Marine Bivalves as a Dietary Source of High-Quality Lipid: A Review with Special Reference to Natural n-3 Long Chain Polyunsaturated Fatty Acids

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REVIEW

Abstract
The most important nutritional feature of mollusks is their lipid composition. Marine bivalves are a good source of high quality lipid, mainly because they concentrate eicosapentaenoic acid and docosahexaenoic acid. Meanwhile, these n-3 Long Chain Polyunsaturated Fatty Acids (LC-PUFAs) are essential in the fight against inflammation, preventing depression, reducing weight and waist size, decreasing liver fat and promoting mental wellbeing. Typically, marine bivalves have a low saturated fatty acid content and a high n-3 LC-PUFA concentration, which is usually absent in most foods. Freshwater bivalves generally contain lower proportions of n-3 PUFA than marine bivalves; the lipid quality also differ in farmed and wild species. The focus of this review is on physiological important n-3 LC-PUFA present in marine bivalves, with an emphasis on the indicators for assessing quality and the effects of heat on bivalve lipid.

Keywords: bivalve; fatty acids; nutritional quality; marine lipid; shellfish.

INTRODUCTION
The term "Mollusk (Mollusc)" was derived from Latin word mollus meaning soft. It is used to describe squids, cuttlefish and animals with reduced shells, characterized by a combination of morphological and anatomical features that distinguish them from all other invertebrates. Considering the vast species of mollusks and the large number of fossil species, they are the largest marine phylum, comprising about 23% of all the named marine organisms (Sadjadi, 2018). The Phylum Mollusca is divided into Scaphopoda, Gastropoda, Cephalopoda, Bivalvia, Polyplacophora, Caudofoveata, Solenogastres, and Monoplacophora (Puglisi et al., 2020). Out of the eight classes of these soft-bodied invertebrates, bivalves (oysters, scallops, mussels, and clams), cephalopods (octopus, squid, and cuttlefish), and gastropods (whelks, sea snail, abalone, and cockle) represent the economically significant mollusks (Venugopal & Gopakumar, 2017). Bivalves and gastropods represent the most studied mollusks in relation to the isolation of natural products and account for approximately 98 percent of the total molluscan species (Núñez-Pons et al., 2015). Bivalves as a group lack some typical molluscan organs such as the radula and the odontophore (Romano et al., 2014; Moruf et al., 2020). Most mollusks from the Class Bivalvia are known to be suspension-feeders, their diets consisting mainly of plankton from the water column, bottom water layer
and deposit-feeders collecting food from the surface of bottom sediments. Thus, planktonic and benthic microalgae, zooplankton, protozoans, including heterotrophic flagellates, ciliates and bacteria are the main components of diet of filter-feeding bivalves (Zhukova et al., 2019). This feeding mode and, consequently, the diet primarily impact the composition of bivalve fatty acids (Van der Heijden et al., 2019).

Two fatty acid molecules found to be important for humans are contained in lipids: n-3 and n-6 fatty acids (Julaecha & Safitri, 2020). The biological effects of these fatty acid molecules are mediated by their mutual interactions. Closely related, these fatty acids act as competing substrates for the same enzymes. Hence, the proportion of n-3 to n-6 fatty acids in a diet may have metabolic implications (Bibus & Lands, 2015). There are two different versions of n-3 called long-chain polyunsaturated fatty Acid (≥C20, LCPUFA) and short-chain polyunsaturated fatty Acid (≤C18, SCPUFA). We focus here on the long-chain n-3, which include eicosapentaenoic acid (EPA, 20:5 n-3), docosapentaenoic acid (DPA, 22:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). The main objective of this review is to summarize the physiological important n-3 LC-PUFA in bivalve mollusks, the indicators for assessing the nutritional quality of bivalve lipids, and the effects of heat on the marine-derived n-3 LCPUFA. Furthermore, a critical analysis of literatures on n-3 LCPUFA is presented to generate a useful reference for food industry and academia.

MATERIALS AND METHODS

Lipid content of marine bivalves

The term “lipid” is sometimes used as a synonym for fats. Meanwhile, fats are a subgroup of lipids called triglycerides. Lipid also encompasses molecules such as fatty acids and their derivatives (including tri-, di-, monoglycerides, and phospholipids), as well as other sterol-containing metabolites such as cholesterol (Domiszewski et al., 2011). Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) are the principal classes of fatty acids in fish lipids (Airaodion et al., 2019). The lipid SFAs are dominated by palmitic acid (C16:0), followed by myristic acid (C14:0), which are more abundant in the warmer water fish than the coldwater fish. The MUFAs are dominated by oleic acid (C18:1), while PUFA composition is the most characteristic trait of fish lipids.

Marine Bivalves are generally characterized by the predominance of essential n-3 PUFA, mainly EPA, DHA and DPA (Figure1), which constitute usually almost half of the total fatty acids (Zhukova, 2014). Researchers have shown that freshwater bivalves generally contain lower proportions of n-3 PUFA than marine bivalves; the lipid quality also differ in farmed and wild species (Moradi et al., 2011). However, the lipid composition of marine bivalves is affected by the taxonomic relationship (species and nutrient habits) and environmental conditions (food availability and physiological conditions) (Prato et al., 2019). In general, the lipid content of most studied marine bivalves ranged between 0.5 and 11.9% (Tan et al., 2019).

Figure 1. n-3 fatty acid content of commercially important marine bivalves (Zhukova, 2019)

Indicators for assessing lipid quality of marine bivalves

Marine bivalves are a good source of high quality lipid, mainly because they concentrate EPA and DHA from phytoplankton (Ricardo et al., 2015). According to published data, the EPA+DHA content of mussels and oysters is
usually higher than that of cockles, scallops and clams (Table 1). The indicators for assessing lipid quality of bivalves are the ratios of n-3/ n-6 and PUFA/ SAFA contained in the mollusks.

n-3/ n-6 ratio: Like n-3s, n-6 fatty acids are polyunsaturated fatty acids. However, the last double bond is six carbons from the omega end of the fatty acid molecule. The most common n-6 fat is linoleic acid, which the body can convert to longer n-6 fats such as arachidonic acid (AA). Like EPA, AA produces eicosanoids. However, the eicosanoids that AA produces are more pro-inflammatory (Calder, 2013).

It is necessary to obtain the ratio of n-3/ n-6 with a healthy balance >1.0 (Bhardwaj et al., 2016). They both have health benefits, but it’s important to get the right balance between them. According to Simopoulos & DiNiccolantonio (2016), a low intake of n-3 fatty acids compared with n-6s may contribute to inflammation and chronic diseases, such as rheumatoid arthritis, diabetes, atherosclerosis, and heart failure. The World Health Organization has recommended that the daily ratio of n-6/n-3 in total human diet should be below 15 and preferably between 5 and 9 (Arinond et al., 2015). In general, all bivalves have an n-3/ n-6 ratio >1.0 (bivalves with higher omega-3/ omega-6 ratios are preferred), indicating that they all meet the nutritional needs of consumers for high quality lipids.

PUFA/ SAFA ratio: The PUFA/ SAFA ratio is another useful indicator for assessing the nutritional quality of food lipids. The PUFA/ SAFA ratio recommended by the Department of Health (1994) is > 0.45. Foods with a PUFA/ SAFA ratio below 0.45 are suitable for consumers in low fat food selection.

Table 1. Lipid quality indicators of commercially important marine bivalves (Tan et al, 2019)

<table>
<thead>
<tr>
<th>Types</th>
<th>Species</th>
<th>Total lipid (%)</th>
<th>Ratios</th>
<th>PUFA/SAFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mussels</td>
<td>Modiolus barbatu</td>
<td>3.00 ± 0.2</td>
<td>2.99 ± 0.14</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>My. Galloprovincialis</td>
<td>2.20 ± 0.30</td>
<td>7.19 ± 0.28</td>
<td>0.85 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Perna viridis</td>
<td>2.00 ± 0.10</td>
<td>1.85 ± 0.01</td>
<td>1.16 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Flexopecten glaber</td>
<td>1.00 ± 0.30</td>
<td>2.65 ± 0.06</td>
<td>0.51 ± 0.14</td>
</tr>
<tr>
<td>Scallops</td>
<td>Pecten maximus</td>
<td>0.80 ± 0.10</td>
<td>15.2 ± 2.52</td>
<td>1.61 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Placopecten magellanicus</td>
<td>0.60 ± 0.10</td>
<td>18.2 ± 0.44</td>
<td>1.69 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Arca noae</td>
<td>1.20 ± 0.00</td>
<td>4.21 ± 0.65</td>
<td>0.68 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>Solen marginatus</td>
<td>1.30 ± 0.10</td>
<td>2.86 ± 0.44</td>
<td>0.68 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Limaria tuberculate</td>
<td>2.80 ± 0.40</td>
<td>5.77 ± 0.21</td>
<td>0.77 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Mastra stultorum</td>
<td>1.30 to 2.90</td>
<td>5.25 to 7.67</td>
<td>1.12 ± 0.05</td>
</tr>
<tr>
<td>Clams</td>
<td>Astarte sulcate</td>
<td>0.50 ± 0.10</td>
<td>3.68 to 3.75</td>
<td>1.07 to 1.34</td>
</tr>
<tr>
<td></td>
<td>Chamelea gallina</td>
<td>0.70 to 1.60</td>
<td>4.28 to 10.80</td>
<td>1.06 to 1.55</td>
</tr>
<tr>
<td></td>
<td>Megangulus zyonoensis</td>
<td>0.90 ± 0.60</td>
<td>9.53 ± 5.91</td>
<td>1.61 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Megangulus venulosus</td>
<td>0.70 ± 0.20</td>
<td>4.09 ± 1.04</td>
<td>1.57 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Laternula elliptica</td>
<td>1.90 ± 2.70</td>
<td>8.20 ± 3.70</td>
<td>0.72 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Ostrea edulis</td>
<td>2.70 ± 0.30</td>
<td>5.31 ± 0.98</td>
<td>0.68 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>Crassostrea hongkongensis</td>
<td>5.00 ± 0.60</td>
<td>6.61 ± 0.50</td>
<td>1.00 ± 0.01</td>
</tr>
<tr>
<td>Oysters</td>
<td>C. madrasensis</td>
<td>3.30 ± 0.30</td>
<td>4.66 ± 0.01</td>
<td>1.39 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>C. virginica</td>
<td>-</td>
<td>2.93 ± 0.49</td>
<td>2.13 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>C. gigas</td>
<td>6.70 ± 1.30</td>
<td>2.78 ± 0.29</td>
<td>1.78 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Tegillarca granosa</td>
<td>1.60 ± 0.30</td>
<td>3.50 ± 0.20</td>
<td>1.60 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>Fulvia mutica</td>
<td>1.50 to 3.00</td>
<td>22.10 ± 4.30</td>
<td>19.40 ± 2.60</td>
</tr>
<tr>
<td>Cockles</td>
<td>Anadara granosa</td>
<td>1.90 ± 1.30</td>
<td>3.80 ± 0.01</td>
<td>1.00 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Cerastoderma edule</td>
<td>-</td>
<td>17.39 ± 4.44</td>
<td>1.62 ± 0.43</td>
</tr>
</tbody>
</table>

Indicators for assessing lipid quality of marine bivalves

Physiological important n-3 LC-PUFA in marine bivalves

An n-3 fatty acid is a multi-double bonded fatty acid where the first double is between the third and fourth carbon atoms from the end of the carbon atom chain. "Short-chain" n-3 fatty acids have a chain of 18 carbon atoms or less, while “long-chain” n-3 fatty acids have a chain of 20 or more. Due to outstanding benefits in human
physiology, there has been considerable interest in marine-derived long chain n-3 PUFAs; EPA, DHA and DPA (Figure 2). These fatty acids have attracted a lot of attention due to their important physiological functions and the inability of the human body to synthesize these compounds (Drouin et al., 2019).

![Figure 2. Structure of some physiological important n-3 LC-PUFA](image)

Most of the beneficial effects of bivalve n-3 LC-PUFA have been attributed to DHA and then to EPA, for which there is growing interest in the independent and shared functions. In most bivalve mollusks, DHA is usually more abundant than EPA; and up to 2–3 times the proportion of EPA is higher in bivalves than in finfish (Moradi et al., 2011). Low intake of dietary EPA and DHA is associated with increased inflammatory processes, general cardiovascular health and risk of the development of Alzheimer’s disease, as well as with poor fetal development, including neuronal, retinal and immune function (Calder, 2018). Low maternal DHA intake may also contribute to increased risk of early preterm birth and asthma in children (Külzow et al., 2016). Consumption of n-3 LC-PUFAs, particularly DHA may enhance cognitive performance relating to learning, cognitive development, memory and rate of fulfilling cognitive tasks (Campoy et al., 2012).

On the other hand, the literature concerning the potential protective effects of n-3 DPA is brief because n-3 DPA represents less than one-third of each EPA and DHA in fish oils (Drouin et al., 2019). In addition, n-3 DPA is not commercially available in sufficient quantity, with high purity (>98%) and at an affordable price to set up in vivo nutritional supplementation studies (Byelashov et al., 2015). n-3 DPA is the only intermediate between EPA and DHA in the n-3 LCPUFA conversion pathway from α-linolenic acid (ALA) present in significant quantities in the diet. Therefore, n-3 DPA serves as a dietary source or biological reservoir of DHA and EPA (Drouin et al., 2019).

Compared to other body organs, the brain has a peculiar composition, with a high content of n-3 DHA, comprising 50% of the total brain lipid and 10–15% of all of the fatty acids, and low contents of other PUFA such as DPA and EPA (Dyall, 2015). However, the total fatty acid composition and DHA content of the brain varies depending on dietary intake, age, gender, and regional distribution/different regions of the brain (Oroc et al., 2019). A low intake of n-3 LCPUFAs lowers the level of DHA and boosts the level of arachidonic acid (AA) in the brain (Larrieu et al., 2012). In the absence of the fatty acid enriched diet, the n-3 LCPUFAs content in the brain is also strongly influenced by aging (Joffre et al., 2016). In contrast to males, the brain of female has been found to contain more DHA, which is associated with elevated levels of hormones that increase the level of fatty acid desaturase mRNA expression (Lin et al., 2016; Ahmmed et al., 2020). Despite the EPA lower concentration in brain compared to DHA, the physiological importance of EPA for brain health cannot be ignored since it is not only providing anti-inflammatory and immunity functions, but also plays an important role in neurotransmission, synaptic plasticity, and sound sleep, which are important parameters for treating patients suffering from depression (Luchtman & Song, 2013).

In terms of food safety, infectious disease, trace element and biotoxin hazards associated with consumption of bivalves have been recognized for many years (Tan and Ransangan, 2017). As a result, sanitary regulations on the cultivation and processing of bivalves have been introduced by most countries (Santos-Ferreira et al., 2020). Based on the recommended daily intake of 500 mg EPA + DHA (Salem and Eggersdorfer, 2015), consumers only need to consume 1.5 to 21 g/day, 4 to 21 g/day or 70 to 150 g/day of clams, oysters or mussels, respectively, to obtain the recommended doses of EPA and DHA (Tan et al., 2019).
Major sources of natural n-3 LC-PUFA

Aquatic food webs are the primary sources of natural n-3 PUFA. PUFA are produced de novo predominantly by unicellular phytoplankton and seaweeds including microalgae (e.g Cryptothecodinium, thraustochytrids, Thraustochytrium, Ulkenia, and Schizochytrium sp.), bacteria (e.g Phaeodactylum tricornutum, Nannochloropsis sp. and Desmodesmus sp) and zooplankton (e.g Calanus finmarchicus and Euphasia superba). They are further transfer from primary producers to consumers on trophic levels of the marine food chains. (Rossoll et al., 2012; Van Huis, 2013; Gong et al., 2017). Members of Bacillariophyceae are abundant in aquatic habitats and are considered as the most important primary producers of n-3 LC-PUFA in marine food chains. The fatty acids reported for different species of Bacillariophyceae are typical for diatoms. Diatoms frequently dominate in seasonal phytoplankton blooms and, accordingly, these algae are the most studied classes of microalgae in terms of their lipids and fatty acids (Zhukova, 2019). Another important source of PUFA for marine mollusks is heterotrophic protists, zooflagellates and ciliates, constituting the links in the food web named the “microbial loop”.

The high cost of extracting algae from biomass is a major limitation in the production of algal oil. Moreso, algal fatty acid profiles are highly sensitive to changes in culture conditions, making it difficult to maintain the quality and quantity of algae oil (Sprague et al., 2016). Furthermore, the natural antioxidants in the microalgae are destroyed during the extraction process, so once the algae oil is extracted, there is additional difficulty in the protection of the algae oil from oxidation (Winwood, 2013). Due to these challenges, the current extraction of n-3 LC-PUFA from cultured microalgae is still limited to laboratory scale and is insufficient to meet the growing demand for n-3 LC-PUFA (Tan et al., 2019). The production volumes of zooplankton are low, and overfishing of zooplankton may have a negative impact on the higher trophic species that depend on this food source, thereby disrupting the balance of the ecosystem (Tan and Ransangan, 2017).

Bivalves are arguably the group among marine invertebrates in which biosynthesis of PUFA has been most extensively investigated, in part due to commercial interest and perceived nutritional value of molluscs as sources of “n-3” for humans (Zhukova, 2014). Therefore, among the potential sources of natural n-3 LCPUFA, bivalve farming appears to be the most promising approach for producing natural n-3 LC-PUFA (Tan et al., 2019). The mass production of bivalves through aquaculture is self-regulating (additional feed not required), highly sustainable and environmentally friendly, as bivalves feed directly from the environment, thereby preventing environmental pollutants associated with feed production and nutrient input into the marine environment (Tan et al., 2019). In addition, bivalve production is also economically relevant as it accounts for 21.42% of the global aquaculture sector and 17.14 million tonnes of farmed bivalves in 2018 (FAO, 2018).

Effect of heat on bivalve n-3 LC-PUFA

Long chain n-3 PUFA are esterified in either triacylglycerides (TAG) or in phospholipid (PLs) form. Fish oil produced from fish waste is an example of fish derived triglyceride. However, temperature affects lipid oxidation status and it is not always possible to maintain the n-3 content unchanged during the processing of the fish oil. Molecular distillation of fish oil, a method to extract contaminants and impurities from fish oil and to boost the stability of n-3 LCPUFA during storage, will turn the fatty acids into the form of ethyl ester (EE). Consequently, most fish oil supplements in the market contain n-3 fatty acid in their EE form, which is less bioavailable than n-3 fatty acid in TAG form (Ahmmed et al., 2020). On the other hand, n-3 LCPUFAs in the PL form are more stable and bioavailable compared to those in the EE and TAG form (Ahn et al., 2018; Ahmmed et al., 2020). Most importantly, only n-3 LCPUFAs in the PL form can cross the blood–brain barrier, and hence that is why enrichment of brain DHA is only possible using supplements with DHA in the PL form (Chouinard-Watkins et al., 2019). Again, n-3 LCPUFAs in the PL form are more resistant to oxidative deterioration compared to that in the form of TAG and EE (Ahmmed et al., 2020).

Several cooking processes, such as boiling, microwaving, grilling, and frying, can affect the lipid oxidation of cooked bivalves compared with those in the raw samples (Domínguez et al., 2014). Boiling (100 °C, 5 min), microwaving (2450 MHz, 13 min), and freezing (150 °C, 15 min) change the composition of fatty acids in fish, while baking (180 °C, 30 min) has only minor effects (Hosseini et al., 2016). Frying has the greatest effect on lipid composition in fish because of water loss, fat uptake, and changes in the profile of fatty acids (Perez-Palacios et al., 2014). The oils used in the frying process had no DHA or EPA, hence oil absorption would reduce the content of these n-3 fatty acids when compared with the other fatty acids (Jayasena et al., 2018). Moradi et al. (2011) indicated that deep-fat frying of fish leads not only to an increase in the total amount of fat but also to an increase in the n-6/n-3 PUFA ratio, limiting the positive effects of the high n-3 PUFA level of raw fish.

It is also worth noting that reheating the cooked food can also result in a loss of n-3 LC-PUFA content, depending on the reheating methods, with microwave reheating results in a significantly higher loss of EPA and DHA content compared to oven heating (Sobral et al., 2018). Therefore, consumption of raw bivalves is the most effective way to obtain omega-3 LC-PUFA from bivalves. For consumers who cannot tolerate raw seafood consumption, oven cooking appears to be an effective way to reduce the loss of n-3 LC-PUFA content.

The presence of PUFA renders the product more susceptible to oxidation during heating. Lipid oxidation reactions are more prone to happen in fish samples because of its high content in PUFA (Shahidi & De Camargo,
Hydroperoxides and other primary products of oxidation are formed mainly from PUFAs (Sobral et al., 2018). Cooking duration seems to contribute more to lipid oxidation than temperature, as roasting (200 °C, 12 min), microwaving (1000 W, 1.5 min), grilling (130 to 150 °C, 5 min), and frying (170 to 180 °C, 4 min) foal steaks increased lipid oxidation, measured by 2-thiobarbituric acid (TBARs) assay (Perez-Palacios et al., 2017).

CONCLUSIONS

Marine bivalves are a good source of high quality lipid, mainly because they concentrate EPA and DHA from phytoplankton. Freshwater bivalves generally contain lower proportions of n-3 PUFA than marine bivalves; the lipid quality also differ in farmed and wild species. Meanwhile, these n-3 LCPUFAs are essential in the fight against inflammation, preventing depression, reducing weight and waist size, decreasing liver fat and promoting mental wellbeing. PUFA/ SAFA values for all marine bivalves are usually above 0.45, suggesting that bivalves are ideal for consumers interested in selecting low-fat food. Furthermore, all marine bivalves have an n-3/ n-6 ratio >1.0, indicating a high quality lipid. On the other hand, since n-3 LC-PUFA is highly sensitive to the oxidation of heat, which can contribute to the generation of harmful free radicals, then the processing technology and preservation of bivalve meat should be a subject of future research.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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