to AOAC method 938.08 (Horwitz 2004). The dried samples were combusted at 500° C for 12 hours.

Protein content and amino acid composition

Free amino acids (FAA) were prepared by homogenizing 0.2 gram of freeze dried material in 9 ml dH_2O and 1 ml 20 mM nor-leucine (N-leu) as an internal standard. The sample was homogenized for 15 seconds using an Ultra turrax (T25 basic, ICA werke), 1 mL 35% sulfosalicylic acid (SSA) was added and the sample was homogenized for another 15 seconds. The sample was centrifuged at 20.000 rpm for 10 minutes, 4°C. The supernatant (0.2 ml) was transferred to an analyzing tube and 0.8 ml lithium citrate buffer (pH 2.2) was added.

Total amino acids were prepared by homogenizing 40 mg of freeze dried material in 700 µL H_2O and 1.2 ml Hydrochloric acid (HCL). 500 µL 20mM N-leu was added as an internal standard. The samples were flushed with N_2 for 10 seconds to prevent oxidation. The sample was hydrolyzed at 105°C for 22 hours. The supernatant (0.1 ml) was dried using N_2 gas and 1.0 ml lithium citrate buffer (pH 2.2) was added.

The amino acids were analyzed chromatographically using an ion exchange column. Buffers with different pH and ionic strength were used according to Spackman *et al* (1958), and compounds were detected by UV-light after post-column derivatization with ninhydrin. The machine used was a Biochrom 30 amino acid analyzer (Biochrom Co, Cambridge, UK). The UV-signals were analyzed with Chromeleon software (Dionex, sunnyvale CA, USA), and compared to A9906 physiological amino acid standard (Sigma Chemicals Co, St Louis, MO, USA).

The formula for calculating the amount of AA from a sample:

$$\left(\left(\frac{nmol\ AA}{nmol\ N-leu}\right)\times(ml\ N-leu\times mM\ N-leu)\right)\div gram\ sample\ =\ \frac{\mu mol\ AA}{gram\ sample}$$

$$\frac{\mu mol\ AA}{gram\ sample}\times molecular\ weight\left(\frac{Gram}{Mol}\right)\div 1000=\frac{mg\ AA}{gram\ Sample}$$

Protein content was calculated from amino acid content using the following formula:

$$\sum Total\ AA\ -\sum Free\ AA=Protein\ bound\ AA$$

Lipid content

The total lipid content was analyzed using a method described by Folch *et al* (1957), the chloroform was substituted with dichloromethane for safety reasons. 1 gram sample was mixed with 20 ml dichloromethane/methanol (2:1 v/v) and mixed for 20-30 minutes. The samples were filtrated and 4 ml 0.9% NaCl was added before the sample was centrifuged at 2000 rpm at 4°C for 10 minutes. The upper, water-soluble layer was removed, and the lower lipid phase was transferred to a round-bottomed flask and dried using an evaporator (RV 10, IKA werke) (400 mbar 40°C). The lipid content was calculated gravimetrically.

Fatty acid composition

The sample was methylated by method described by Stoffel *et al* (1959), with some modifications. Dichloromethane/methanol was added to make an concentration of 10mg lipid per ml. 100 µL of sample was mixed with 900 µL dichloromethane and 2 ml 2% H_2SO_4 in methanol. The mixture was then boiled for one hour. 3.5 ml heptane and 3.5 ml 5% NaCl was added. The heptane phase was transferred to glass tubes and dried using N_2 gas. The sample was resuspended in 100 µL heptane and transferred to analyzing tubes.

The FA profile was determined by gas chromatography, using an Agilent 6890N with a 7683B autoinjector and flame ionization injector (FID) A predefined temperature program was used (50°C for two minutes, then a 10°C per minute increase till 150°C, 2°C per min till 205°C and 15°C till 255°C followed by stabilization for 10 minutes). Some samples were run on a slightly different program to see if separation could be improved, but the FA composition did not differ between the two programs. The FA profile was compared to known standards: PUFA 1, 2 and 3 from Sigma (Sigma Chemicals Co, St Louis, MO, USA).

Simulated digestion

Simulated digestion was performed according to Dragnes (2008). Enzymatic hydrolysis was performed using the following enzymes: pepsin (Sigma P6887), trypsin (Sigma T4665), and chymotrypsin (Sigma C4129). The relationship between the sample and amount of enzyme used was 1:250 for all enzymes (0.05g enzyme: 12.5g sample). Samples were mixed with water and pH was adjusted to 2 to simulate conditions in the stomach. Pepsin was added and the sample was kept at 37°C on a magnetic stirrer for two hours. To simulate the intestinal phase the pH was adjusted to 6.5 before trypsin and chymotrypsin was added. The mixture was incubated for an additional 2.5 hours. The samples were frozen and freeze dried for later use.

ACE-assay

ACE-assay was performed according to Dragnes (2008). 200 mg of digested sample and 10 ml of borate buffer (pH 8.3) was mixed. Dilution series of 2.0, 1.2, 1.0, 0.8, 0.6, 0.4, 0.2 and 0.1µg/µl was prepared in borate buffer and 25µl was transferred to a 48 well titer plate, giving end concentrations of 50, 30, 25, 20, 15, 10, 5 and 2.5 µg. 100 µL of N-hippuryl-Histidyl-Leucine-tetrahydrate (HHL) was added to each well. Borate buffer was used as a standard. The plate was incubated for 30 minutes at 37°C.
After incubation, 50 µL (1mU/10µL) of Angiotensin converting enzyme (ACE) was added, and the plate was incubated for additional 30 minutes. 215 µL of 1M HCl was added to stop the reaction.

HPLC

The method used was a modified version of Wu *et al* (2002). The analysis was performed using a Waters 2695 separation module equipped with a Waters 2996 photodiode detector (Waters Corporation, MA, USA), using a Symmetry Shield reverse phase column (Waters 20 x 3.0 mm id 3.5 µm). The mobile phases used were 0.05% trifluoroacetic acid (TFA) in dH_2O and acetonitrile (ACN) at 30°C. A 2 minute linear gradient was used, flow rate was set at 2.0 ml/min. UV-detection was set at 228nm, and injection volume was 10µL Identification of HA and HHL was performed using the software Millennium (Waters Corporation, MA, USA).

ACE-inhibition was determined as IC_{50}, or the amount of material that would inhibit 50 percent of the enzyme activity.

Statistics

Statistics were performed using SPSS 19.0 (SPSS Inc, Chicago, Il, USA). An independent T-test was used to compare two variables. A P-value of less than 0.05 was considered significant for this test. The variances were equal in all samples.

Results

Shell and edible content

Table 1 - Shell weight and weight of edible tissue in blue mussels and scallops. Weight is the average for each subgroup ± standard deviations.

	Blue mussels, raw	Blue mussels, cooked	Scallops, raw	Scallops, cooked
		Average weight per batch (g)		
Number of batches	5	5	5	5
Weight with shell	486.0 ± 24.8	485.6 ± 25.4	531.6 ± 33.7	526.5 ± 24.8
Weight without shell	175.5 ± 43.3	128.3 ± 31.5	73.8 ± 3.7	66.2 ± 6.7
Yield %	35.9 ± 8.2	26.5 ± 6.3	13.9 ± 0.4	12.6 ± 1.3

Mussels contained more edible tissue than scallops, and the difference in yield was greater between raw and cooked mussels than scallops.

Proximate composition

Table 2 shows the proximate composition in wet weight mussels and scallops. There was a significant difference between water, protein, and lipids between raw and steamed mussels. In scallops there was a small but significant difference in water, protein and ash after preparation. The difference between the two species (raw samples) was significant for all variables.

Table 2 - Water, protein, lipids and ash in wet weight mussels and scallops (n=5). Values are given as mean ± standard deviations. ¤ indicates significant difference between raw and cooked mussels, * indicates a significant difference between raw mussels and raw scallops, † indicates a significant difference between raw scallops and cooked scallops.

	Blue mussels, raw	Blue mussels, cooked	Scallops, raw	Scallops, cooked
		g/100g		
Water	82.2 ± 1.4	78.0 ± 0.8 ¤	78.0 ± 0.6 *	76.0 ± 0.4 †
Protein	5.2 ± 0.2	8.1 ± 0.2 ¤	11.4 ± 0.2 *	12.8 ± 0.1 †
Lipids	1.3 ± 0.2	1.8 ± 0.3 ¤	0.5 ± 0.2 *	0.6 ± 0.1
Ash	1.4 ± 0.1	1.3 ± 0.0	1.6 ± 0.0 *	1.7 ± 0.0 †

Amino acids

In mussels free AA was significantly reduced and total AA was significantly increased after preparation. The change in sulfur-containing AA was non-significant. In scallops there was almost no significant difference in AA content between raw and prepared samples. The only difference was a small but significant change in free alanine and total cysteine after preparation.

Free amino acids are presented in table 3. Total amino acids are presented in table 4.

Table 3 – Free amino acids in raw and prepared blue mussels and scallops (n=5). Values are given as a mean ± standard deviations and in mg/g sample (dry weight). ¤ indicates significant difference between raw and cooked mussels, * indicates a significant difference between raw mussels and raw scallops, † indicates a significant difference between raw scallops and cooked scallops. n.a = not analyzed. n.d = not detected. EAA = essential amino acids.

	Blue mussels raw	Blue mussels, cooked	Scallops, raw	Scallops, Cooked
EAA		mg/g		
Histidine	1.3 ± 0.2	1.1 ± 0.2	n.d	n.d
Isoleucine	0.6 ± 0.0	0.5 ± 0.1 ¤	n.d	n.d
Leucine	0.9 ± 0.1	0.7 ± 0.1 ¤	n.d	n.d
Lysine	2.9 ± 0.9	2.4 ± 0.2	n.d	n.d
Methionine	1.1 ± 0.1	0.7 ± 0.1 ¤	n.d	n.d
Phenylalanine	0.8 ± 0.1	0.6 ± 0.1 ¤	n.d	n.d
Threonine	2.0 ± 0.2	1.4 ± 0.0 ¤	n.d	n.d
Tryptophan	n.a	n.a	n.a	n.a
Valine	1.2 ± 0.1	0.8 ± 0.1	n.d	n.d
Sum EAA	10.7 ± 0.6	8.2 ± 0.5 ¤	n.d	n.d
Non-EAA				
Arginine	3.9 ± 1.0	4.3 ± 0.5	14.4 ± 3.8 *	13.5 ± 2.8
Cysteine	0.6 ± 0.2	0.4 ± 0.1	n.d	n.d
Glutamine	2.1 ± 0.1	2.0 ± 0.3	n.d	n.d
Glycine	11.5 ± 0.4	9.7 ± 0.5 ¤	67.5 ± 2.2 *	66.2 ± 2.9
Tyrosine	1.4 ± 0.1	1.1 ± 0.1 ¤	n.d	n.d
Alanine	15.2 ± 0.8	10.7 ± 0.9 ¤	3.7 ± 0.9 *	2.6 ± 0.4 †
Taurine	26.5 ± 1.8	22.1 ± 0.9 ¤	44.3 ± 4.4 *	47.3 ± 2.6
B-Alanine	1.1 ± 0.2	0.9 ± 0.1	n.d	n.d
Aspartic acid	1.7 ± 0.3	1.9 ± 0.1	n.d	n.d
Glutamic acid	6.0 ± 3.4	6.1 ± 0.4	n.d	n.d
Proline	3.2 ± 0.3	2.6 ± 0.4 ¤	n.d	n.d
Serine	5.1 ± 1.1	4.1 ± 0.4	n.d	n.d
Ornithine	0.3 ± 0.0	0.3 ± 0.1 ¤	n.d	n.d
Sum Non EAA	78.5 ± 4.1	66.2 ± 3.4 ¤	129.9 ± 5.8 *	129.6 ± 4.7
Sum AA	89.2 ± 4.2	74.3 ± 3.9 ¤	129.9 ± 5.8 *	129.6 ± 4.7
Sulfuric AA	28.1 ± 0.7	23.3 ± 0.4 ¤	44.3 ± 4.4 *	47.3 ± 2.6
UREA	67.2 ± 5.4	49.1 ± 9.4 ¤	n.d	n.d
NH3	0.5 ± 0.4	0.2 ± 0.1	n.d	n.d

Table 4 - Free amino acids in raw and prepared blue mussels and scallops (n=5). Values are given as a mean ± standard deviations and in mg/g sample (dry weight). ¤ indicates significant difference between raw and cooked mussels, * indicates a significant difference between raw mussels and raw scallops, † indicates a significant difference between raw scallops and cooked scallops. n.a = not analyzed. n.d = not detected. EAA = essential amino acids.

	Blue mussels raw	Blue mussels, cooked	Scallops, raw	Scallops, Cooked
EAA		mg/g		
Histidine	8.6 ± 0.3	9.6 ± 0.3 ¤	9.3 ± 0.3 *	9.4 ± 0.3
Isoleucine	14.6 ± 0.7	18.1 ± 0.8 ¤	24.6 ± 1.0 *	24.9 ± 1.1
Leucine	23.9 ± 1.2	29.7 ± 1.4 ¤	46.5 ± 1.5 *	48.3 ± 1.9
Lysine	30.0 ± 0.9	36.2 ± 1.7 ¤	49.8 ± 1.5 *	51.0 ± 1.6
Methionine	8.6 ± 0.4	7.8 ± 3.9	17.1 ± 0.7 *	17.8 ± 0.9
Phenylalanine	11.9 ± 6.0	17.9 ± 0.7	21.8 ± 0.6 *	22.0 ± 1.0
Threonine	20.2 ± 0.9	24.0 ± 1.0 ¤	25.1 ± 1.0 *	26.1 ± 1.1
Tryptophan	n.a	n.a	n.a	n.a
Valine	13.1 ± 6.6	19.3 ± 0.9	23.8 ± 1.2 *	23.3 ± 1.4
Sum EAA	130.9 ± 12.3	162.6 ± 9.0 ¤	217.9 ± 7.8 *	222.9 ± 10.1
Non-EAA				
Arginine	26.0 ± 1.3	32.2 ± 1.8 ¤	54.9 ± 4.3 *	55.8 ± 4.1
Cysteine	3.8 ± 0.3	4.9 ± 0.4 ¤	2.5 ± 0.2 *	3.2 ± 0.3 †
Glycine	29.5 ± 1.3	32.0 ± 0.9 ¤	91.2 ± 2.4 *	89.8 ± 3.0
Tyrosine	12.1 ± 3.4	16.3 ± 0.8 ¤	17.7 ± 0.8 *	18.6 ± 1.0
Alanine	30.7 ± 1.7	31.7 ± 1.3	35.5 ± 1.2 *	36.0 ± 1.9
Taurine	26.0 ± 2.4	22.8 ± 2.4 ¤	45.5 ± 3.4 *	46.8 ± 2.4
Aspartic acid	27.3 ± 0.8	33.9 ± 1.3 ¤	43.4 ± 1.7 *	44.9 ± 1.6
Glutamic acid	52.0 ± 3.2	60.4 ± 2.2	93.1 ± 3.4 *	96.4 ± 3.7
Proline	17.3 ± 1.1	19.9 ± 1.8 ¤	15.5 ± 1.0 *	15.8 ± 1.2
Serine	22.4 ± 1.0	26.3 ± 0.8 ¤	28.4 ± 1.2 *	29.3 ± 1.3
Sum Non EAA	247.0 ± 29.2	280.3 ± 7.9 ¤	427.9 ± 16.0 *	437.3 ± 17.6
Sum AA	377.9 ± 34.6	442.9 ± 15.3 ¤	645.8 ± 23.7 *	659.6 ± 27.2
Sulfuric AA	38.3 ± 3.2	35.4 ± 3.3	65.2 ± 4.4 *	67.7 ± 3.2
UREA	n.d	n.d	n.d	n.d
NH3	4.0 ± 0.4	4.0 ± 0.2	5.5 ± 0.3 *	5.5 ± 0.1

Lipid content

The lipid content in raw blue mussels ranged from 1.09 to 1.77 g/100g. Steamed mussels had a slightly higher fat percentage than raw, ranging from 1.41 to 2.27 g/100g. The difference between raw and prepared samples was significant. The amount of fat in scallops was very low, 0.52 and 0.58 g/100g in raw and prepared scallops, respectively. The difference between raw and prepared was not significant.

Palmitic acid (C16:0) and palmitoleic acid (C16:1 n-7) accounted for a significant proportion of the FA in mussels, over 30 percent combined. The other big contributors were EPA (C20:5 n-3) and DHA (C22:6 n-3), which accounted for 22 and 12 percent of total fatty acids, respectively. Scallops also had a large percentage of palmitic acid, but little palmitoleic acid. EPA and DHA contributed to about 45 percent of the total FA.

There was a small, but significant difference in certain FA after processing in mussels. Total amount of SFA, MUFA, PUFA, n3 and LC-n3 was not significantly affected by processing. In scallops, C22:5n3 and C22:6n3 was significantly increased after processing, which resulted in a significant increase in PUFA, n3 and LC-n3 Most FA was significantly different when the two species were compared. Preparation had little effect on FA composition in either mussels or scallops.

Lipid composition is presented in table 5 and 6, whereas mean amount of lipids in wet sample are found in table 2.

Table 5 – Main FA composition in raw and prepared mussels and scallops. Composition is expressed as g/100g. # n6/n3 is described as a ratio between the two types of FA. * indicates a significant difference between raw mussels and raw scallops, † indicates a significant difference between raw scallops and cooked scallops.

	Blue mussels, raw	Blue mussels, cooked	Scallops, raw	Scallops cooked
			FA g/100g lipids	
SFA	21.4 ± 0.4	21.4 ± 0.4	27.9 ± 2.9 *	28.2 ± 0.4
MUFA	23.1 ± 0.6	22.5 ± 1.0	9.4 ± 2.6 *	8.6 ± 0.8
PUFA	45.7 ± 1.3	46.6 ± 2.5	48.3 ± 2.2 *	51.9 ± 0.7 †
n3	37.7 ± 0.9	38.7 ± 1.7	48.3 ± 2.1 *	52.0 ± 0.7 †
Lc-n3	36.4 ± 0.9	37.4 ± 1.7	45.5 ± 2.0 *	49.1 ± 0.9 †
n6/n3#	1:4 ± 0.2	1:4 ± 0.3	1:20 ± 0.1 *	1:20 ± 0.1

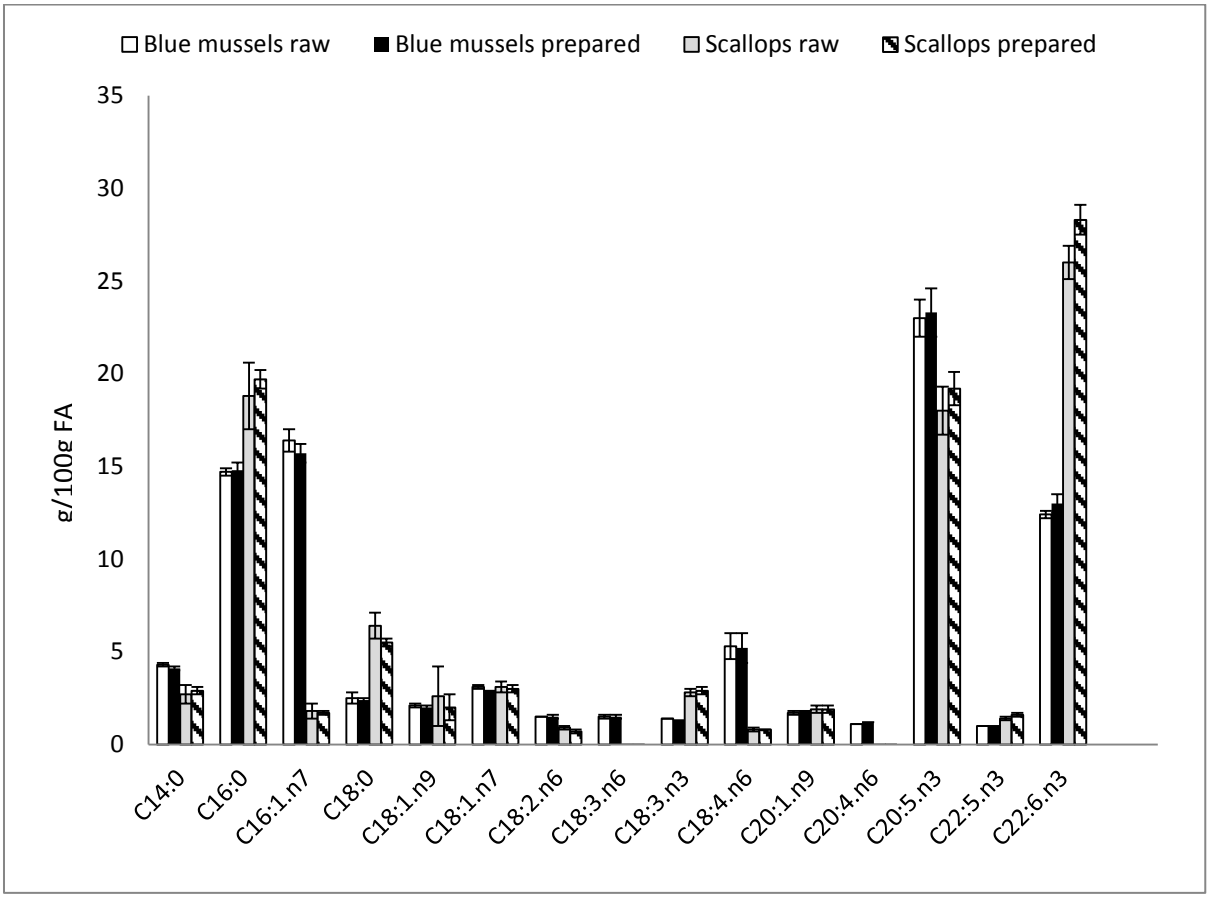

Figure 2 - Main FA composition in raw and prepared mussels and scallops. Composition is expressed as g/100g lipids.

ACE-inhibition

Mussels

Table 7 – Values for IC_{50} 1mU, mussels and scallops freeze dried sample. ¤ indicates significant difference between raw and cooked mussels, * indicates a significant difference between raw mussels and raw scallops.

	Blue mussels, raw	Blue mussels, cooked	Scallops, raw	Scallops, cooked
			IC_{50} µg/mU	
Freeze dried	4.1 ± 0.2	3.5 ± 0.3 ¤	2.1 ± 0.5 *	1.9 ± 0.4

In mussels there was a significant difference between raw and prepared samples. The difference in IC_{50} between raw and prepared scallops was not significant.

Discussion

Water and ash

Different effect from the two types of food preparation techniques was clearly seen, where boiling had a larger effect on composition than quick panfrying. Studies looking at grilling of other food items has seen a larger change in composition than in this study, but samples are usually subjected to higher temperatures and for a longer time than used in this study (Weber, Bochi *et al.* 2008). There was a small, but significant loss of water after preparation of scallops. There was a significant difference in ash and AA content between raw and prepared scallops in the wet samples, but there was no significant difference between raw and prepared dry samples.

The difference in ash content between raw and prepared mussels was not significant in the wet samples, in the dry samples there was a significant loss of ash after preparation. When comparing mussels and scallops it appears that ash content and water loss during preparation was connected. Losses of water lead to a loss of inorganic water soluble compounds (WSC), which resulted in a loss of total ash content. Boiling of other types of seafood has been shown

to have similar effect, loss of moisture and (WSC) leads to an increase in relative lipid and amino acid content (Weber, Bochi *et al.* 2008).

Amino Acids

Protein content was higher in scallops than in mussels, this is consistent with data from NIFES who state 10 and 18 g/100g protein for mussels and scallops, respectively (Nifes 2012a; Nifes 2012b). Amount of protein bound AA in dry samples was 288.6 mg/g in raw mussels and 368.6mg/g in cooked mussels whilst in scallops protein bound AA was 515.9mg/g and 529.9mg/g in raw and cooked respectively. Loss of water soluble compounds during food preparation means that a higher relative amount of sample will consist of insoluble compounds such as protein-bound AA.

Scallops and mussels had similar relative amount of essential and sulfur containing AA. Scallops contained more essential AA and sulfur AA per 100g since they have a higher total amount of AA. Both species have all eight analyzed essential AA and can therefore be considered a high quality protein source. As the ninth AA (tryptophan) is destroyed during acid hydrolysis, no results for this AA could be analyzed.

Blue mussels contained a wide variety of free AA. They are commonly consumed as a whole organism, and contain a larger variation of AA than food items that mostly consists of muscle tissue. The large amount of urea and other waste products not commonly found in muscle are other signs that the AA are from a whole organism. In wet sample there was approximately 1.2g urea per 100 g edible raw mussels, and 1.0g per 100g cooked mussels. Preparation of blue mussels led to a loss of most of the free AA, and an increase in total AA in dry samples. The total AA is mostly bound in muscle tissue and organs so the increase is likely to be due to loss of water during preparation. Loss of water and WSC such as free AA are observed in other marine food items (Chiou, Tsai *et al.* 2004)

FAA from scallops consists mainly of glycine and taurine, some arginine and small amounts of alanine. Scallops contain more protein, total AA, free AA and taurine than mussels, a difference that was significant when comparing the raw samples. Glycine and taurine have been shown to be important for osmoregulation in other bivalves and are probably important

for osmoregulation in scallops (Hosoi, Kubota *et al.* 2003). Scallops contained more taurine than mussels and the amount was not affected by preparation. The preparation of scallops led to little loss of water which may explain the good retention of taurine. Loss of glycine and taurine is observed in other processing methods used on seafood (Larsen, Stormo *et al.* 2007).

There are several possible explanations for the low protein content in mussels found in this study compared to other studies. One is methodical differences. The most commonly used method for protein determination is the Kjeldahl method based on total nitrogen. As mussels contain a large amount of non-protein nitrogen (i.e. FAA and urea), a method based on analysis of total nitrogen would probably over-estimate the protein content. Another possible explanation is seasonal variation. In blue mussels nitrogen excretion peaks prior to spawning, and declines during fall and early winter. Stress, food availability and salinity will also affect utilization of protein reserves (Elizabeth 2007). Bivalves have poor ability to change intracellular levels of fluid, and must therefore adjust cellular volume by changing the concentration of certain amino acids and other small molecules. As direct uptake of FAA is limited the main source of FAA is whole body protein. Changes in salinity can lead to a major loss of nitrogen, and may lead to stress and increased mortality in a bivalve population (Elizabeth 2007). Other species of bivalve mollusks are known to lose large amounts of protein after spawning, and will rebuild the nitrogen storages during the fall and winter (Davis and Wilson 1983).

Taurine

When calculated for water loss mussels and scallops contained around 500 and 1000 mg taurine respectively per 100 g edible tissue; a 100g serving would provide far more taurine than what is normally consumed in an average Western diet. The amounts found in this study correspond well other studies investigating taurine content in food (Laidlaw, Grosvenor *et al.* 1990; Dragnes, Larsen *et al.* 2009; Wójcik, Koenig *et al.* 2010). Taurine content in dry samples decreased significantly after steaming of blue mussels, and was not affected by frying of scallops. In wet samples the relative taurine content increased in both species due to loss of water during processing.

Marine bivalves are generally the food group containing the highest amount of taurine (Simpson, Allen *et al.* 1959; Allen 1961). Taurine and other free amino acids are used for osmoregulation and taurine content will therefore depend on the mollusk growth conditions such as salinity (Zurburg and De Zwaan 1981; Burg and Ferraris 2008).

Average intake of taurine varies greatly depending on diet. One British study estimated the average daily intake to be around 58mg/day for non-vegans, while an American study estimated average intake among non-vegans to be 123mg/day (Rana and Sanders 1986; Stapleton, Charles *et al.* 1997). There is no recommended daily intake for taurine.

Taurine has shown beneficial effects in the prevention of stroke and CVD in SHR and stroke prone hypertensive rats (SHRSP). Feeding the SHRSP taurine-rich fish protein in a salt reduced diet resulted in a reduction in BP and incidence of stroke decreased from 80 to 10 percent. (Yamori, Taguchi *et al.* 2010). These findings are supported by other SHR studies (Nandhini and Anuradha 2004; Hu, Xu *et al.* 2009), and studies on fructose induced hypertension in rat models (Rahman, Park *et al.* 2011). There are different suggestions for BP lowering mechanisms of taurine. Production of NO, increased kallikrein system activity, reduction in epinephrine and norepinephrine and indirect improvement by improving glucose metabolism are mechanisms that have been suggested (Anitha Nandhini and Anuradha 2002; Wójcik, Koenig *et al.* 2010).

A study comparing two Japanese populations of Okinawan ancestry, one in Japan and one in Brazil, revealed a higher rate of obesity and hypertension in the Brazilian population. Fish intake, taurine excretion, and plasma EPA and DHA were far lower in the Brazilian population (Moriguchi, Moriguchi *et al.* 2004). Other epidemiological studies have shown an inverse relationship between urinary taurine excretion and CVD risk and mortality (Yamori, Liu *et al.* 2009). In female Japanese high school students higher intakes of taurine was associated with lower levels of plasma glucose and an improved lipid profile (Ishikawa, Arai *et al.* 2010).

Lipids

Lipid content in bivalves varies seasonally and depends on food availability, reproductive status and growth. Spawning especially has a large impact on lipid levels as lipids are used in the production of eggs that are released in the water, resulting in a loss of lipids (Narváez, Freites *et al.* 2008).

Average amount of lipids per 100g blue mussels was 1.3 g and 1.8 g in raw and prepared mussels, respectively. This is consistent with values of 1g/100g from NIFES (2012a). Lipid content in scallops was lower than expected when compared to values from NIFES 1g/100g (2012b). This might be due to varying lipid-content as a result of gametogenesis as all shells contained roe. In other species of scallops, gonad size is inversely related to lipid content in muscle. The lowest lipid amount (0.36 g/100g) for this species was observed close to spawning (Beltrán-Lugo, Maeda-Martínez *et al.* 2006). This is supported by observations of increased lipid content in gonads in *Pecten maximus* near spawning (Pazos, Román *et al.* 1997). Although some individual FAs changed significantly after processing there was no difference in saturated, monounsaturated or polyunsaturated fat between raw and prepared mussels. In scallops EPA and DHA was significantly increased after processing, which resulted in a significant increase in PUFA, n-3 and LC-PUFA when comparing raw and prepared samples. Saturated and monounsaturated fat was not affected by preparation. The change in individual FAs was small in both species and could be a result of the small sample size used in this study. There was a significant difference between raw mussels and raw scallops for all major groups of FAs.

The high amount of EPA and DHA in mussels was typical for fat profiles of food from marine sources. 37 % of FA in mussels was n-3, with EPA being the biggest contributor. FA composition is also consistent with NIFES (2012a). One exception is EPA and DHA composition, EPA was higher and DHA was lower in this study compared to NIFES data, total amount of n-3 was similiar.

The lipid profile in scallops was typical of marine species, with EPA and DHA contributing to about 45 percent of the total FA. The percent of DHA was very high at 27%. Lipid composition in scallops varies seasonally. The highest levels of PUFA are found during sexual maturity in June-July, which coincides with the biggest algae bloom. (Besnard, Lubet

et al. 1989). Most of the individual shells in this paper had roe, which is expected since scallops spawn in the late fall in Norway. This could explain the relatively high relative amount of PUFA found in this study, as well as the low lipid content. Other studies support the observation that there is a selective incorporation of certain FAs in eggs of scallops, but also notes that diet has a great influence on FA composition (Pazos, Román *et al.* 1997).

A 100g serving of prepared scallops would provide 111mg EPA and 164mg DHA, while an equivalent serving of prepared mussels would provide 417mg EPA and 230mg DHA. Despite scallops having a relative higher amount of total FA as n-3 PUFA, mussels is a better source of n-3 PUFA as they contain more fat. Both mussels and scallops can be considered a good sources of EPA+DHA as a 100g serving would satisfy daily requirements.

A minimum of 250 mg EPA+DHA per day is advised. For people with coronary heart disease and for secondary prevention of myocardial infarction approximately 1 g EPA+DHA per day is recommended (Elmadfa and Kornsteiner 2009). For prevention against hypertension the recommended amount may be even higher. With some sources claiming that around 3 grams are needed to have an significant effect (Campbell, Dickinson *et al.* 2012). This is supported by studies investigating hypertriglyceridemia where the recommended amount is 2-4 g/day (Kris-Etherton, Harris *et al.* 2002).

ACE-inhibitors

Preparation significantly improved the IC_{50} value of mussels, but did not affect scallops significantly. Scallops had better ACE inhibition per gram sample, but also contained more protein per gram. Raw scallops had on average more than twice as much protein as raw mussels. The IC_{50} value for raw scallops was almost half of the IC_{50} of raw mussels. When IC_{50} value per mg protein was calculated the difference between samples was not significant. It appears that the most important factor for ACE inhibition in this study was the amount of protein. This is consistent with studies on cod muscle, which show an increase in ACEI activity after heat treatment and enzymatic hydrolysis. But no difference between samples after simulated gastrointestinal digestion (Dragnes 2010).

However, in this the amount and composition of peptides formed as a result of the enzymatic hydrolysis was not determined. It is therefore not possible to determine whether one of the species contains more potent ACEI peptides than the other. The use of different cooking methods and large difference in protein content between species makes it hard to compare blue mussels to scallops in regard to ACE-inhibition.

Effects of ACE-inhibitors on animal and human models

While *in vitro* experiments are useful in the search for biologically active compounds, it's not certain that the results can be reproduced in *in vivo* experiments. Much work has been done on antihypertensive peptides in both animal and human studies. Spontaneously hypertensive rats are commonly used to evaluate the effect of antihypertensive peptides. Several studies have shown antihypertensive effects of peptides in rat models (Liu, Tung *et al.* 2011). Animal studies have also shown that lactopeptides pass the gastrointestional barrier and accumulate in organs (Masuda, Nakamura *et al.* 1996). The lowest dose with an observed effect in human subjects with mild hypertension was 3.07mg/d lactotripeptides. SBP was lowered 4.4 mmHg and DBP was lowered by 2.8 mmHg after 10 weeks compared to a placebo drug. As much as 52.5 mg/day was used in a safety study, SBP decreased by 4.1 mmHg and DBP decreased by 1.8 mmHg. A clear dose-effect relationship has not been discovered (Boelsma and Kloek 2009). In some human trials VPP and IPP has shown promising results (Seppo, Jauhiainen *et al.* 2003; Nakamura, Mizutani *et al.* 2011; Turpeinen, Ehlers *et al.* 2011), while other studies have shown no effect.

Two recent studies tested the effect of IPP and VPP on hypertensive subjects in Denmark and the Netherlands, and no effects on BP were found. Lack of effect in these populations could be due to genetics or diet, and it is possible that the high consumption of dairy in these countries might limit the effect of dairy derived peptides (Engberink, Schouten *et al.* 2008; Usinger, Jensen *et al.* 2010). While several human studies have promising results there are conflicting results. There might be a large variation between populations with regards to effect of ACE-inhibitors. In a meta-analysis it was found that the effect of IPP and VPP was more evident in Asian subjects than Caucasian (Cicero, Gerocarni *et al.* 2010). Difference in *in vitro* and *in vivo* effects might be due to gastrointestinal degradation of the peptide and

factors preventing the peptide reaching the target organ (Martinez-Maqueda, Miralles *et al.* 2012).

ACE-inhibitory peptides have been isolated from a number of marine sources, both from muscle protein and waste products. Salmon, sardine, bonito, tuna, Pollock sea bream, pelagic thresher, fish sauce and yellowfin sola have all shown ACE-inhibition *in vitro*. One of the most potent ACE-inhibitor found in fish was a tripeptide isolated from bonito with a IC_{50} value of 0,32 µM. (Howell and Kasase 2010) The ultrafiltrate of dried bonito was also able to reduce BP in 62% of borderline and mildly hypertensive subjects using 1,5 g/d (Fujita, Yamagami *et al.* 2001). Sardine peptides was effective at reducing BP and improved glucose tolerance by improving insulin sensitivity in SHR models (Otani, Ninomiya *et al.* 2009). The tripeptide Leu–His–Pro was isolated from shrimp *(Acetes chinensis)* protein. The peptide was tested on SHR and was found to have a strong antihypertensive effect. SBP was reduced by 31 and 18 mmHg using dosages at 6mg/kg and 2mg/kg respectively (Cao, Zhang *et al.* 2010).

Recently peptides from jellyfish peptides (JPC) were administered to renovascular hypertensive rats (RVH). SBP and DBP were significantly reduced in the hypertensive rats, while there was no effect on BP in normotensive rats. Earlier tests has determined the IC_{50} value of JPC to be 43 µg/ml. Measurements of AngII showed no changes in plasma concentration, but concentration of AngII in kidneys decreased significantly (Zhuang, Sun *et al.* 2012). A Dutch study found a significant reduction in BP with intake of milk containing lactotripeptides, but no effect on plasma ACE (van der Zander, Jakel *et al.* 2008). As mentioned previously this result can occur from a RAAS activity reduction in various organs, which can happen without reducing plasma RAAS activity (Geleijnse and Engberink 2010). Different ACEI peptides have varying affinity for certain organs in the body. They can therefore have a antihypertensive effect by reducing AngII activity in kidneys, without having any effect on plasma ACE (Zhuang, Sun *et al.* 2012).

A study on marine collagen peptides on Chinese subjects showed a significant reduction in DBP and SBP compared to controls. Renal function, insulin sensitivity, glucose and lipid metabolism also seemed to improve in the group receiving the peptides. The subjects tested had type 2 diabetes and primary hypertension (Zhu, Li *et al.* 2010).

Several studies in rats have shown a strong anti-hypertensive effect of ACE-inhibitory peptides. In humans the results have been more uncertain, ranging from a strong antihypertensive effect to none at all. The influence of genetics and diet is unknown. Further research is needed to investigate a difference in effect between populations, and mechanisms to explain such an effect

Conclusion

Changes in proximate composition were clearly affected differently by the two preparation methods. Quick panfrying of scallops had little effect on AA, lipids, water and ash. Normally one would use butter or oil, which might have given a different result. Blue mussels were affected by steaming which led to a loss of water, ash and some free AA. This resulted in an increase in lipids and protein bound AA per 100 grams.

Both species have a good lipid profile with high relative amount of n-3 PUFA, although the total amount of fat was relatively low. Around 100g fried scallops or 50 g cooked mussels are needed to satisfy daily marine n-3 PUFA requirements for most individuals.

The amount of taurine in both species was very high and could possibly be a component of a diet that has the goal of reducing hypertension.

There was ACE-inhibiting activity found in both species. Scallops had the best ACE-inhibitory activity and this was not affected by food preparation methods. Mussels had a lower inhibitory effect, but cooking improved the activity. However when considering the protein content both species performed equally well and there was no significant improvement after preparation. It’s difficult to predict what, if any, effect the ACE-inhibitors present could have *in vivo* as this depends on several variables. Identifying the various fractions with ACE-inhibiting activity and animal studies are needed to identify the usefulness of peptides from mussels and scallops.

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