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Review

Bioactive compounds from marine mussels and their effects on human health

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ABSTRACT

The consumption of marine mussels as popular seafood has increased steadily over the past decades. Awareness of mussel derived molecules, that promote health, has contributed to extensive research efforts in that field. This review highlights the bioactive potential of mussel components from species of the genus *Mytilus* (*e.g. M. edulis*) and *Perna* (*e.g. P. canaliculus*). In particular, the bioactivity related to three major chemical classes of mussel primary metabolites, *i.e.* proteins, lipids, and carbohydrates, is evaluated. Within the group of proteins the focus is mainly on mussel peptides *e.g.* those obtained by bio-transformation processes, such as fermentation. In addition, mussel lipids, comprising polyunsaturated fatty acids (PUFAs), are discussed as compounds that are well known for prevention and treatment of rheumatoid arthritis (RA). Within the third group of carbohydrates, mussel polysaccharides are investigated. Furthermore, the importance of monitoring the mussel as food material in respect to contaminations with natural toxins produced by microalgae is discussed.

Contents

Abbreviations: AA, amino acid; AMP, antimicrobial peptide; ASP, amnesic shellfish poisoning; AZP, azaspiracid shellfish poisoning; CFP, ciguatera fish poisoning; COX, cyclooxigenase; DHA, docosapentaenoic acid; DSP, diarrhetic shellfish poisoning; EPA, eicosapentaenoic acid; HAB, harmful algal bloom; LO, lipoxygenase; NSP, neurotoxic shellfish poisoning; PSP, paralytic shellfish poisoning; PUFA, polyunsaturated fatty acid.

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1. Introduction

It is predicted that, approximately 2,210,000 distinct life forms exist in the ocean, from which only around 190,000 species have been catalogued so far (Mora, Tittensor, Adl, Simpson, & Worm, 2011). The phylum Mollusca represents one of the largest and most diverse groups of marine animals. The Bivalvia, a large class with around 20,000 species (Chapman, 2009) within Mollusca, includes some of the best known invertebrates such as clams, oysters, scallops, and mussels and is represented at all depths and in all marine environments. Molluscan shells, including those from bivalves, have been used as tools, containers, religious symbols, and decorations since ancient times. Large populations, particularly those living in coastal areas, *e.g.* aboriginal groups, have relied on these animals for a substantial portion of their diet (Brusca & Brusca, 1990). Apart from the New Zealand green-lipped mussel, few reports available in the public domain deal with the traditional use of mussels against diseases. The sauce from a decoction of *Mytilus edulis*, for instance, is traditionally used in China for its immune strengthening properties and to treat liver and kidney dysfunctions, as well as impotence and menoxenia (Li & Ding, 2006). Nowadays, molluscan shellfish, including bivalves, are harvested commercially and are of considerable relevance for aquaculture industries worldwide. Farmed marine mussels from the *Mytilidae* family, comprising genera, such as *Mytilus* and *Perna*, are popular in human diet, providing high levels of proteins, omega-3 polyunsaturated fatty acids (PUFAs), iodine, and carbohydrates.

Considering the close relation between food and health, bioactive mussel components have proven to play a vital role for the development of functional foods, defined as food with specific beneficial health effect beyond simple nutrition, or nutraceuticals, describing a union between nutrition and pharmaceutics (Bernal, Mendiola, Ibanez, & Cifuentes, 2011; Haller, 2010; Lordan, Ross, & Stanton, 2011). Moreover, relatively high volumes of mussel wastes in aquaculture and processing, prompted researchers to focus on this underexplored source for bioactives (Harnedy & FitzGerald, 2012; Kim & Mendis, 2006). Over the past decades, bioactive properties of mussel components have been investigated by many researchers and several dietary supplements, containing mussel extracts, have been brought to the market. For example Lyprinol®, a dietary supplement product containing the lipid extract of the green-lipped mussel, *Perna canaliculus*, is sold almost worldwide as an anti-inflammatory and anti-arthritic remedy. Hence, the importance of marine mussels as source for bioactive substances, such as *e.g.* antimicrobial, anti-inflammatory, as well as anti-cancer agents, is increasing rapidly. In this review article, we focus on mussel primary metabolites comprising peptides, lipids, and carbohydrates considering their bioactive properties, as well as different classes of shellfish toxins and their impact on human health.

1.1. Literature search and data evaluation concerning mussel bioactives

This review covers literature up to January 2013 and is based on the combination of surveys in three scientific databases, *i.e.* SciFinder Scholar (Chemical Abstracts Service-http://www.cas.org/products/sfacad/index.html), ISI Web of Knowledge (Thomson Reuters-http://www.webofknowledge.com), and Scopus (Reed Elsevier-http://www.scopus.com). The two most abundant mussel genera, *i.e. Mytilus* and *Perna,* were applied as keywords and the retrieved references were further refined focusing on reported bioactivity. Fig. 1 gives an overview on the number of publications dealing with mussel bioactives corresponding to three major primary metabolite classes, *i.e.* proteins/peptides/amino acids, lipids, and carbohydrates, as well as miscellaneous metabolites. Furthermore, selected publications were evaluated according to the type of bioactivity in relation to metabolite classes, revealing that most studies deal with antimicrobial mussel peptides or anti-inflammatory mussel lipids (Fig. 2).

1.2. Marine mussel species of interest: morphology, geographical distribution, and habitat

Marketed worldwide as live, frozen or processed seafood, marine mussels are native to both, northern and southern hemispheres. The mussel industry is split into two production techniques, *i.e.* bottom mussels, naturally grown on the seabed and harvested by specialised dredging equipment, and rope mussels, cultivated on rope structures in aquaculture (Gosling, 1992). In their natural environment mussels have to adapt to parameters, such as salinity, wave exposure, substrate, zone, height, temperature, and water quality. Most species tolerate a wide range of salinity. However, at very low salinities the mussel growth is limited, which leads to smaller sizes (Almada-Villela, 1984). Mussels, occurring in low and mid intertidal areas, prefer sheltered places where individuals are usually attached to hard surfaces, such as rocky substrates. In order to adhere to boulders, cobbles, or pebbles they use their byssal threads which are proteinaceous silk-like fibres, also known as the mussel's beard (Lee, Messersmith, Israelachvili, & Waite, 2011). The most limiting parameter for the distribution of marine mussels is the temperature, as some species prefer colder while some prefer warmer waters. Furthermore, the content of active metabolites varies with season, life cycle, and habitat (Freites, Fernandez-Reiriz, & Labarta, 2002).

Commercially most relevant marine mussel species belong to the two genera of *Mytilus* and *Perna*. *Mytilus* species occur in temperate waters of Europe, Asia, and America, whereas *Perna* species are cultured in warmer waters such as Thailand, the Philippines, China, and New Zealand (Gosling, 1992). Within the genus *Mytilus*, the marine mollusc *M. edulis* is commonly known as blue or black mussel (Fig. 3A and B) due to the colour of its shell (size up to

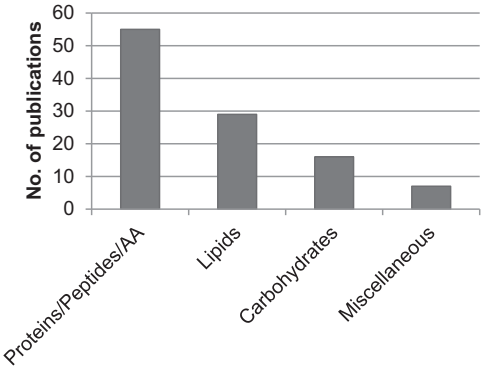

Fig. 1. Comparison of number of selected publications dealing with bioactives from marine mussels categorised in four classes, *i.e.* proteins/peptides/amino acids (AA), lipids, carbohydrates, and miscellaneous.

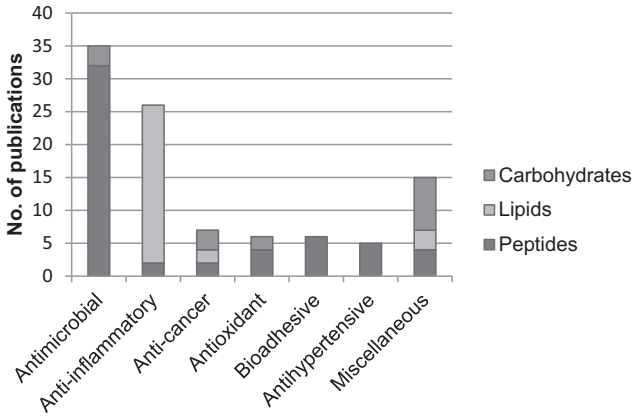

Fig. 2. Evaluation of number of publications which report bioactive properties related to three major classes of mussel compounds. Peptides, lipids, and carbohydrates are differentiated in different shades of grey.

100 mm in length). It is mostly cultured in Canada, USA, Europe, and Africa. Another common edible mussel, *M. galloprovincialis*, originates from the Mediterranean sea. Interestingly, it is not possible to distinguish *M. edulis* and *M. galloprovincialis* based solely upon morphological characteristics (Gosling, 1992). Moreover, hybrids between the blue mussel and the Mediterranean mussel have been reported in Ireland, western France, southwest England, and north Scotland (Gosling, Doherty, & Howley, 2008). Concerning the genus *Perna*, major aquaculture mussel species include *P. viridis*, the Asian green mussel, and *P. canaliculus*, the green-lipped mussel which is endemic to New Zealand (Fig. 3C and D). The latter one is an integral dietary part of the indigenous Maori culture (Maori name: kuku) and is the basis of an important aquaculture and processing industry serving both export and domestic markets. Besides its green lip, it is characterised by a bright green stripe around the posterior ventral margin of the shell (size up to 240 mm in length) (Wakimoto et al., 2011).

2. The bioactive potential of metabolites derived from marine mussels

Most research groups focus on the evaluation of the bioactive potential of extracts, hydrolysates, or purified components derived from whole mussel meat, single organs, cell compartments, or blood. Mussel shells, produced in large amounts as terrestrial waste from aquaculture processing plants, are composed of organo minerals such as aragonite and calcite as well as shell matrix proteins (Gosling, 1992; Marin, Luquet, Marie, & Medakovic, 2007). Since there is low evidence for bioactivity reported for shell components, this review will not focus on this part of the mussel.

2.1. Bioactive proteins, peptides, and amino acids from marine mussels

2.1.1. Generation of bioactive proteinaceous metabolites

Mussels contain a large portion of muscle tissue with considerably high content of protein. Interestingly, the number of research publications reporting significant bioactivity exerted by high molecular weight proteins is very low. However, one of the rare examples for bioactive proteinaceous macromolecules is pernin (60 kDa), found in the cell-free haemolymph (plasma) of *P. canaliculus*. It is a self-aggregating glycosylated protein, consisting of 497 amino acids, resembling an anti-thrombin peptide, known from terrestrial leeches. However, the anti-thrombin activity found for this mussel-derived protein is only weak (Scotti, Dearing, Greenwood, & Newcomb, 2001).

To exert significant bioactivity, complex protein macromolecules usually need to be split into shorter chains of amino acids (peptides) either by processing techniques, such as fermentation or by gastrointestinal digestion. Since ancient times, fermentation has been used as a method for food preservation, by controlling the growth and multiplication of a number of pathogens. Even today, it is popular as an affordable technology to enhance food safety but also to improve digestibility, taste and flavour of foods (Motarjemi, 2002; Visessanguan, Benjakul, Riebroy, & Thepkasikul, 2004). Fermented fish and shellfish sauces are used as nutritional condiments in various cuisines, *e.g.* in African and south east Asian countries. During fermentation, bioactive peptides or amino acids are enzymatically produced from large precursor or parent proteins which usually show only weak or no bioactivity. The underlying biochemical process of fermentation, which depends on the temperature, pH, and time, is called hydrolysis or proteolysis, respectively. The cleavage of proteins is either catalysed by endogenous and/or exogenous enzymes which can be inactivated by heat treatment in order to terminate the process. Although favoured as a low cost option, the use of endogenous proteases which are

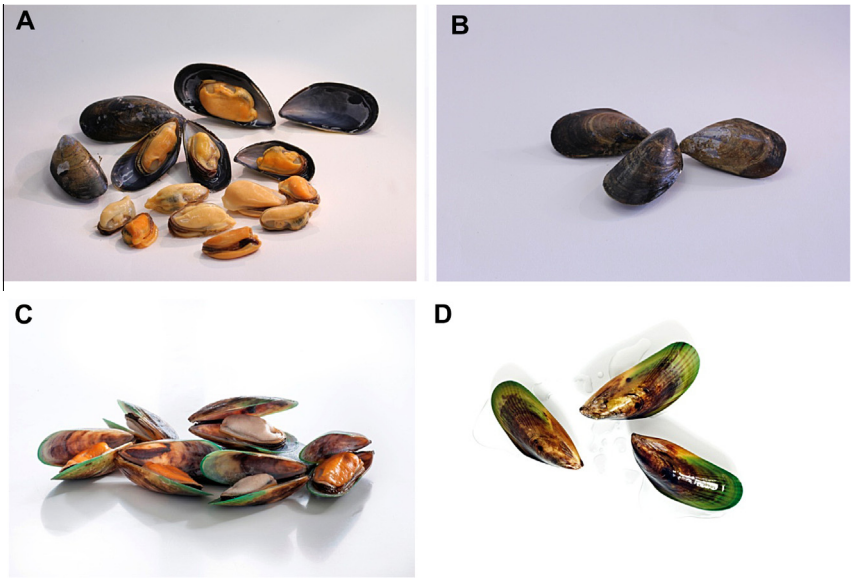

Fig. 3. Pictures of *M. edulis* (A, whole mussel; B, shells) and *P. canaliculus* (C, whole mussel; D, shells).

already present in food matrices, such as shellfish meat, has the disadvantage of long time periods required to obtain desired bioactive peptides. The production of fermented blue mussel sauces (FBMSs) requires six to twelve months of fermentation (Jung, Rajapakse, & Kim, 2005). Usually, this so-called autoproteolysis takes place in brine or salt solution (above 20% NaCl, w/w) to avoid microbial contamination during long time periods. The downside of these fermented products is the high salt content, which is unfavourable especially for consumers with risk factors for cardiovascular diseases. However, electrodialysis can be used as a method to reduce the amount of salt (Park, Je, & Kim, 2005).

Despite higher costs, commercial enzymes are preferred to endogenous proteases, due to faster reaction rates and much shorter fermentation time frames. By the use of food-grade enzymes such as alcalase, neutrase, papain, trypsin, Protamex®, and Flavourzyme®, precursor proteins can be cleaved within an average of 30 min of hydrolysis (Dai, Zhang, Zhang, & Lu, 2012). Moreover, the use of well-characterised commercial enzymes allows a better control over protein breakdown, molecular weight, and composition of generated peptides.

2.1.2. Purification techniques and characterisation of proteinaceous metabolites

To gain insights into the putative health effects of proteinaceous metabolites, these are either investigated in the form of hydrolysate mixtures from fresh, homogenised mussels or as isolated and purified amino acids, proteins, or peptides. Besides desired bioactive peptides or amino acids, generated hydrolysates also contain non-proteinaceous components or inactive protein macromolecules. Methods of choice to purify hydrolysate mixtures and to obtain specific peptide classes, according to their molecular weight, include *e.g.* centrifugation or ultrafiltration using appropriate membranes. To achieve further separation these procedures are followed by gel and ion exchange chromatography techniques, as well as RP-HPLC (Ngo, Vo, Ngo, Wijesekara, & Kim, 2012; Silva, Park, & Hubinger, 2010). However, separation is not always beneficial in respect to bioactivity. In some cases, mixtures of peptides, amino acids, and sugars show higher bioactivity (*e.g.* antioxidant activity) than single purified peptides (Sarmadi & Ismail, 2010).

Some peptides, such as antimicrobial peptides (AMPs; see below) are naturally available in the mussel. Hence, there is no need for time-consuming processes to break down larger proteins to obtain these bioactive peptides. As highlighted by Charlet and coworkers (1996), peptide extraction and purification protocols for AMPs generally include the suspension of homogenised mussel meat, blood or haemolymph in acidic aqueous solutions. In further bioactivity-guided steps the mixture is usually centrifuged and the supernatant is subjected to solid phase extraction followed by RP- and gel permeation HPLC (Charlet et al., 1996). In order to check the purity and/or molecular weight of peptides electrophoresis (*e.g.* SDS–PAGE) or ESI–QTOF tandem mass analyses are performed. In a final step, primarily automated Edman degradation or sequencing is used to elucidate amino acid sequences (Charlet et al., 1996; Jung & Kim, 2009). To date, the 3D structures of only very few mussel peptides have been completely resolved by NMR techniques, *i.e.* the AMPs MGD-1 (Yang et al., 2000) and mytilin B (Roch, Yang, Toubiana, & Aumelas, 2008). Furthermore, knowledge about characteristic structural features, *e.g.* special loop regions, gives further insights into requirements necessary for observed bioactivities (Romestand, Molina, Richard, Roch, & Granier, 2003). Despite the availability of advanced molecular techniques such as expressed sequence tag analyses and gene cloning, the identification of further bioactive mussel peptides and the investigation of their sequential properties, as well as structural features, still appear as new area of research.

2.1.3. Potential health benefits and biological properties of proteinaceous metabolites

In general, bioactive peptides derived from marine mussels contain 5–40 amino acid residues. Depending on the amino acid sequence and structural properties, major biological effects of mussel peptides include antimicrobial, antihypertensive, and anticoagulant activities (Je, Park, Byun, Jung, & Kim, 2005; Jung & Kim, 2009; Löfgren, Miletti, Steindel, Bachère, & Barracco, 2008). An overview on bioactive mussel proteins/peptides/amino acids is given in Table 1.

2.1.3.1. Antimicrobial peptides (AMPs). In the field of molecular cell biology, AMPs comprise the most studied group of peptides from marine mussels. Only recently, AMPs have attracted the attention of medicinal chemists for exploitation as novel drug candidates in the treatment of infectious diseases in humans (Otero-Gonzalez et al., 2010; Sperstad et al., 2011). As mentioned above, AMPs are natural peptides (non-enzymatically hydrolysed), which are expressed by the mussel itself as part of its haemolymph, the specific innate immunodefense system. AMPs might be the reason why mussels seem to be less affected by diseases, compared to other bivalve molluscs (Gestal et al., 2008). AMPs show antifungal, antibacterial, or antiviral effects (Charlet et al., 1996; Mitta, Hubert, Noel, & Roch, 1999; Yasin et al., 2000) and act via binding to microorganisms, by means of electrostatic interaction with cell wall or membrane residues, promoting their elimination through different mechanisms (Jenssen, Hamill, & Hancock, 2006). However, apart from the disruption of membrane permeability causing cytoplasmic membrane lysis, a detailed mode of action of isolated mussel peptides has not been reported yet (Nakajima et al., 2003). These types of amphipathic, cationic peptides defined by a molecular weight <10 kDa, are also widespread among insects (Hancock & Scott, 2000; Steiner, Hultmark, Engstrom, Bennich, & Boman, 1981). According to their secondary structure, AMPs can be categorised into three major groups, *i.e.* (i) linear α-helical peptides, (ii) cysteine-rich peptides containing β-sheets and disulfide bonds, and (iii) peptides with an over-representation of certain amino acids (Yasin et al., 2000; Zasloff, 2002).

Among different species of marine mussels, AMPs have primarily been identified in two species of the *Mytilus* genus and one species of the *Perna* genus. AMPs comprise multiple isoforms in six families, *i.e.* defensins, mytimycins, myticins, mytilins, big defensins, and mytimacins, which mostly belong to the group of cysteine-rich peptides usually showing several disulfide bonds (Charlet et al., 1996; Gerdol, De Moro, Manfrin, Venier, & Pallavicini, 2012; Löfgren, Smânia, Smânia, Bachère, & Barracco, 2009; Mitta, Vandenbulcke, Hubert, Salzet, & Roch, 2000).

About 15 years ago, isolation and characterisation of AMPs from marine mussels (Table 1) began with the identification of mytimycin (6.5 kDa), defined by twelve cysteine residues, as a first solely antifungal molecule from the blood of *M. edulis* (Charlet et al., 1996; Sonthi, Toubiana, Pallavicini, Venier, & Roch, 2011). Further isolated AMPs are generally smaller (3.7–4.5 kDa), positively charged and amphiphilic. The two representatives defensin A and B (Table 1), for instance, were purified from *M. edulis* comprising six cysteine residues (Charlet et al., 1996). The defensins MGD-1 and MGD-2 (Mitta et al., 2000; Romestand et al., 2003) and the family of myticins (A, B, and C), found in *M. galloprovincialis*, show eight cysteine residues engaged in four intramolecular disulfide bonds (Hubert, Noel, & Roch, 1996; Mitta et al., 1999). Furthermore, five AMP isoforms of mytilins were identified (A, B, C, D and G1). Mytilin A and B were first isolated from *M. edulis* (Charlet et al., 1996) and mytilin B, C, D, and G1 originate from *M. galloprovincialis* (Mitta et al., 2000).

Apart from mytimycin which exerts only antifungal effects, members of all *Mytilus* AMP families show a broad-spectrum

Table 1
Overview on bioactive proteins, peptides, and amino acids derived from marine mussels.

Biological activity - name of bioactive protein/peptide	Sequence and molecular weight	Mussel species	Origin/product	Reference(s)
Antioxidant				
Mussel-derived radical scavenging peptide (MRSP)	HFGDPFH (962 Da)	ME	Fermented sauce	Rajapakse et al. (2005)
N.g.	LVGDEQAVPAVCVP (1.59 kDa)	MC	*In vitro* gastrointestinal digest	Jung et al. (2007)
N.g.	FGHPY (620 Da)	ME	Fermented sauce	Jung et al. (2005)
Antimicrobial				
Mytilus defensin A	GFGCPNDYCHRHCKSIPGRXGGYCGGXHRLRCTCYR (~4 kDa)	ME	Blood	Charlet et al. (1996)
Mytilus defensin B	GFGCPNDYPCHRHCKSIPGRYGGYCGGXHRLRCTC— (~4 kDa)	ME	Blood	Charlet et al. (1996)
MGD-1	GFGCPNNYQCHRHCKSIPGRCGGYCGGWHRLRCTCYRCG (4.4 kDa)	MG	Hemocytes	Hubert et al. (1996), Mitta et al. (1999), and Yang et al. (2000)
MGD-2	GFGCPNNYACHQHCKSIRGYCGGYCASWFRLRCTCYRCG (4.4 kDa)	MG	Hemocytes	Mitta et al. (2000), Mitta et al. (1999), and Yang et al. (2000)
Mytilin A	GCASRCKAKCAGRRCKGWASASFRGRCYCKCFRC (~4 kDa)	ME, MG	Hemocytes	Charlet et al. (1996), Löfgren et al. (2009), and Mitta et al. (2000)
Mytilin B	SCASRCKGHCRARRCGYYVSVLYRGRCYCKCLRC (4.0 kDa)	ME, MG	Hemocytes	Charlet et al. (1996) and Mitta et al. (2000)
Mytilin C	SCASRCKSRCRARRCRYYVSVRYGGFCYCRC- (4.2 kDa)	MG	Hemocytes	Mitta et al. (2000)
Mytilin D	GCASRCKAKCAGRRCKGWASASFRRRCYCKCFRC (3.9 kDa)	MG	Hemocytes	Mitta et al. (2000)
Mytilin G1	VVTCGSLCKAHCTFRKCGYFMSVLYHGRCYCRCLLC (~4 kDa)	MG	Hemocytes	Mitta et al. (2000)
Myticin A	HSHACTSYWCGKFCGTASCTHYLCRVLHPGKMCACVHCSR (4.5 kDa)	MG	Hemocytes	Padhi and Verghese (2008)
Myticin B	HPHVCTSYYCSKFCGTAGCTRYGCRNLHRGKLCFCLHCSR (4.6 kDa)	MG	Hemocytes	Padhi and Verghese (2008)
Myticin C	QSVACRSYYCSKFCGSAGCSLYGCYLLHPGKICYCLHCSR (4.4 kDa)	MG	Hemocytes	Balseiro et al. (2011) and Padhi and Verghese (2008)
N.g.	N.g. (9.7 kDa)	PV	Gill homogenate	Chandran et al. (2009)
Anti-inflammatory				
	15 Essential and non-essential amino acids	MG	Proteic extract	Badiu et al. (2010)
Antihypertensive				
N.g.	EVMAGNLYPG (Partial sequence – N-terminal region; 6.5 kDa)	ME	Fermented sauce	Je et al. (2005)
Antifungal				
Mytimycin	DCCRKPFRKHCWDCTAGTPYYGYSTRNIFGCTC— (6.5 kDa)	ME	Blood	Charlet et al. (1996)
Anticoagulant				
N.g.	EADIDGDGQVNYEEFVAMMTSK (2.5 kDa)	ME	Edible part	Jung & Kim (2009)
Anti-thrombin				
Pernin	Protein composed of 497 amino acids (60 kDa)	PC	Cell-free haemolymph	Scotti et al. (2001)
Adhesive for surgical applications				
	Adhesive protein	ME	Byssus	Ninan et al. (2007)

ME, *M. edulis*; MC, *M. coruscus*; MG, *M. galloprovincialis*; PC, *P. canaliculus*; PV, *P. viridis; N.g., not given.*

activity against Gram-positive (*e.g. S. aureus*) and Gram-negative (*e.g. E. coli*) bacteria in liquid growth inhibition assays (Charlet et al., 1996; Hubert et al., 1996; Mitta et al., 2000). As in the case of mytilin B and D (Mitta et al., 2000), myticin B (Mitta et al., 1999), and defensin MGD-1 (Mitta et al., 1999), these antibacterial effects are accompanied with considerable antifungal activity (*e.g.* against *Neurospora crassa* and *Fusarium oxysporum*). Mytilin A has also been tested against fish viral pathogens and protozoan parasites (Carriel-Gomes et al., 2007; Löfgren et al., 2008). However, in both cases it was only active at near cytotoxic concentrations.

Concerning AMPs originating from *Perna* species, less information is available in the literature (Table 1). To our best knowledge, only one AMP (9.7 kDa) was reported from the gill extract of *P. viridis*, the greenshell mussel occurring in the region of Hong Kong. This peptide exhibited distinct antibacterial (against *S. aureus*) and antifungal (against *A. flavus*) activity by comparing the zone of inhibition with either erythromycin or fluconazole as positive control using a standardised single disc method (Chandran, Rameshkumar, & Ravichandran, 2009).

In summary, AMPs have been mostly characterised by environmental biologists to evaluate the mussel's physiological functions. Since the emergence of resistance developed by microorganisms against state-of-the-art treatment options, AMPs from marine mussels might also be considered as a valuable source for therapeutics. In aquaculture, AMPs have been proposed as natural antimicrobial agents for the treatment of infectious diseases in marine species (Balseiro et al., 2011). Moreover, AMPs show a great potential as a natural antimicrobial food additive for human

consumption. However, further target-oriented research is needed to overcome negative aspects, such as bitter taste (Cho, Unklesbay, Hsieh, & Clarke, 2004), possible interactions with other food components, or allergies (Ngo, Wijesekara, Vo, Van Ta, & Kim, 2011).

2.1.3.2. Antioxidant and antihypertensive peptides. Several studies, on fermented or similarly processed marine mussels, focus on the investigation of health beneficial bioactive properties of the derived peptides. Major effects include antihypertensive and antioxidant activity, as well as radical scavenging capacity. For example, Korean researchers identified an antihypertensive and two antioxidant peptides from the sauce of fermented *M. edulis* (Je et al., 2005; Jung et al., 2005; Rajapakse, Mendis, Jung, Je, & Kim, 2005). One of these purified peptides (6.5 kDa; N-terminal amino acid sequence EVMAGNLYPG; Table 1) exhibits angiotensin I converting enzyme (ACE) inhibition with an IC_{50} value of 19.34 μg/ml (= 2.98 μM) in a spectrophotometric assay (Cushman & Cheung, 1971; Je et al., 2005). ACE converts angiotensin I into angiotensin II. Since the latter is known to constrict blood vessels to cause an increased blood pressure, blocking ACE is considered as a valuable strategy in hypertension.

For this peptide, effective blood pressure lowering effects were confirmed in an *in vivo* model using spontaneously hypertensive rats. Further two isolated peptides which have a much smaller size of 962 Da and 620 Da, respectively (Table 1), were found to exhibit radical scavenging properties in a lipid peroxidation inhibitory assay. The first one (MRSP; 962 Da; sequence HFGDPFH; Table 1) was able to scavenge a variety of radicals (superoxide, hydroxyl, carbon-centred, DPPH) with IC_{50} values in a range between 21 and 96 μM, whereas no IC_{50} values were reported for the latter one (620 Da; FGHPY; Table 1). In terms of lipid peroxidation, both peptides showed a strong inhibition which was higher than that observed for the natural antioxidant α-tocopherol, the reference substance (Jung et al., 2005; Rajapakse et al., 2005). Another potent antioxidant mussel peptide with the amino acid sequence LVGDEQAVPAVCVP, characterised by a low molecular weight of 1.6 kDa, was identified from *M. coruscus* using an *in vitro* gastrointestinal digestion system (Jung et al., 2007). This peptide showed higher protective activity against lipid peroxidation in a linoleic acid model system than vitamin C and α-tocopherol.

In another study, Dai and co-workers (2012) analysed the ACE inhibiting effect of *M. edulis* protein hydrolysates obtained by enzymatic processing using six different food-grade proteases. As a result, the hydrolysates produced by alcalase (peptide molecular weights <1000 Da) revealed the highest inhibition on ACE with an IC_{50} value of 66.3 μg/ml. The contained antioxidant or antihypertensive peptides are suggested to find further applications in food preservation as an alternative to synthetic additives or in pharmaceutical industries to avoid lipid peroxidation (Jung et al., 2005; Rajapakse et al., 2005).

2.1.3.3. Other biological effects related to marine mussel proteins and peptides. Besides AMPs, antioxidant and antihypertensive peptides, mussels provide a source for further bioactive proteinaceous compounds. For example, an oligopeptide with a potent dose-dependent anticoagulant activity has been isolated from *M. edulis* (Jung & Kim, 2009). This oligopeptide, named *M. edulis* anticoagulant peptide (MEAP), is characterised by a molecular weight of 2.5 kDa. MEAP is able to prolong the blood clotting time (thrombin time as well as the activated partial thromboplastin time) and interacts with key blood coagulation factors, such as the inhibition of activation of FX in the intrinsic tenase complex (FIXa/VIIIa/PLs) and the conversion of FII to FIIa in the prothrombinase complex (FXa/FVa/PLs) (Jung & Kim, 2009). Furthermore, a recent publication reports anti-inflammatory properties for extracts from *M. galloprovincialis* containing fifteen essential and non-essential amino acids quantified by GC–MS (Badiu, Luque, Dumitrescu, Craciun, & Dinca, 2010). In this work, Badiu and colleagues observed enhanced dermal and epidermal neoformation, in an *in vivo* model for skin burns, that suggests further development of mussel derived proteic extracts for therapeutic applications.

Another part of the mussel which has already been intensively investigated in the direction of applied research is its byssus. Since mussels grow in intertidal zones they are exposed to harsh waves. These circumstances have driven the development of strong adhesive byssal threads in order to brave the elements. The adhesive power of mussels has been the subject of numerous studies trying to synthetically mimic these characteristics in polymers used for surgical applications, technology, and industry (Brubaker & Messersmith, 2012; Lee et al., 2011). Adhesive protein extracts from *M. edulis* byssus, for instance, have shown promising results as moisture-resistant biocompatible curing agents. In an experimental model, they were shown to form strong bonds between two overlapping strips of the porcine small intestinal submucosa (Ninan, Stroshine, Wilker, & Shi, 2007). These findings will clearly drive future efforts to develop new mussel-inspired materials, especially for wet adhesion necessary for surgical applications to coat, heal, or seal tissues.

2.2. Bioactive lipids and non-polar components from marine mussels

2.2.1. Isolation, purification and characterisation of lipid metabolites

Among the three major groups of mussel primary metabolites, lipids have so far shown the highest potential for the commercial development of health beneficial functional foods or dietary supplements. Mussel lipid extracts and fractions can be obtained by solvent extraction and are purified by chromatographic separation. The increasing instability during purification processes limits the investigation of single lipid components, hence analyses mostly focus on the characterisation of lipid extracts or fractions rather than pure compounds.

Mussel lipid extracts are usually obtained by extraction of fresh or freeze-dried mussel meat. By means of enzymatic (*e.g.* using lipase or protease) or chemical (*e.g.* KOH) hydrolysis, complex lipids are cleaved to obtain single fatty acids. In general, normal phase column chromatography is subsequently used to fractionate the crude extracts into major lipid classes, *i.e.* sterol esters, triglycerides, free fatty acids, sterols, and phospholipids. In some cases, purification and structural analysis is also pursued and achieved by chromatographic techniques such as preparative TLC (McPhee et al., 2010). GC–FID or GC–MS techniques are the most suitable methods for the characterisation of fatty acids present in mussel oils. Classically, lipid classes have to be hydrolysed and methylated to obtain fatty acid methyl esters (FAMEs). Subsequently, identification of lipid components is carried out by comparison to known standards.

2.2.2. Bioactive marine oils from the New Zealand green-lipped mussel *P. canaliculus*

Several anti-inflammatory and anti-arthritic dietary supplements, which contain mussel lipids, are available commercially. The best known products are Seatone® and Lyprinol®. Their development was inspired by the observation of the New Zealand Maori population living in coastal areas that consume a high amount of green-lipped mussels (*P. canaliculus*) in their diet. These people develop osteoarthritis (OA) to a much lesser extent than inland Maoris. In 1976, Seatone® was launched as the first commercially available anti-arthritic green-lipped mussel product (McFarlane & Croft, 1980). In early years, stability problems of this freeze-dried mussel extract strongly affected the level of anti-inflammatory activity and raised concerns among consumers and industry. To overcome these issues, 3% tartaric acid was added directly after shucking the mussel as an antioxidant and metal chelator, which

resulted in consistently high anti-inflammatory effects (Halpern, 2000; Whitehouse et al., 1997). Subsequently, the formulation method was improved and in 1998, Lyprinol® was brought to the market as another anti-inflammatory marine mussel product. It contains the oil of *P. canaliculus* obtained by supercritical fluid extraction (CO_2-SFE) of the stabilised, freeze-dried mussel powder formulated with olive oil and vitamin E as an antioxidant (Singh et al., 2008; Whitehouse et al., 1997). By using this technique and CO_2 as the extracting medium, the material is not exposed to high temperatures, hence a mild extraction is achieved without activating lipid degrading enzymes such as phospholipases and lipoxygenases (Wakimoto et al., 2011). Since there are no organic solvents present during this extraction process, it is considered as food-friendly.

Lipid classes present in mussel oil comprise sterol esters, triglycerides, free fatty acids (saturated and unsaturated), carotenoids, sterols and polar lipids (Sukumaran et al., 2010). In total there are 90 fatty acid components reported for Lyprinol® (Murphy, Mooney, Mann, Nichols, & Sinclair, 2002). Omega-3 polyunsaturated fatty acids (PUFAs) with 13% eicosapentaenoic acid (EPA) and 21% docosapentaenoic acid (DHA) are found as major ingredients (Murphy et al., 2002). Furthermore, novel anti-inflammatory omega-3 PUFAs, *i.e.* 5,9,12,15-octadecatetraenoic acid, 5,9,12,16-nonadecatetraenoic acid, 7,11,14,17-eicosatetraenoic acid, and 5,9,12,15,18-heneicosapentaenoic acid have been purified by normal and reversed phase chromatography and identified by GC–MS (Singh et al., 2008; Treschow et al., 2007). Fig. 4 gives an overview on the main and novel PUFA structures present in *P. canaliculus* oils. The nomenclature of these fatty acids is based on the number of carbon atoms and the number of their double bonds. Basically, PUFAs are categorised in the omega-6 and the omega-3 class. Omega-6 PUFAs are found mainly in plants and are contained in vegetable oils, whereas omega-3 PUFAs are found in fish and shellfish and occur to a lesser extent in plants. In terms of bioactivity, the omega-3 PUFAs originating from fish and shellfish sources are considered to be more efficient in their biological activity than those found in plants (Chan & Cho, 2009).

The anti-inflammatory mode of action of the green-lipped mussel oil has been linked to its ability to inhibit the production of inflammatory mediators by affecting key enzymes in the arachidonic acid (AA) cascade (Fig. 5) (McPhee et al., 2007; McPhee et al., 2010). AA is metabolised via well characterised pathways including cyclooxygenase (COX) and lipoxygenase (LO) enzymes. As a result, pro-inflammatory (*e.g.* prostaglandins), chemotactic, broncho-constricting (*e.g.* leukotrienes), or potential tumour promoting (*e.g.* 5-hydroxyeicosatetraenoic acid (5-HETE)) agents are produced. Due to their structural similarity to AA (Fig. 4), mussel PUFAs like EPA and DHA can act as competitive substrates for these key enzymes and thus reduce inflammatory responses. For example, the production of prostaglandin E_2 (PGE_2) is inhibited by Lyprinol® with an IC_{50} of 1.2 μg/ml (Whitehouse et al., 1997). Furthermore, the efficacy of Lyprinol® for the treatment of chronic airway inflammation was demonstrated in a study with patients with atopic asthma (Emelyanov et al., 2002).

As opposed to conventional therapeutic treatment options such as non-steroidal anti-inflammatory drugs (NSAIDs), the use of the above discussed mussel oil products, as natural remedies against arthritis, causes very few side effects. Rapid-acting NSAIDs are well known for their adverse effects in the gastro-intestinal system due to non-selective inhibition of COX enzymes, related deficiency in cytoprotective prostaglandins, and subsequently reduced mucous production leading *e.g.* to the development of gastric ulcers (Toki, Aoki, Katsumi, & Takahashi, 2007). In contrast, green-lipped mussel preparations demonstrate *in vivo* gastro-protective effects (Rainsford & Whitehouse, 1980). Lyprinol® does neither affect platelet aggregation nor the natural resistance of the stomach mucosa in humans, which is related to the involvement of the inducible COX-II rather than COX-I (Whitehouse et al., 1997). *P. canaliculus* lipid extracts inhibit COX-I and COX-II by 12% and 25%, respectively (McPhee et al., 2007). Hence, these mussel oils are mainly suitable for the treatment of chronic inflammation. This further explains that the mentioned mussel products show no or modest activity on the carrageenan-induced paw oedema assay in rats, an experiment which is typically used to evaluate acute inflammation (Whitehouse et al., 1997).

In a recent study, McPhee and co-workers (2007) compared *in vitro* COX-inhibiting effects of Lyprinol® and the total lipid extracts obtained from *P. canaliculus* and *M. edulis* before and

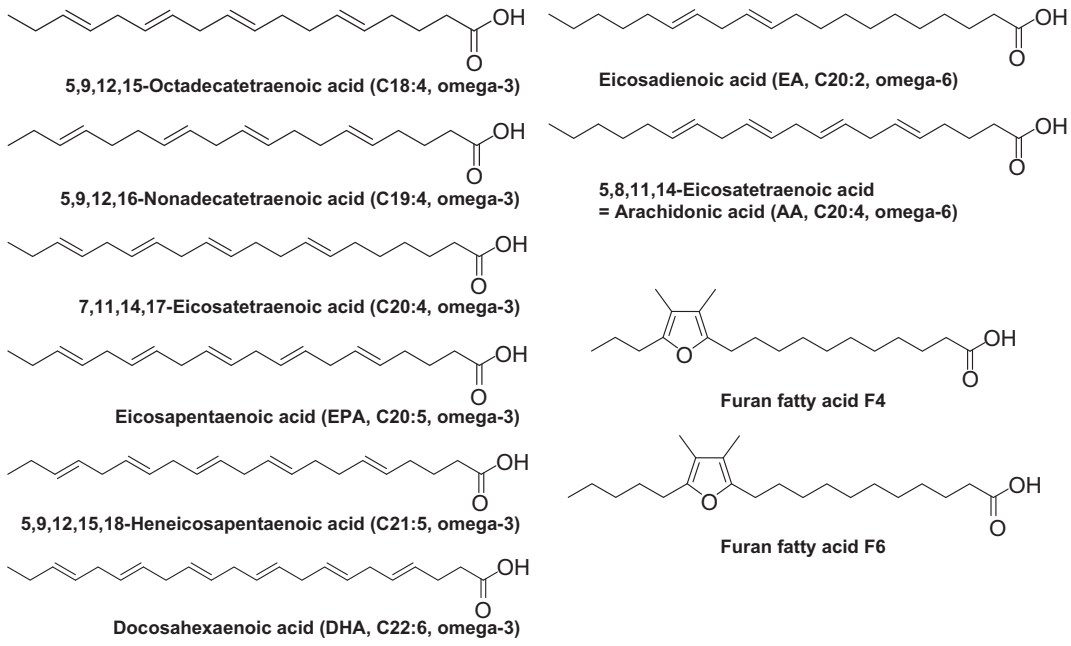

Fig. 4. Structures of *P. canaliculus* PUFAs (omega-3) and novel anti-inflammatory furan fatty acids in comparison to omega-6 PUFAs eicosadienoic acid (EA) and arachidonic acid (AA).

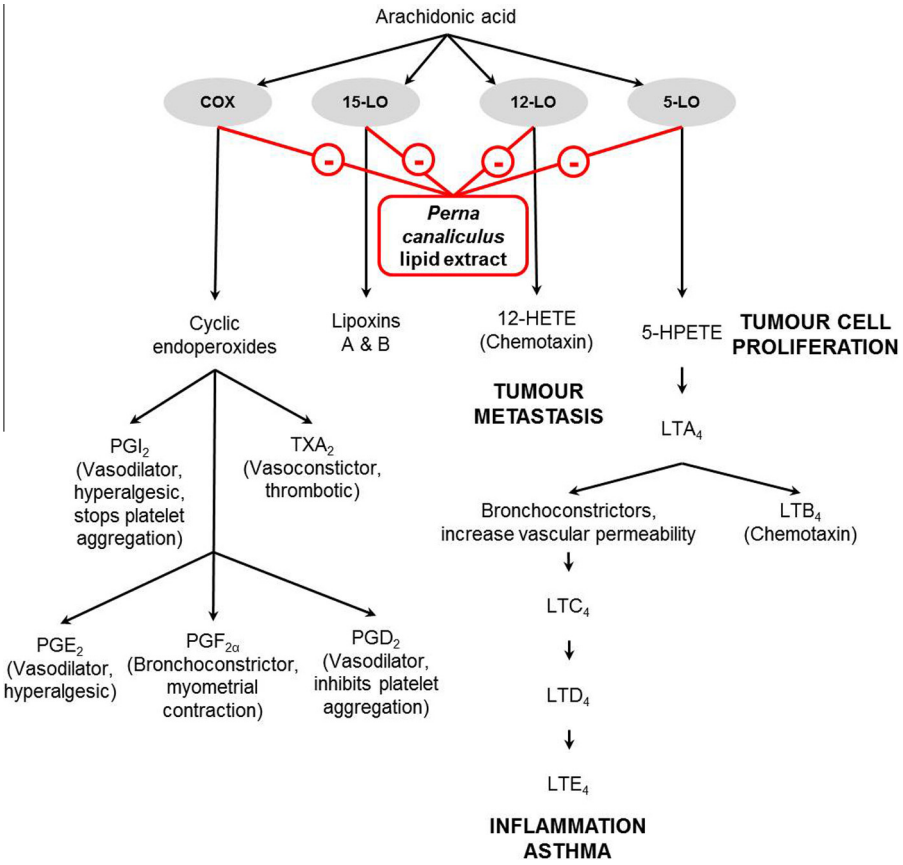

Fig. 5. Down-regulation of the key enzymes in the arachidonic acid cascade by lipid extract of *P. canaliculus* resulting in a reduced formation of inflammatory mediators (figure modified from Halpern, 2000). HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LT, leukotriene; PGI_2, prostacyclin; TX, thromboxane.

after KOH-, protease-, or protease-lipase-hydrolysis. The non-hydrolysed total lipid extracts of both *P. canaliculus* as well as *M. edulis* showed a moderate COX inhibition. Related to an increase of free fatty acids, a strong COX inhibition was observed after saponifying the extracts by KOH hydrolysis. Except for the higher lipid contents in *P. canaliculus*, there were no significant differences observed between the two mussel species. Interestingly, also Lyprinol® has demonstrated a 10-fold higher inhibition of both COX isoforms after hydrolysis (McPhee et al., 2007).

A recent study provided mechanistic insights into the anti-inflammatory effects of Lyprinol® in the arachidonic acid cascade. Lee and co-workers (2008) investigated protein expression profiles of splenocytes in rats with adjuvant-induced arthritis (AIA). In AIA rats consuming Lyprinol® orally, they found that six metabolism-related proteins are down-regulated while malate dehydrogenase (MDH), a key enzyme in gluconeogenesis, is up-regulated. Hence, the level of glucose necessary for Major-Histocompatibility-Complex I (MHC-I) activation is decreased, which underlines the anti-inflammatory mechanism of Lyprinol®, since an activated MHC-I is involved in the development of autoimmune diseases. These findings indicate that Lyprinol®'s anti-inflammatory activity is mediated by multiple mechanisms, which have still not been fully evaluated.

Since their launch in 1976, effects of nutritional green-lipped mussel supplement products on osteoarthritis (OA), rheumatoid arthritis (RA), asthma, and cancer have been studied in various clinical trials and discussed in numerous review papers (Brien, Prescott, Coghlan, Bashir, & Lewith, 2008; Doggrell, 2011; Gibson & Gibson, 1998; Gibson, Gibson, Conway, & Chappell, 1980; Lau et al., 2004; Sukumaran et al., 2010). Within its main field of application, *i.e.* in patients suffering from OA or RA, outcomes of most studies have proven a reduced amount of pain and stiffness related to the intake of mussel preparations (Halpern, 2000). Apart from orally applied Lyprinol® capsules, topical preparations, such as skin cream containing mussel lipids, also seem to be effective against OA and RA (Chandler, 2005a; Chandler, 2005b; Kenneth, Waite, & Downs, 2009; Mulye & Assoulin, 2012; Williams & Sansom, 2008). However, to our knowledge no comparative study has been conducted to evaluate the efficacy of orally versus topically applied mussel lipids.

2.2.3. Bioactive marine oils from Mytilus species

In contrast to lipids from *Perna* species, the amount of information available, on mussel lipids from *Mytilus* species, is comparably small. In general, *Mytilus* oils are known to contain similar major long chain omega-3 PUFAs (EA, EPA and DHA) as *Perna* oils, however, in considerably lower yields (McPhee et al., 2007; McPhee et al., 2010). Anti-inflammatory effects were found for both non-hydrolysed *M. edulis* crude lipid extracts and hydrolysed triglyceride fractions (Christie, 1982; McPhee et al., 2010). Especially after saponification of the crude extracts, containing EPA and DHA at a percentage of 37% of total fatty acids, inhibition of leukotriene production was observed in a neutrophil 5-LO assay *in vitro* and in an AIA rat model (McPhee et al., 2010). Combined with the 5-LO inhibition the total free fatty acid fractions show a selective *in vitro* inhibition of COX-II (McPhee et al., 2007). Moreover, since there were no negative side effects observed in animal models, the findings of McPhee and colleagues suggest the potential of *M. edulis* lipid extracts or fractions as anti-inflammatory agents.

Further *Mytilus* species reported to contain bioactive lipids comprise the Korean mussel, *M. coruscus* and the Mediterranean

mussel, *M. galloprovincialis*. Different *M. coruscus* lipid extracts obtained by suspension in organic solvents (MeOH, chloroform, hexane) or solvent mixtures (MeOH:chloroform, MeOH:hexane) were analysed for their potential to induce apoptosis of several cancer cells including human prostate, breast, lung, and liver cancer cell lines. As a result, the hexane extract was found to possess the highest *in vitro* anti-tumour effects by inducing apoptosis of human prostate cancer cells (Kim et al., 2011). Interestingly, the most active fraction of the hexane extract of this Korean mussel was found to contain a remarkably high amount of EPA (33.4%). In another study, Badiu and colleagues investigated the wound healing potential of *M. galloprovincialis* lipid extracts obtained from extractions with chloroform:MeOH (1:2; v/v). A positive effect on burnt skin was confirmed in an animal model using Wistar rats, suggesting further development of these extracts for skin-care products (Badiu et al., 2008).

2.2.4. Isolated single lipid components from marine mussels

Bioactivity studies on marine lipids are usually carried out on extracts or fractions rather than pure compounds. As mentioned before, this might be due to the increasing instability of fatty acids, for instance, during isolation processes. In a very recent study, Wakimoto and colleagues (2011) detected unstable anti-inflammatory and antioxidant furan fatty acids (Fig. 4) in the green-lipped mussel, *P. canaliculus*. After semisynthetic stabilisation, one of the furan fatty acid ethyl esters showed an even higher anti-inflammatory potential than EPA ethyl ester in an *in vivo* model of AIA (Wakimoto et al., 2011).

Another rare example for an isolated single lipid compound is lysolecithin. This hydrophilic phospholipide belongs to the class of phosphatidylcholines and was identified as the anti-histaminic and anti-inflammatory component in *P. canaliculus* lipid fractions in the mid 1980s (Kosuge, Tsuji, Ishida, & Yamaguchi, 1986). However, no further studies have been undertaken to pursue these results.

2.3. Bioactive carbohydrates from marine mussels

2.3.1. Structural characteristics and analysis of mussel carbohydrates

The group of mussel carbohydrates is primarily represented by polysaccharides. These sugars are composed of monosaccharides, which are connected via glycosidic bonds forming linear or branched macromolecules (average molecular weight 1.5×10^6 Da). It is distinguished between homo- (one type of monosaccharide) and heteropolysaccharides (different types of monosaccharides). In some cases, sugar components are covalently linked to polypeptide side chains of cell wall proteins. These so-called glycoproteins are important for the invertebrate's immune system (Smital & Kurelec, 1998). In general, carbohydrates can be extracted from the mussel, by using different organic solvents. In most cases, they are obtained by hot-water extraction, followed by further purification steps, including anion-exchange and gel-permeation chromatography (Miller, Dodd, Ormrod, & Geddes, 1993).

Techniques, such as dialysis and lyophilisation, allow a coarse fractionation of the sugar components. Isolation and purification is usually achieved by the use of molecular-sieve or affinity chromatography (Ovodova et al., 1992). An array of different techniques such as complete hydrolysis, periodate oxidation, methylation analysis, Fourier transform infrared spectroscopy (FTIR), and NMR, are applied in a final step to elucidate the structure of the carbohydrate of interest.

2.3.2. Bioactivities related to mussel carbohydrates

Among the three major bioactive primary metabolite classes from mussels, the least attention has been paid to the group of carbohydrates, hence only very few research articles are available in the literature. In 1992, Ovodova and co-workers reported a high immunomodulating activity for mytilan, a branched bioglycan isolated from *Crenomytilus grayanus*, a marine mussel originating from Japan. Mytilan is a non-covalently linked complex of 95% polysaccharide and 5% protein with lectin (carbohydrate-binding) properties. The polysaccharide as the carbohydrate part consists of α-D-glucan similar to glycogen, an equivalent to starch which is known as an energy storing molecule in plants. Sonication of mytilan resulted in a mixture of glucose, maltose, and malto-oligosaccharides. Further NMR-based analyses of mytilan revealed an even higher degree of branching than glycogen (Ovodova et al., 1992).

In another study, a glycogen was isolated from *P. canaliculus* and investigated in an *in vivo* model in rats with a carrageenin-induced footpad oedema where it was given *i.v.* and showed a dose-dependent anti-inflammatory activity (Miller et al., 1993). However, this activity could no longer be observed after treatment with KOH or proteinase K, suggesting that the anti-inflammatory properties are mediated by proteinaceous moieties associated with the macromolecule glycogen (Miller et al., 1993).

Recently, Xu and colleagues (2008) published a work on the isolation and characterisation of the antioxidant polysaccharide MP-I (composed of glucose monomers) from *M. coruscus*. This was found to be an α-(1→4)-D-glucan, branched with a single α-D-glucose at the C6 position every eighth residue along the main chain (Xu et al., 2008). This molecule (1.35×10^6 Da) exhibited a protective effect on acute liver injury in mice (given intraperitoneally). MP-1 has been shown to inhibit lipid peroxidation, which is involved in tissue damage during hepatic failure. The observed bioactivities render MP-I as a beneficial marine mussel carbohydrate, which may find pharmaceutical applications in humans (Xu et al., 2008). Cheng and co-workers (2010) have evaluated the impact of several extraction methods (by using water, acidic, or alkaline extracting media) on the antioxidant activity of mussel polysaccharides. A dose-dependent antioxidant activity was detected for all mussel extracts in an *in vitro* spectrophotometric assay, with water and alkaline extracts being the most active (Cheng, Yu, & Zhang, 2010).

2.4. Miscellaneous bioactive compounds from marine mussels

Our literature survey suggests that the most bioactive marine mussel compounds can be classified as typical primary metabolites, such as the discussed proteins, lipids, or carbohydrates. However, MytiLec, a sugar-binding protein (lectin) isolated from the mussel *M. galloprovincialis*, is discussed here under miscellaneous compounds, due to its carbohydrate-binding properties. Recently, this compound was discovered by Japanese researchers as a 17 kDa α-D-galactose-binding lectin comprising a novel primary structure which is expected to promote research not only in the field of glycobiology (Fujii et al., 2012), but also in pharmacology. MytiLec belongs to the humoral defence factors of the mussel similar to AMPs (Casas, Comesana, Cao, & Villalba, 2011). In terms of bioactivity, it exhibits a dose-dependent cytotoxic effect on human Burkitt's lymphoma Reji cells (Fujii et al., 2012). These results give insights into possible anti-cancer applications and will also help to reveal the physiological role of this novel galactose-binding lectin.

3. Biotoxins affecting marine mussels

Besides the beneficial effects and bioactives that mussel components may yield, it is vital to also consider potential harmful biotoxins that may be present in mussels. Mussels in common with other bivalve molluscs are filter-feeders. They can filter up to eight

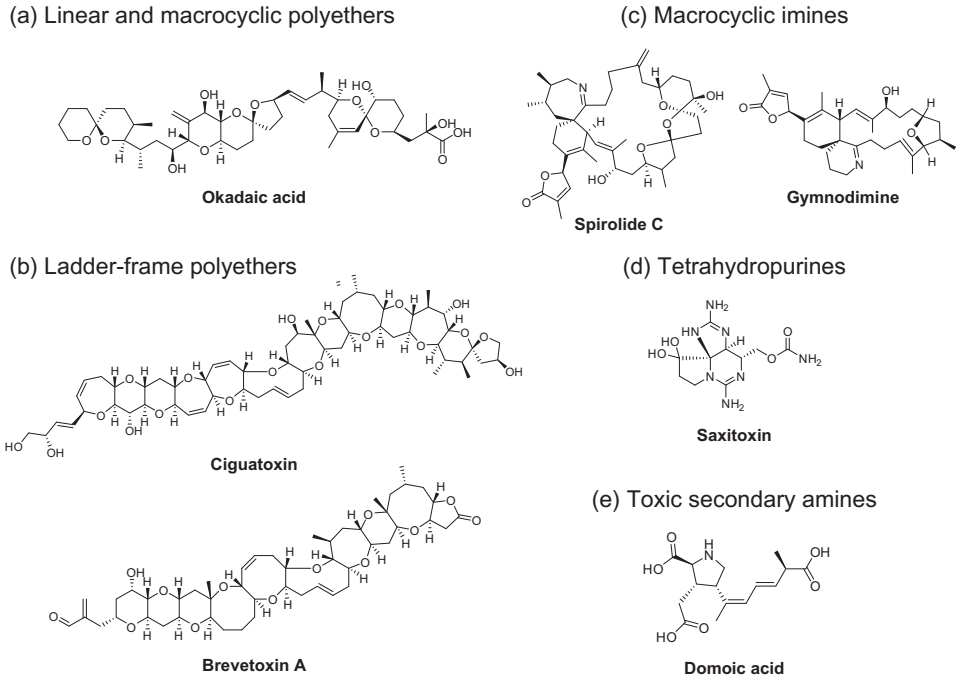

Fig. 6. Chemical structures of shellfish biotoxins: (a) okadaic acid; (b) ciguatoxin and brevetoxin A; (c) spirolide C and gymnodimine; (d) saxitoxin; and (e) domoic acid.

litres of sea water per hour whereby they derive their nutrition by passing water over their gills and extract and sort particles of food during this process (Jones, Richards, & Southern, 1992). They selectively choose filtered particles based on size and eject non-edible particles as pseudo-faeces before they enter the digestive tract. A large component of the ingested particles comprise of nutrient rich eukaryotic microalgae, mostly diatoms and dinoflagellates (Newell & Shumway, 1993). In marine environments there are a small but significant group of microalgae referred to as harmful algal bloom (HAB) species that cause injury to human health or socioeconomic interests, or to components of aquatic ecosystems (Anderson, Cembella, & Hallegraeff, 2012). Mussels are fairly non-discriminatory towards the species of microalgae that they ingest. Their target feed can include a number of different HAB species that contain various compounds that are toxic to humans, and filter feeding shellfish including mussels are a significant source by which these toxins find a pathway through the food chain at concentrations that can cause human illness.

HAB toxins have typically been classified based on the illness that they produce in human consumers; amnesic shellfish poisoning (ASP), ciguatera fish poisoning (CFP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), paralytic shellfish poisoning (PSP), and azaspiracid shellfish poisoning (AZP). In addition, there are other toxins that have yet to demonstrate adverse effects (acute or chronic) in humans including the Cyclic Imines Group, Pectenotoxins (PTX) Group and Yessotoxins (YTX) Group (UNESCO., 2005). Within these groups, there are a large number of toxins differing in their structures, solubility and mode of action. In addition, a wide range of metabolites is found in shellfish due to biotransformation through enzymatic reactions.

The majority of human diseases associated with HAB toxins appear to be acute phenomena, although some can cause prolonged chronic disease (*e.g.* ASP). Toxins from the PSP, DSP and ASP groups are considered to have a worldwide distribution and their causative species are also known to have global distribution (Ciminiello & Fattorusso, 2004). The micro-algae responsible for the remaining toxins are less cosmopolitan and their toxins are more restricted geographically. The distribution can however at best be described as patchy in a biogeographical sense, and this is compounded by variation in biotransformation of the parent toxin into chemical analogues that may be more or less toxic as they pass through the food web. There is also evidence of transfer of HABs by anthropogenic and non-anthropogenic vectors and this can result in toxins appearing in previously toxin free areas so the potential of toxic HAB species is to be regarded as potentially worldwide (Bolch & de Salas, 2007).

The classification of toxins, based on symptomology, has been greatly advanced with the introduction of high-resolution analytical separation and detection technologies including HPLC combined with MS and NMR technologies. This has resulted in several prominent groups of toxins, which can be summarised as (a) linear and macrocyclic polyethers, *e.g.* okadaic acid or dinophysistoxins; (b) ladder-frame polyethers, *e.g.* ciguatoxins and brevetoxins; (c) macrocyclic imines, *e.g.*, spirolides and gymnodimine; (d) tetrahydropurines, *e.g.*, saxitoxin and analogues; and (e) toxic secondary amines, including domoic acid (Fig. 6).

At present, there are no practical solutions to removing these toxins from shellfish other than allowing them to naturally depurate by metabolic processes within the shellfish growing area. This can take between several weeks to several months depending on the level of toxin and environmental parameters. The primary preventive tool for intoxications with natural toxins is the monitoring of toxin levels in algae in the harvesting areas. Based on the presence of toxins, restrictions can be placed and harvesting of shellfish forbidden if levels of toxin are too high.

4. Conclusion and outlook

Within the past decades, marine mussels, in particular species of the genera *Mytilus* and *Perna* have attracted research interest for their primary metabolites. In addition, as highlighted in this review, monitoring the mussel for possible contaminations with harmful biotoxins is emerging as a subject of great importance for public health and product development. Driven by both industry and academia, prospecting a tremendous potential in the devel-

opment of functional foods and nutraceuticals, research has been mainly directed towards the bioactive potential of proteins, lipids, and carbohydrates. As opposed to natural product drug discovery programmes, which are usually aiming at the discovery of small molecules or secondary metabolites, the investigation of bioactive primary metabolites is a research area scattered over several scientific disciplines, often accompanied by a lack of target-oriented strategies for analytics, isolation, and characterisation procedures for compounds of interest. To overcome these issues it might be of relevance to cross frontiers between chemistry and food industry in order to successfully use the gained knowledge about bioactives from marine mussels. As witnessed in a growing number of cases and especially in light of current European legislations concerning aquaculture waste regulations a huge commercial interest facilitates such endeavours.

Since protein accounts for the major part of mussel meat, it is not surprising that proteins, peptides and amino acids make up the most significant group of bioactives found among mussels. The advantages of this are reflected in a win–win situation for both industry and academia. Considering prospering aquaculture businesses producing large volumes of mussel waste materials, the generation of bioactive proteinaceus metabolites helps to exploit these waste materials and offers sources for the development of functional foods or nutraceuticals. However, challenges such as stability, bitter taste and the development of suitable food-grade formulation methods require interdisciplinary expertise.

In terms of commercial applications, the investigation of mussel lipids for instance as anti-arthritic remedies has shown much faster progress compared to mussel proteins. The bioactive potential of mussel carbohydrates has been explored to a lesser extent in comparison to the other two classes of metabolites, however existing studies point out promising immune-modulating or antioxidant bioactivities, especially for mussel-derived polysaccharides. In summary, primary metabolites from marine mussels of the genus *Mytilus* and *Perna* have shown promising results. Hence, they represent invaluable sources for the development of functional foods, food ingredients, or pharmaceuticals. Further advances in processing and analytical technologies, as well as a more interdisciplinary research focus, are expected to promote a straightforward and targeted development of health beneficial products in the near future.

Acknowledgements

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References

Almada-Villela, P. C. (1984). The effects of reduced salinity on the shell growth of small *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom, 64*(01), 171–182.

Anderson, D. M., Cembella, A. D., & Hallegraeff, G. M. (2012). Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. *Annual Review of Marine Science, 4*, 143–176.

Badiu, D. L., Balu, A. M., Barbes, L., Luque, R., Nita, R., Radu, M., et al. (2008). Physico-chemical characterisation of lipids from *Mytilus galloprovincialis* (L.) and *Rapana venosa* and their healing properties on skin burns. *Lipids, 43*(9), 829–841.

Badiu, D. L., Luque, R., Dumitrescu, E., Craciun, A., & Dinca, D. (2010). Amino acids from *Mytilus galloprovincialis* (L.) and *Rapana venosa* molluscs accelerate skin wounds healing via enhancement of dermal and epidermal neoformation. *The Protein Journal, 29*(2), 81–92.

Balseiro, P., Falco, A., Romero, A., Dios, S., Martinez-Lopez, A., Figueras, A., et al. (2011). *Mytilus galloprovincialis* myticin C: A chemotactic molecule with antiviral activity and immunoregulatory properties. *PLoS ONE, 6*(8), e23140.

Bernal, J., Mendiola, J. A., Ibanez, E., & Cifuentes, A. (2011). Advanced analysis of nutraceuticals. *Journal of Pharmaceutical and Biomedical Analysis, 55*(4), 758–774.

Bolch, C. J. S., & de Salas, M. F. (2007). A review of the molecular evidence for ballast water introduction of the toxic dinoflagellates *Gymnodinium catenatum* and the *Alexandrium tamarensis* complex to Australasia. *Harmful Algae, 6*(4), 465–485.

Brien, S., Prescott, P., Coghlan, B., Bashir, N., & Lewith, G. (2008). Systematic review of the nutritional supplement *Perna canaliculus* (green-lipped mussel) in the treatment of osteoarthritis. *QJM: Monthly Journal of the Association of Physicians, 101*(3), 167–179.

Brubaker, C. E., & Messersmith, P. B. (2012). The present and future of biologically inspired adhesive interfaces and materials. *Langmuir, 28*(4), 2200–2205.

Brusca, R. C., & Brusca, G. J. (1990). *Invertebrates*. Massachusetts USA: Sinauer Associates, Inc., Sunderland.

Carriel-Gomes, M. C., Kratz, J. M., Barracco, M. A., Bachere, E., Barardi, C. R., & Simoes, C. M. (2007). *In vitro* antiviral activity of antimicrobial peptides against *Herpes simplex* virus 1, adenovirus, and rotavirus. *Memórias do Instituto Oswaldo Cruz, 102*(4), 469–472.

Casas, S. M., Comesana, P., Cao, A., & Villalba, A. (2011). Comparison of antibacterial activity in the hemolymph of marine bivalves from Galicia (NW Spain). *Journal of Invertebrate Pathology, 106*(2), 343–345.

Chan, E. J., & Cho, L. (2009). What can we expect from omega-3 fatty acids? *Cleveland Clinic Journal of Medicine, 76*(4), 245–251.

Chandler, A.M. (2005a). *Combinations of hyaluronic acid and polyunsaturated fatty acids*. WO 2005112910 A1 20051201.

Chandler, A.M. (2005b). *Treatment for asthma and arthritis and other inflammatory diseases*. WO 2005112960 A1 20051201.

Chandran, B., Rameshkumar, G., & Ravichandran, S. (2009). Antimicrobial activity from the gill extraction of *Perna viridis* (Linnaeus, 1758). *Global Journal of Biotechnology & Biochemistry, 4*(2), 88–92.

Chapman, A. D. (2009). *Numbers of living species in Australia and the world* (2nd ed.). Canberra: Australian Biological Resources Study.

Charlet, M., Chernysh, S., Philippe, H., Hetru, C., Hoffmann, J. A., & Bulet, P. (1996). Innate immunity. Isolation of several cysteine-rich antimicrobial peptides from the blood of a mollusc, *Mytilus edulis*. *The Journal of Biological Chemistry, 271*(36), 21808–21813.

Cheng, S., Yu, X., & Zhang, Y. (2010). Extraction of polysaccharides from *Mytilus edulis* and their antioxidant activity *in vitro*. *Shipin Gongye Keji, 31*, 132–134.

Cho, M. J., Unklesbay, N., Hsieh, F. H., & Clarke, A. D. (2004). Hydrophobicity of bitter peptides from soy protein hydrolysates. *Journal of Agricultural and Food Chemistry, 52*(19), 5895–5901.

Christie, W. W. (1982). *Lipid Analysis*. Oxford, UK: Pergamon Press.

Ciminiello, P., & Fattorusso, E. (2004). Shellfish toxins – Chemical studies on northern adriatic mussels. *European Journal of Organic Chemistry, 2004*(12), 2533–2551.

Cushman, D. W., & Cheung, H. S. (1971). Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochemical Pharmacology, 20*(7), 1637–1648.

Dai, Z.-Y., Zhang, Y.-P., Zhang, H., & Lu, Y.-B. (2012). Preparation and characterisation of mussel (*Mytilus edulis*) protein hydrolysates with angiontensin-I-converting enzyme (ACE) inhibitory activity by enzymatic hydrolysis. *Journal of Food Biochemistry, 36*(1), 66–74.

Doggrell, S. A. (2011). Lyprinol – Is it a useful anti-inflammatory agent? *Evidence-based Complementary and Alternative Medicine: eCAM, 2011*, 307121–307127.

Emelyanov, A., Fedoseev, G., Krasnoschekova, O., Abulimity, A., Trendeleva, T., & Barnes, P. J. (2002). Treatment of asthma with lipid extract of New Zealand green-lipped mussel: A randomised clinical trial. *The European Respiratory Journal, 20*(3), 596–600.

Freites, L., Fernandez-Reiriz, M. J., & Labarta, U. (2002). Fatty acid profiles of *Mytilus galloprovincialis* (Lmk) mussel of subtidal and rocky shore origin. *Comparative Biochemistry and Physiology, 132*(2), 453–461.

Fujii, Y., Dohmae, N., Takio, K., Kawsar, S. M., Matsumoto, R., Hasan, I., et al. (2012). A lectin from the mussel *Mytilus galloprovincialis* has a highly novel primary structure and induces glycan-mediated cytotoxicity of globotriaosylceramide-expressing lymphoma cells. *The Journal of Biological Chemistry, 287*(53), 44772–44783.

Gerdol, M., De Moro, G., Manfrin, C., Venier, P., & Pallavicini, A. (2012). Big defensins and mytimacins, new AMP families of the Mediterranean mussel *Mytilus galloprovincialis*. *Developmental and Comparative Immunology, 36*(2), 390–399.

Gestal, C., Roch, P., Renault, T., Pallavicini, A., Paillard, C., Novoa, B., et al. (2008). Study of diseases and the immune system of bivalves using molecular biology and genomics. *Reviews in Fisheries Science, 16*(S1), 133–156.

Gibson, R. G., Gibson, S. L., Conway, V., & Chappell, D. (1980). *Perna canaliculus* in the treatment of arthritis. *The Practitioner, 224*(1347), 955–960.

Gibson, S. L. M., & Gibson, R. G. (1998). The treatment of arthritis with a lipid extract of *Perna canaliculus*: A randomized trial. *Complementary Therapies in Medicine, 6*(3), 122–126.

Gosling, E. (1992). *The Mussel Mytilus: Geology, Physiology, Genetics and Culture*. Amsterdam: Elsevier.

Gosling, E., Doherty, S., & Howley, N. (2008). Genetic characterisation of hybrid mussel (*Mytilus*) populations on Irish coasts. *Journal of the Marine Biological Association of the United Kingdom, 88*(2), 341–346.

Haller, C. A. (2010). Nutraceuticals: Has there been any progress? *Clinical Pharmacology & Therapeutics, 87*(2), 137–141.

Halpern, G. M. (2000). Anti-inflammatory effects of a stabilized lipid extract of *Perna canaliculus* (Lyprinol). *Allergie et Immunologie, 32*(7), 272–278.

Hancock, R. E., & Scott, M. G. (2000). The role of antimicrobial peptides in animal defenses. *Proceedings of the National Academy of Science of the United States of America, 97*(16), 8856–8861.

Harnedy, P. A., & FitzGerald, R. J. (2012). Bioactive peptides from marine processing waste and shellfish: A review. *Journal of Functional Foods, 4*(1), 6–24.

Hubert, F., Noel, T., & Roch, P. (1996). A member of the arthropod defensin family from edible Mediterranean mussels (*Mytilus galloprovincialis*). *European Journal of Biochemisty, 240*(1), 302–306.

Je, J. Y., Park, P. J., Byun, H. G., Jung, W. K., & Kim, S. K. (2005). Angiotensin I converting enzyme (ACE) inhibitory peptide derived from the sauce of fermented blue mussel. *Mytilus edulis. Bioresource Technology, 96*(14), 1624–1629.

Jenssen, H., Hamill, P., & Hancock, R. E. W. (2006). Peptide antimicrobial agents. *Clinical Microbiology Reviews, 19*(3), 491–511.

Jones, H. D., Richards, O. G., & Southern, T. A. (1992). Gill dimensions, water pumping rate and body size in the mussel *Mytilus edulis* L. *Journal of Experimental Marine Biology and Ecology, 155*(2), 213–237.

Jung, W.-K., & Kim, S.-K. (2009). Isolation and characterisation of an anticoagulant oligopeptide from blue mussel, *Mytilus edulis*. *Food Chemistry, 117*(4), 687–692.

Jung, W.-K., Qian, Z.-J., Lee, S.-H., Choi, S.-Y., Sung, N.-J., Byun, H.-G., et al. (2007). Free radical scavenging activity of a novel antioxidative peptide isolated from *in vitro* gastrointestinal digests of *Mytilus coruscus*. *Journal of Medicinal Foods, 10*(1), 197–202.

Jung, W.-K., Rajapakse, N., & Kim, S.-K. (2005). Antioxidative activity of a low molecular weight peptide derived from the sauce of fermented blue mussel, *Mytilus edulis*. *European Food Research and Technology, 220*(5), 535–539.

Kenneth, B., Waite, R.L., & Downs, B.W. (2009). *Nutragenomics and genomic customization of formulation components for treatment of diseases*. WO 2009155585 A1 20091223.

Kim, E.-K., Kim, Y.-S., Lee, S.-J., Jeon, Y.-J., Ahn, C.-B., Kim, Y.-T., et al. (2011). Effect of partially purified lipid from the mussel *Mytilus coruscus* on apoptosis in cancer cells. *Journal of the Korean Society for Applied Biological Chemistry, 54*(1), 59–65.

Kim, S.-K., & Mendis, E. (2006). Bioactive compounds from marine processing byproducts - A review. *Food Research International, 39*(4), 383–393.

Kosuge, T., Tsuji, K., Ishida, H., & Yamaguchi, T. (1986). Isolation of an antihistaminic substance from green-lipped mussel (*Perna canaliculus*). *Chemical & Pharmaceutical Bulletin, 34*(11), 4825–4828.

Lau, W. C. S., Chiu, P. K. Y., Chu, M. Y., Cheng, I. Y. W., Tang, W. M., Man, R. Y. K., et al. (2004). Treatment of knee osteoarthritis with Lyprinol, lipid extract of the green-lipped mussel - a double-blind placebo-controlled study. *Progress in Nutrition, 6*(1), 17–31.

Lee, B. P., Messersmith, P. B., Israelachvili, J. N., & Waite, J. H. (2011). Mussel-inspired adhesives and coatings. *Annual Review of Materials Research, 41*, 99–132.

Lee, C. H., Butt, Y. K. C., Wong, M. S., & Lo, S. C. L. (2008). A lipid extract of *Perna canaliculus* affects the expression of pro-inflammatory cytokines in a rat adjuvant-induced arthritis model. *European Annals of Allergy and Clinical Immunology, 40*(4), 148–153.

Li, P., & Ding, X. (2006). The manufacture and nutritional analysis of the functional natural sauce from the decoction of *Mytilus edulis*. *Zhongguo Tiaoweipin, 2*, 17–19.

Löfgren, S. E., Miletti, L. C., Steindel, M., Bachère, E., & Barracco, M. A. (2008). Trypanocidal and leishmanicidal activities of different antimicrobial peptides (AMPs) isolated from aquatic animals. *Experimental Parasitology, 118*(2), 197–202.

Löfgren, S. E., Smânia, A., Smânia, E. d. F. A., Bachère, E., & Barracco, M. A. (2009). Comparative activity and stability under salinity conditions of different antimicrobial peptides isolated from aquatic animals. *Aquaculture Research, 40*(16), 1805–1812.

Lordan, S., Ross, R. P., & Stanton, C. (2011). Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. *Marine Drugs, 9*(6), 1056–1100.

Marin, F., Luquet, G., Marie, B., & Medakovic, D. (2007). Molluscan shell proteins: Primary structure, origin, and evolution. In G. P. Schatten (Ed.). *Current Topics in Developmental Biology* (80, pp. 209–276). Academic Press.

McFarlane, S. J., & Croft, J. E. (1980). *Pharmaceutical preparations containing a mollusk extract*. EP 10061 A1 19800416.

McPhee, S., Hodges, L. D., Wright, P. F., Wynne, P. M., Kalafatis, N., Harney, D. W., et al. (2007). Anti-cyclooxygenase effects of lipid extracts from the New Zealand green-lipped mussel, *Perna canaliculus*. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology, 146*(3), 346–356.

McPhee, S., Hodges, L. D., Wright, P. F., Wynne, P. M., Kalafatis, N., & Macrides, T. A. (2010). Prophylactic and therapeutic effects of *Mytilus edulis* fatty acids on adjuvant-induced arthritis in male Wistar rats. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 82*(2–3), 97–103.

Miller, T. E., Dodd, J., Ormrod, D. J., & Geddes, R. (1993). Anti-inflammatory activity of glycogen extracted from *Perna canaliculus* (NZ green-lipped mussel). *Agents Actions, 38*, C139–142.

Mitta, G., Hubert, F., Dyrynda, E. A., Boudry, P., & Roch, P. (2000). Mytilin B and MGD2, two antimicrobial peptides of marine mussels: gene structure and expression analysis. *Developmental & Comparative Immunology, 24*(4), 381–393.

Mitta, G., Hubert, F., Noel, T., & Roch, P. (1999). Myticin, a novel cysteine-rich antimicrobial peptide isolated from haemocytes and plasma of the mussel *Mytilus galloprovincialis*. *European Journal of Biochemisty, 265*(1), 71–78.

Mitta, G., Vandenbulcke, F., Hubert, F., Salzet, M., & Roch, P. (2000). Involvement of mytilins in mussel antimicrobial defense. *The Journal of Biological Chemistry, 275*(17), 12954–12962.

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G. B., & Worm, B. (2011). How many species are there on earth and in the ocean? *PLoS Biology, 9*(8), e1001127.

Motarjemi, Y. (2002). Impact of small scale fermentation technology on food safety in developing countries. *International Journal of Food Microbiology, 75*(3), 213–229.

Mulye, N., & Assoulin, D. (2012). *Topical formulations for administration of omega-3 fatty acids*. WO 2012075093 A2 20120607.

Murphy, K., Mooney, B., Mann, N., Nichols, P., & Sinclair, A. (2002). Lipid, FA, and sterol composition of New Zealand green lipped mussel (*Perna canaliculus*) and Tasmanian blue mussel (*Mytilus edulis*). *Lipids, 37*(6), 587–595.

Nakajima, Y., Ishibashi, J., Yukuhiro, F., Asaoka, A., Taylor, D., & Yamakawa, M. (2003). Antibacterial activity and mechanism of action of tick defensin against Gram-positive bacteria. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1624*(1-3), 125–130.

Newell, C. R., & Shumway, S. E. (1993). Grazing of natural particulates by bivalve molluscs: A spatial and temporal perspective. In R. F. Dame (Ed.). *Dame, Bivalve filter feeders in estuarine and coastal ecosystem processes* (33, pp. 85–148). Berlin: Springer-Verlag.

Ngo, D.-H., Wijesekara, I., Vo, T.-S., Van Ta, Q., & Kim, S.-K. (2011). Marine food-derived functional ingredients as potential antioxidants in the food industry: An overview. *Food Research International, 44*, 523–529.

Ngo, D. H., Vo, T. S., Ngo, D. N., Wijesekara, I., & Kim, S. K. (2012). Biological activities and potential health benefits of bioactive peptides derived from marine organisms. *International Journal of Biological Macromolecules, 51*(4), 378–383.

Ninan, L., Stroshine, R. L., Wilker, J. J., & Shi, R. (2007). Adhesive strength and curing rate of marine mussel protein extracts on porcine small intestinal submucosa. *Acta Biomaterialia, 3*(5), 687–694.

Otero-Gonzalez, A. J., Magalhaes, B. S., Garcia-Villarino, M., Lopez-Abarrategui, C., Sousa, D. A., Dias, S. C., et al. (2010). Antimicrobial peptides from marine invertebrates as a new frontier for microbial infection control. *FASEB Journal, 24*(5), 1320–1334.

Ovodova, R. G., Glazkova, V. E., Mikheiskaya, L. V., Molchanova, V. I., Isakov, V. V., Ovodov, Y. S., et al. (1992). The structure of mytilan, a bioglycan-immunomodulator isolated from the mussel *Crenomytilus grayanus*. *Carbohydrate Research, 223*, 221–226.

Padhi, A., & Verghese, B. (2008). Molecular diversity and evolution of myticin-C antimicrobial peptide variants in the Mediterranean mussel, *Mytilus galloprovincialis*. *Peptides, 29*(7), 1094–1101.

Park, P.-J., Je, J.-Y., & Kim, S.-K. (2005). Amino acid changes in the Korean traditional fermentation process for blue mussel, *Mytilus edulis*. *Journal of Food Biochemistry, 29*(1), 108–116.

Rainsford, K. D., & Whitehouse, M. W. (1980). Gastroprotective and anti-inflammatory properties of green lipped mussel (*Perna canaliculus*) preparation. *Arzneimittel-Forschung, 30*(12), 2128–2132.

Rajapakse, N., Mendis, E., Jung, W. K., Je, J. Y., & Kim, S. K. (2005). Purification of a radical scavenging peptide from fermented mussel sauce and its antioxidant properties. *Food Research International, 38*(2), 175–182.

Roch, P., Yang, Y., Toubiana, M., & Aumelas, A. (2008). NMR structure of mussel mytilin, and antiviral-antibacterial activities of derived synthetic peptides. *Developmental & Comparative Immunology, 32*(3), 227–238.

Romestand, B., Molina, F., Richard, V., Roch, P., & Granier, C. (2003). Key role of the loop connecting the two beta strands of mussel defensin in its antimicrobial activity. *European Journal of Biochemisty, 270*(13), 2805–2813.

Sarmadi, B. H., & Ismail, A. (2010). Antioxidative peptides from food proteins: A review. *Peptides, 31*(10), 1949–1956.

Scotti, P. D., Dearing, S. C., Greenwood, D. R., & Newcomb, R. D. (2001). Pernin: A novel, self-aggregating haemolymph protein from the New Zealand green-lipped mussel, *Perna canaliculus* (Bivalvia: Mytilidae). *Comparative Biochemistry and Physiology, Part B, 128*(4), 767–779.

Silva, V. M., Park, K. J., & Hubinger, M. D. (2010). Optimization of the enzymatic hydrolysis of mussel meat. *Journal of Food Science, 75*(1), C36–42.

Singh, M., Hodges, L. D., Wright, P. F., Cheah, D. M., Wynne, P. M., Kalafatis, N., et al. (2008). The CO_2-SFE crude lipid extract and the free fatty acid extract from *Perna canaliculus* have anti-inflammatory effects on adjuvant-induced arthritis in rats. *Comparative Biochemisty and Physiology, Part B, 149*(2), 251–258.

Smital, T., & Kurelec, B. (1998). The chemosensitizers of multixenobiotic resistance mechanism in aquatic invertebrates: A new class of pollutants. *Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis, 399*(1), 43–53.

Sonthi, M., Toubiana, M., Pallavicini, A., Venier, P., & Roch, P. (2011). Diversity of coding sequences and gene structures of the antifungal peptide mytimycin (MytM) from the Mediterranean mussel, *Mytilus galloprovincialis*. *Marine Biotechnology, 13*(5), 857–867.

Sperstad, S. V., Haug, T., Blencke, H. M., Styrvold, O. B., Li, C., & Stensvag, K. (2011). Antimicrobial peptides from marine invertebrates: Challenges and perspectives in marine antimicrobial peptide discovery. *Biotechnology Advances, 29*(5), 519–530.

Steiner, H., Hultmark, D., Engstrom, A., Bennich, H., & Boman, H. G. (1981). Sequence and specificity of two antibacterial proteins involved in insect immunity. *Nature, 292*(5820), 246–248.

Sukumaran, S., Pittman, K. B., Patterson, W. K., Dickson, J., Yeend, S., Townsend, A., et al. (2010). A phase I study to determine the safety, tolerability and maximum tolerated dose of green-lipped mussel (*Perna canaliculus*) lipid extract, in

patients with advanced prostate and breast cancer. *Annals of Oncology, 21*(5), 1089–1093.

Toki, M., Aoki, K., Katsumi, N., & Takahashi, S. I. (2007). NSAID and its effect on prostaglandin. *Japanese Journal of Clinical Medicine, 65*(10), 1807–1811.

Treschow, A. P., Hodges, L. D., Wright, P. F., Wynne, P. M., Kalafatis, N., & Macrides, T. A. (2007). Novel anti-inflammatory omega-3 PUFAs from the New Zealand green-lipped mussel, *Perna canaliculus*. *Comparative Biochemistry and Physiology, Part B, 147*(4), 645–656.

UNESCO. (2005). Report of the joint FAO/IOC/WHO ad hoc expert consultation on biotoxins in bivalve molluscs. In: *IOC/INF-1215*, pp. 8, Oslo, Norway, 26–30 September 2004.

Visessanguan, W., Benjakul, S., Riebroy, S., & Thepkasikul, P. (2004). Changes in composition and functional properties of proteins and their contributions to Nham characteristics. *Meat Science, 66*(3), 579–588.

Wakimoto, T., Kondo, H., Nii, H., Kimura, K., Egami, Y., Oka, Y., et al. (2011). Furan fatty acid as an anti-inflammatory component from the green-lipped mussel *Perna canaliculus*. *Proceedings of the National Academy of Sciences of the United States of America, 108*(42), 17533–17537.

Whitehouse, M. W., Macrides, T. A., Kalafatis, N., Betts, W. H., Haynes, D. R., & Broadbent, J. (1997). Anti-inflammatory activity of a lipid fraction (lyprinol) from the NZ green-lipped mussel. *Inflammopharmacology, 5*(3), 237–246.

Williams, C.E., & Sansom, A.J. (2008). *Oil extract from marine materials rich in fatty acid amides including N-acylethanolamines*. WO 2008075978 A2 20080626.

Xu, H., Guo, T., Guo, Y. F., Zhang, J., Li, Y., Feng, W., et al. (2008). Characterisation and protection on acute liver injury of a polysaccharide MP-I from *Mytilus coruscus*. *Glycobiology, 18*(1), 97–103.

Yang, Y. S., Mitta, G., Chavanieu, A., Calas, B., Sanchez, J. F., Roch, P., et al. (2000). Solution structure and activity of the synthetic four-disulfide bond Mediterranean mussel defensin (MGD-1). *Biochemistry, 39*(47), 14436–14447.

Yasin, B., Pang, M., Turner, J. S., Cho, Y., Dinh, N. N., Waring, A. J., et al. (2000). Evaluation of the inactivation of infectious *Herpes simplex* virus by host-defense peptides. *European Journal of Clinical Microbiology & Infectious Diseases, 19*(3), 187–194.

Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature, 415*(6870), 389–395.