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Nutritional Analysis of Oleaginous Algae (Review)

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Abstract—*Microalgae are single-celled and in the form of microscopic plants are called "phytoplankton". Microalgae have the ability to synthetize many compounds, some of which have been recognized as a source of functional ingredients for nutraceuticals with positive health effects. Promya et al., (2008) reported that algae are significant source of human food, especially in Asia and cultivated for nutrition and pigments for supplemental use as human food and animal feed. Nutritional value of Cladophora show that it contains significant amounts of protein and carotenoids, which are essential for human and fish nutrition (Khuantrairong and Traichaiyaporn, 2009; Traichaiyapornet al., 2010). Epifanio, (1979) studied that the nutritional value of a microalgal diet contains both essential macro- and micronutrients to the target animal consumer. Microalgae provide many phytonutrients, including in particular PUFAs e. g. Eicosapentaenic acid (EPA), arachidonic acid (AA) and docosahexaenoicacid (DHA), which are known to be essential for various marine animals (Nichols, 2003). Microalgae contain 30-40% protein and 10-20% lipids in the late-logarithmic growth phase (Renaud et al., 1999 and Brown, 1997). Lipid membranes contain sterols such as fucosterol and β-sitosterol (Fahyet al., 2005) that also have reported health benefits (Arul et al., 2012). Most microalgal species contain 7-34% EPA and 0. 2-11% DHA (Brown. 1997). The brain is a structural-lipid rich organ that uses highly unsaturated fatty acids, particularly AA and DHA, for structure and function (Crawford et al., 1997; Crawford and Sinclair, 1972). It is usual to consider that AA and DHA are synthesized from their parent precursors, linoleic (LA; 18: 2n26) and alph-linolenic (ALA; 18: 3n23) acids.*

INTRODUCTION

Microalgae are most abundant primary unicellular producers found in all the aquatic systems such as, freshwater, seawater, hypersaline lakes, brackish, wastewater, municipal sewage, industrial effluents and even in deserts and arctic ecosystems[(Dibenedetto and Colucci 2015; Guccione et al. 2014; Pérez-Martínez et al. 2010; Pittman et al. 2011)]. Microalgae can also grow in extreme environments; it could be produced on agricultural and non-agricultural lands. Microalgae (including the cyanobacteria) are established commercial sources of high-value chemicals such as β-carotene, astaxanthin, docosahexaenoic acid, eicosahexaenoic acid, phycobilin pigments and algal extracts for use in cosmetics. Microalgae are also increasingly playing a role in cosmaceuticals, nutraceuticals and functional foods (Borowitzka 2010), long-chain polyunsaturated fatty acids (Kyle et al. 1992; Ratledge 2004; Mendes et al. 2009)

Some of the most significant groups of algae are green algae (Chloraphyceae), red algae (Rhodophyceae), diatoms (Bacillariphyceae), and brown algae (Phaeophyceae). Microalgae contain lipids and fatty acids as membrane components, metabolites, storage products, and sources of energy. Microalgae which include algal strains, diatoms, and cyanobacteria have been found to contain high levels of lipids (over 30%). Algal oils have been found to be very high in unsaturated fatty acids. Some of these unsaturated fatty acids that are found in different algal species include: arachidonic acid, eicospentaenoic acid, docasahexaenoic acid, gamma-linolenic acid, and linoleic acid. When comparing the lipid yield of algae to vegetable sources, algae can produce between 20,000 and 100,000 liters per hectare. Lipid accumulation in algae usually occurs during times of environmental stress, including growth under nutrient deficient conditions. The lipid and fatty acid contents of microalgae differ according to the culture conditions. It has been found that in some cases the lipid content can be enhanced by imposing nitrogen starvation. Biochemical studies have indicated that acetyl-CoA carboxylase (ACCase), a biotin containing enzyme that catalyzes an early step in fatty acid biosynthesis, might be involved in the control of the lipid accumulation process. In light of this fact, it might be possible to increase lipid production rates by increasing the activity of this enzyme by genetically engineering the microalgae.

The three most important classes of micro-algae in terms of abundance are the diatoms (Bacillariophyceae), the green algae (Chlorophyceae), and the golden algae (Chrysophyceae). Green algae are especially abundant in fresh water. The main storage compound of these algae is starch, although oils can also be produced. The fresh water green algae *Haematococcuspluvialis* is commercially important as a source for astaxanthin, Chlorella vulgaris as a supplementary food product, and the halophilic algae Dunaliella species as a source of β-carotene. The golden algae are similar to the diatoms and produce oils and carbohydrates. The blue-green algae (cyanobacteria) are found in a variety of habitats and are often known for their toxic water polluting products. Microalgae (single-celled eukaryotic organisms) are the

primary natural producers of LC-PUFA. These organisms offer a promising vegetative and non-polluted resource for biotechnology and bioengineering of LC-PUFA production as an alternative to fish oil.

Nutritional value and Biosynthesis in Microalgae

The biosynthesis of omega-3 fatty acids (EPA & DHA) occurs through series of reactions which can be divided into two distinct steps. First is the de novo synthesis of oleic acid (18: 1 n-9) from acetate. This is followed by conversion of oleic acid (18: 1 n-9) to linoleic acid (LA, 18: 2 n- linolenicacid (ALA, 18: 3 n-3). It was followed by a number of subsequent stepwise desaturation and elongation steps to form an n-3 PUFA family including EPA. Nearly all biological systems, including microorganisms, insects, higher plants and animals, are capable of de novo fatty acid synthesis from acetate to short chain fatty acids, with oleic acid (18: 1 n-9) as the major product. The biosynthesis starts with the carboxylation of acetyl-CoA to form acetate or pyruvate by the action of glycolytic enzymes. Then acetyl-CoA is converted into malonyl-CoA, which is used to drive a condensation reaction to extend the acyl group to stearic acid (18: 0) and desaturate to oleic acid (18: 1 n-9).

Oleic acid (18: 1 n-9) is further desaturated by a D12 desaturase to form linoleic acid (18: 2 n- -linolenic acid (18: 3 n3). Then, the n-9, n-6 and n-3 fatty acid families are formed from their precursors by a series of desaturation and elongation reactions. The three parent fatty acids-oleic acid, LA and ALA-compete with each other for the D6 desaturase. The affinity of the enzyme to the substrate and the amount of substrate available determine which metabolic pathway is predominant (Gurr 1985). Generally, the first D6 desaturation is the limiting step and ALA has the highest affinity for D6 desaturase followed by LA and oleic acid.

Microalgae are single-celled and in the form of microscopic plants are called “phytoplankton”. Microalgae have the ability to synthetize many compounds, some of which have been recognized as a source of functional ingredients for nutraceuticals with positive health effects. Promya et al. 2008 reported that algae are significant source of human food, especially in Asia and cultivated for nutrition and pigments for supplemental use as human food and animal feed. Epifanio (1979) studied that the nutritional value of a microalgal diet contains both essential macro- and micronutrients. Microalgae provide many phytonutrients, including in particular PUFAs – e. g. EPA, arachidonic acid (AA) and DHA, which are known to be essential for various marine animals (Nichols, 2003). Microalgae contain 30-40% protein and 10-20% lipids in the late-logarithmic growth phase (Renaud et al. 1999 and Brown, 1997). Lipid membranes contain sterols such as fucosterol and β-sitosterol (Fahy et al. 2005) that also have reported health benefits (Arul et al. 2012). Most microalgal species contain 7-34% EPA and 0. 2-11% DHA (Brown,1997).

The major fatty acids of *P. cruentum* were found to be 16: 0, 18: 2, 20: 4 (n-6) and 20: 5 (n-3) under all conditions tested. An increase in cell concentration resulted in an increase in the proportions of the 18: 2 and 20: 4 fatty acids accompanied by a decrease in the 20: 5 acid. Arachidonic acid (AA; 20: 4n26) and docosahexaenoic acid (DHA; 22: 6n23) during fetal development. Postnatal enteral and parenteral nutrition of preterm infants do not mimic intrauterine conditions. The brain is a structural-lipid rich organ that uses highly unsaturated fatty acids, particularly AA and DHA, for structure and function (Crawford et al. 1997). It is usual to consider that AA and DHA are synthesized from their parent precursors, linoleic (LA; 18: 2n26) and a-linolenic (ALA; 18: 3n23) acids.

Eicosapentaenic acid (EPA, 20: 5 ω3) and docosahexaenoic acid (DHA, 22: 6 ω3) are the important ω3 PUFA, while arachidonic acid (AA, 20: 4 ω6), is a vital ω6 PUFA. EPA and DHA are important in treatment of arthrosclerosis, cancer, rheumatoid arthritis, psoriasis and diseases of old age such as Alzheimer’s and age-related macular degeneration (Drevon et al, 1993; Simopoulos et al, 1999). AA and DHA are of special importance in the brain and blood vessels, and are considered essential for pre- and post-natal brain and retinal development (Crawford, 2000). The eicosanoids, such as prostaglandins, prostacyclins and leukotrienes, derived from ω3 PUFA are also important in new-born and infant development, modulatory vascular resistance and wound healing (Simopoulos et al,1999; Nettleton, 1995). Microalgae are prized for their micronutritional value, PUFA, containing immuno-stimulant substances, fight cancer, protect us from UV radiation and treat joint ailments, to mention but a few applications. Others have explored their use in bioremediation – the removal of heavy metals from polluted soil and water and some have even tried them for biological CO_2 fixation in flue gas of industrial plants (Moore, 2001).

The PUFAs 20: 5(n3) and 22: 6(n-3) are essential fatty acids (EFAs) for the survival and growth of many juvenille aquaculture organisms (Langdon and Walcock, 1981; Levine and Sulkin, 1984; Enright et al., 1986). The importance of polyunsaturated fatty acids (PUFA) in human nutrition and disease prevention was scientically recognized three decades ago. The long-chain omega 3-PUFA, namely eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), may be acquired mainly from seafoods or derived from -linolenic acid by a series of chain elongation and desaturation (Sprecher, 1995). EPA and DHA are synthesized mainly by both uni- and multicellular marine plants such as phytoplankton and algae (Pigott and Tucker, 1987).

Among algae of alpine environment there could be strains particularly rich in long chain polyunsaturated fatty acids (LC-PUFA). Indeed, the chlorophyte (*Trebuxiophyceae*) *Parietochlorisincisa* isolated from *Mt. Tateyama*, Japan, was found to be the richest plant source of the pharmaceutically valuable LC-PUFA, arachidonic acid (AA, 20: 4o6). The algae are also extremely rich in triacylglycerols (TAG), which reaches 43% (of total fatty acids) in the logarithmic phase and up to 77% in the stationary phase. In contrast to most algae whose TAG are made of mainly saturated and monounsaturated fatty acids, TAG of *P. incisa* are the major lipid class where AA is deposited, reaching up to 47% in the stationary phase. Except for the presence of AA, the PUFA composition of the chloroplastic lipids resembled that of green algae, consisting predominantly of C_{16} and C_{18} PUFAs. The composition of the extrachloroplastic lipids is rare, including phosphatidylcholine (PC), phosphatidylethanolamine (PE) as well as diacylglyceryltrimethylhomoserine (DGTS). PC and PE are particularly rich in AA and are also the major depots of the presumed precursors of AA, l8: 3o6 and 20: 3o6, respectively. Long-chain polyunsaturated fatty acids (LC-PUFAs) of the omega-3 family are quite abundant in microalgae. For example, *Porphyridiumcruentum* (Cohen et al. 1988), *Nannochloropsis sp.* (Seto et al. 1984; Sukenik and Carmeli, 1989), *Phaeodactylumtricornutum* (Yongmanitchai and Ward, 1992; Molina Grima et al. 1999) and *Monodussubterraneus* (Cohen, 1994) were studied for their potential to produce eicosapentaenoic acid (EPA, 20: 5o3). Likewise, *Crypthecodiniumcohnii*(Jiang et al. 1999) and *Chroomonassalina* (Henderson and Mac Kinlay, 1992) contain docosahexaenoic acid (DHA, 22: 6o3). However, ω-6 LC-PUFAs are relatively rare. High contents of 20: 3 ω 6 were not found in any organism. Arachidonic acid (AA, 20: 4 ω 6) is almost excluded from the lipids of fresh water algae and in most marine species it does not account for more than a few per cent of total fatty acids (Thompson, 1996). The only microalga reported to produce AA in significant quantities is the unicellular rhodophyte*P. cruentum* (Cohen, 1990). Under logarithmic growth, the major PUFA of this alga is EPA, but under unfavorable conditions, AA predominated, reaching 40% of total fatty acids. In other rhodophytes such as *Gracilaria sp*., the proportion of AA can be as high as 60% of total fatty acids; however, the dry weight content does not exceed 0. 2% (Araki et al. 1990). When present, LC-PUFAs are predominantly located in the polar membranal lipids, whereas triacylglycerols (TAG) generally contain very little PUFAs (Cohen, 1999). The capacity for AA accumulation makes *P. incisa* one of the best candidates for large-scale production of AA. This PUFA was shown to be a major component of brain cell membranes, as well as of breast milk (Hansen et al. 1997). It was thus suggested that the diet of preterm infants that are not breast-fed should be implemented with AA by supplementation of new born milk formula (Carlson et al. 1993; Boswell et al. 1996). The finding that most AA of *P. incisa* is deposited in TAG is of practical value since TAG are the preferred chemical form for the introduction of AA into baby form

Lipid extraction Freeze-dried samples of *P. incisa* biomass were extracted with methanol containing 10% DMSO, by warming to 40°C for 5 min and stirring at 40°C for another hour. The mixture was centrifuged, the supernatant removed and the pellet was re-extracted with a mixture of hexane and ether (1: 1, v/v). Diethyl ether, hexane and water were added to the combined supernatants, so as to form a ratio of 1: 1: 1: 1 (v/v/v/v). The mixture was shaken and then centrifuged for 5 min at 35100 rpm and the upper phase was collected. The water phase was re-extracted twice with a mixture of diethyl ether: hexane (1: 1, v/v). The organic phases were combined and evaporated to dryness. The diethyl ether utilized in the extractions and the lipid analysis was peroxide-free and contained 0. 01% but ylatedhydroxytoluene (BHT). 4. 3. Lipid analysis Polar lipids were separated by two-dimensional TLC using a solvent system of chloroform: methanol: water (65: 25: 4, v/v/v) for the first direction and of chloroform: methanol: 1 - ethylpropylamine: concentation ammonia (65: 35: 0. 5: 5, v/v/v/v) for the second direction. Neutral lipids were resolved with petroleum ether: diethyl, ether: acetic acid (80: 20: 1, v/v/v). MGDG and DGDG eluted from the silica gel plates were separated to their constituent molecular species by reversed-phase HPLC as previously described (Khozin et al. 1997). Fatty acid analysis Freeze-dried cells, lipid extracts, or individual lipids were transmethylated with 2% H2SO4 in methanol at 70 C for 1 h. Heptadecanoic acid was added as an internal standard. Gas chromatographic analysis was performed according to Cohen et al. (1993). Fatty acid methyl esters were identified by co-chromatography with authentic standards (Sigma Co., St Louis) and by comparison of their equivalent chain length (Ackman, 1969). The data shown represent mean values with a range of less than 5% for major peaks (over 10% of fatty acids) and 10% for minor peaks, of at least two independent samples, each analysed in duplicate.

Conclusion

Microalgae are used as food: They are rich in Protein, Vitamin, Polyunsaturated fatty acid and Minerals. e. g*Chorella* (high protein and lipid content) and *porphyra* (soups, salads and vegetables). Microalgae are commercially available. Microalgae are greatest productivity of EPA and the EPA content of its biomass was enhanced under mixotrophic conditions in the presence of acetic acid. Nutritional deficiencies in a diet can be avoided by the use of mixed algae diets. The future use of EPA for treatment of various diseases/ disorders would require extensive clinical trials. Microalgae contain significant amounts of protein and carotenoids, which are essential for human and fish nutrition.

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