

Extraction and Isolation of Polyunsaturated Fatty Acids from Microalgae *Tetraselmis*.Sp

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ABSTRACT: Omega 3 fatty acids are long chain polyunsaturated fatty acids (LC – PUFA) which are essential for normal growth and prolonged health of higher eukaryotes. Nutritionally, eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) are the most important component of the omega 3 fatty acids family, as they have significant human health benefits such as brain functioning, reducing cardiovascular, neurons and immune system related diseases. Microalgae are primary source of EPA and DHA. EPA is polyunsaturated fatty acids that act as a precursor for prostaglandin – 3 (which inhibits platelet aggregation), thromboxane – 3, and leukotriene – 5 groups. The other marine sources such as fish and krill do not produce omega 3 fatty acids, but rather accumulate it from the algae and their prey consume in their natural environment. The microalgae *Tetraselmis* sp. is a potential source of economically valuable eicosapentaenoic acid and docosahexanoic acid. Thus this paper explain the extraction of omega 3 fatty acids directly from the microalgae through cell disruption method.

KEYWORDS: Omega 3 fatty acids, eicosapentaenoic acid, docosahexanoic acid, polyunsaturated fatty acids (PUFA), extraction, isolation, cell disruption method.

I. INTRODUCTION

Microalgae, also called phytoplankton by biologists, are very small Plant-like organisms between 1-50 micrometres in diameter without roots or leaves. Together with the seaweeds (microalgae or aquatic plants), microalgae are part of the so called *aquatic biomass*. Microalgae are very common and occur both in freshwater and seawater where they form basis for most food chains. Most species contain chlorophyll, use sunlight as an energy source and convert Carbon dioxide (CO₂) into biomass. In this process of photosynthesis the algae produce oxygen (O₂). On a global scale microalgae produce more than 75% of the oxygen required for animal and humans. Normally microalgae are not visible by naked eye, but if the water is eutrophic, massive algal blooms occur, changing the water in a green, brown, blue, or orange liquid mass. The biodiversity of microalgae is enormous and they represent an almost untapped resources. It has been estimated that about 200,000-800,000 species in many different genera exist of which about 50,000 species are described in literature. Most of these microalgae species produce unique products like toxins, carotenoids, antioxidant, fatty acids, enzymes, polymers, peptides and sterol. Microalgae are indispensable in the commercial rearing of various species of marine animals as a food source for all growth stages of bivalve molluscs, larval stages of some crustacean species, and very early growth stages of some fish species. The genetic analysis and ranking of all microalgae is still in progress. Many algae contain a considerable amount of high-quality oils, partly consisting out of omega-3 and omega-6 fatty acids, which are used as raw material for food supplements. Omega-3 fatty acids in fish originate from microalgae. Long chain polyunsaturated fatty acids (LC-PUFAs) such as

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eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are considered essential omega-3 fatty acids in human nutrition. In marine microalgae, EPA and/or DHA are allegedly involved in the regulation of membrane fluidity and thylakoid membrane functioning.

DHA, produced from algae, is a vegetarian source of docosahexaenoic acid, DHA. DHA is a long-chain polyunsaturated omega-3 fatty acid and is important for brain, eye and heart health throughout the lifecycle. DHA has several applications including infant formulas, products for pregnant and nursing women, food and beverage products and dietary supplements. EPA is a polyunsaturated fatty acid (PUFA) that acts as a precursor for prostaglandin-3 (which inhibits platelet aggregation), thromboxane-3, and leukotriene-5 groups. Omega-3 fatty acids can be found in fish, such as salmon, tuna, and halibut, other seafood including algae and krill, some plants, and nut oils. DHA is the primary compound of the human brain and retina. Cold-water oceanic fish oils are rich in DHA. Most of the DHA in fish and complex organisms originates in photosynthetic and heterotrophic microalgae, and becomes increasingly concentrated in organisms, as they move up the food chain. As of today DHA is commercially produced from microalgae. EPA is obtained in the human diet by eating oily fish or fish oil— e.g., cod liver, herring, mackerel, salmon, menhaden and sardine. It is also found in human breast milk. Fish do not naturally produce EPA, but obtain it from the algae they consume. It is available to humans from some non-animal sources. Microalgae are being developed as a commercial source. EPA is not usually found in higher plants. Microalgae, and supplements derived from it, are excellent alternative sources of EPA and other fatty acids, since fish often contain toxins due to pollution. Many marine microalgae strains have oil contents of between 10–50%, (w/w) and produce a high percentage of total lipids (up to 30–70% of dry weight)

II. MATERIALS AND METHOD

SELECTION OF MICROALGAE

The microalgae selected for the production of high content of omega 3 fatty acids is *Tetraselmis sp.* This microalga contains high quantity of EPA (eicosapentaenoic acid) which is the primary source of omega 3 fatty acids.

PREPARATION OF CULTURE MEDIUM

The microalgae can be cultured in various environment. This microalgae (*Tetraselmis sp.*) is cultured in marine water by using various chemicals such as NaNO₃ (150µl), NaH₂PO₄ (150µl), trace metals (150µl) and small quantity of vitamin in a well sterilized conical flask.

GROWTH AND CONDITION

Microscopic analysis of culture was done through scanning electron microscope (SEM) . The cultures were grown in 1 litre Erlenmeyer flasks with culture medium that have been prepared using marine water at 30°C temperature in the photo-incubator. Grown under 3–4 Klux intensity of fluorescent white light at 24 h of light. The cultures were hand shaken three to four times daily to avoid sticking. Glass wares and media were always sterilized prior to inoculation. Experiments were carried out in triplicates.

LYOPHILIZATION OF MICROALGAE

The process in which the creation of stable preparation of biologic substance by rapid freezing and dehydration of frozen product under vacuum. The prepared microalgae culture is transferred to centrifuge tubes (15ml) and frozen at -20 degree celsius. This microalga is lyophilised by using freeze dryer apparatus.

CELL DISRUPTION METHOD

It is a method or process of removing biomolecules inside a cell.

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FREEZING

Lyophilized microalgae were frozen in liquid nitrogen and then thawed, three times. Lyophilized microalgae were suspended in 0.5 mL methanol and beaten twice for 30 s at 60 Hz.

SONICATION (ULTRASONIC LYSIS)

Sonication is the act of applying sound energy to agitate particles in a sample, for various purposes. Ultrasonic frequencies (>20 kHz) are usually used, leading to the process also being known as ultrasonication or ultra-sonication. The goal of ultrasonic lysis is to disrupt parts of the cell wall or the complete cell to release biological molecules. Lyophilized microalgae were sonicated for 15 min after addition of 4ml methanol, 2ml chloroform, and 0.4 ml water.

EXTRACTION

Steps will be followed to extract EPA by using cell disruption method.

EPA EXTRACTION

Add 4 ml methanol to 100 mg of lyophilized microalgae. 2ml chloroform and 0.4ml water is added and kept in vortex, mixed for 30s for 2 times. These tubes were centrifuged at 2000 rpm for 10 minutes. Using a micropipette, pipette out the upper layer and the lower layer was transferred into a clear tube.

RE – EXTRACTION

The remaining solids were re – extracted with 4ml chloroform and methanol (1:1).

COLLECTION OF CHLOROFORM PHASE

Again vortex for 5 sec and then sonicated for 10 min. Centrifuge for 5 min at 1,200 x g. Now collect the chloroform phase using pipette. Evaporate the chloroform from the tube in a nitrogen gas stream and store it under -20°C.

TRANSESTERIFICATION

Add 3ml methanol containing 5% sulphuric acid to the tube and close tightly. Vortex for 5 min. Incubate samples for 3 hr at 70°C in a water bath. During this reaction fatty acids are methylated to fatty acid methyl ester (FAMES).

ANALYSIS OF EPA CONTENT

This analysis of EPA content is done by quantification of FAMES using Gas Chromatography. The fatty acid methyl esters (FAMES) obtained were separated by gas chromatography with cold on-column injection and flame ionization detection. The assay of FAME was analysed using a gas chromatography equipped with a split/splitless injector, and detector Hewlett Packard EL-980 flame ionization detection (FID). FID system was used to separate and quantify each FAME component. FAME was separated using HP-88 capillary column (100 m x 0.25 mm id x 0.2 µm of film thickness) (Agilent J&W Scientific GC Column, USA). Chromatography data was recorded and integrated using chemstation software.

III. RESULT

The amount of total lipids extracted from *Tetraselmis sp.* was used as an indication of the efficiency of the cell disruption method used. No significant differences could be detected between the different cell-disruption methods. This indicates that the cell wall is penetrated or dissolved by the solvents used, so it does not need to be destroyed for optimum extraction. However, when looking at the first extraction only, bead beating led to more efficient extraction of lipid, although the difference was rather small (7.5%). This result is consistent with the result of Lee et al. [6], who extracted once only. During the second extraction, a smaller amount of lipids is extracted when bead beating is used as a cell-disruption method. Ideally, one extraction only is sufficient to determine the complete lipid profile of the microalgae. This is, however, only possible if there is no selective extraction of specific lipid classes or fatty acids during the first or second extraction.

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