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Micro-organismes producteurs de lipides

Recent developments in the commercial production of DHA and EPA rich oils from micro-algae

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Abstract – The regular intake of marine omega 3's DHA and EPA has been scientifically established as providing a wide range of health benefits. This paper reviews recent developments in the commercial production of DHA and EPA rich oils from micro-algae. The selection of suitable micro-algae species is discussed. The complexities of producing algal oil rich in marine omega 3's is examined in terms of both upstream and downstream production.

Keywords: Marine omega 3 / DHA / EPA / commercial production / selection

Résumé – Progrès récents dans la production commerciale d'huiles riches en DHA et d'EPA issues de micro-algues. Il est démontré scientifiquement que la consommation régulière de DHA et EPA, oméga-3 d'origine marine, fournit une large palette de bénéfices santé. Cet article propose une revue des récents progrès intervenus dans la production commerciale d'huiles riches en DHA et d'EPA issues de micro-algues. La sélection des espèces adaptées de miro-algues est discutée. Les difficultés rencontrées pour la production d'huile algale riche en oméga-3 d'origine marine est étudiée à la fois en termes de production amont et avale.

Mots clés : Oméga-3 d'origine marine / DHA / EPA / production commerciale / sélection

Introduction

The marine omega-3 fatty acids DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) have a wide range of scientifically established health benefits attributed to their consumption. As it is believed that the main dietary source of DHA and EPA, *i.e.* fish oil, has already reached maximum global production, the search is now on for a sustainable alternative. A recent commercial development of this century has been the production of oils rich in DHA and EPA from micro-algae. These algal oils are already established for use in infant formula and are now increasingly being used in health supplements and enriched food products.

The micro-algae used to produce these algal oils are derived from the oceans. Strains have been developed using conventional methods that maximise oil yields. Algal oils have the benefit of being produced in a carefully controlled environment, as well as being suitable for those following a vegetarian diet, and having excellent sustainability credentials. Algal oil production is expensive, but refinements in the process and economies of scale have attenuated the final cost. Whilst there remains a considerable gap between the cost of algal oils and fish oils, the recent, inexorable rises in the cost of high quality fish oils means this gap is being considerably reduced.

There remains a considerable gap in the actual versus recommended intake of DHA and EPA in most of the world. Conservative estimates show this gap cannot be fulfilled by marine sources alone. The only realistic terrestrial alternative are cereal oils containing the necessary marine organism-derived genes to produce DHA and EPA, however these are not currently commercially available. Hence algal oil is likely to provide an increasing proportion of our DHA and EPA needs in Europe in the future.

DHA (C22:6 n-3) is a long chain omega-3 fatty acid that is found throughout the human body. More specifically, it is a major structural lipid in the brain and the retina of the eye and is a key component of the heart. Circulating levels of DHA and EPA are an essential part of the body's defense mechanisms and have a key role in reducing inflammation and nullifying reactive oxygen species (ROS) at the cellular level.

EPA (C20:5 n-3) is also a long chain omega-3 fatty acid. The major health benefit of EPA is its role as an eicosanoid; playing an important role in regulating inflammation and

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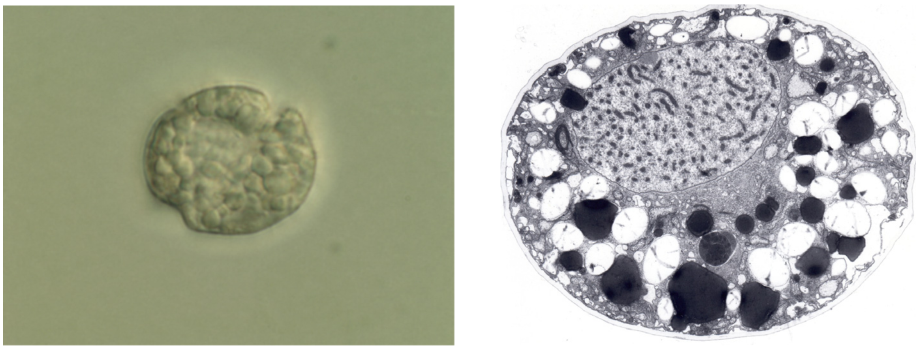

Fig. 1. Photomicrograph of the micro-algae *Crypthecodinium cohnii* courtesy of Casey Lippmeier of DSM Nutritional Products and David L. Spector of Cold Spring Harbor Laboratory, Laurel Hollow, NY, USA respectively.

immunity in humans. It is widely accepted that EPA-derived eicosanoids are effective in helping manage heart disease and other chronic and inflammatory processes.

1 Why are micro-algae used to produce food ingredients?

Micro-algae have a higher growth rate and higher biomass density in comparison to land-based crops. They are a source of rare, key bioactive nutrients normally only found in the marine environment and provide an alternative to extraction from fish.

The fermentation substrates used in micro-algal fermentation are readily available and derived from renewable resources (glucose syrup is a popular choice as the major constituent!). Micro-algal fermentations have other environmental benefits too, in that they do not compete for land space and some can be used to fix carbon dioxide.

Numerous phototrophic algae are able to adapt their metabolism to heterotrophic conditions – indeed that is the only way they can function when the sun's rays are not available!

2 Selecting EPA and DHA producing micro-algae

Currently, the most common micro-algae used for the production of DHA rich algal oil and biomass are from the marine members of the families *Thraustochytriaceae and Crypthecodiniaceae*. The Thraustochytrids include the genera *Schizochytrium* and *Ulkenia*, whereas *Crypthecodinium* is a genus of the family Crypthecodiniaceae. Members of these genera are widely dispersed in the oceans of the world. The selection of suitable heterotrophic micro-algae depends on their ability to inexpensively produce large quantities of DHA from glucose in large stainless steel vessels (*i.e.* absence of light). Autotrophic algae require a source of light, using the light of the sun to drive their metabolism. This seriously limits the design of the fermenter, particularly the size, which in turn, limits the yield. Open pond systems, on the other hand, are optimally suited for growing autotrophic algae.

Thraustochytrids are heterotrophs with a high oil content (typically 50–77% on a dry weight basis). The oil is >90% triacylglcyeride, rich in DHA and has a low cholesterol content. The precise taxonomy of Thraustochytrids remains a matter of some debate. They are found in the *kingdom Chromista* and *phylum Labyrinthulomycota*. In fact, they are often found in association with decomposing plants/algal matter and their nutrition is primarily saprotrophic (Armenta and Valentine, 2013).

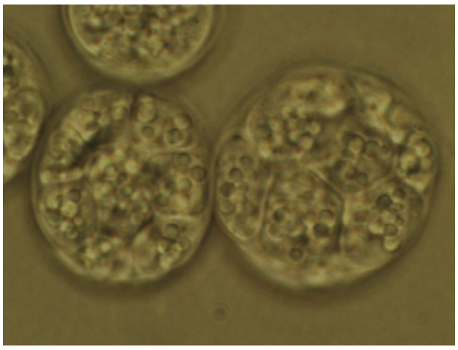

Fig. 2. Photomicrograph of the micro-algae *Schizochytrium* courtesy of Casey Lippmeier, DSM Nutritional Products.

In practice, the strains of micro-algae in use today in the production of DHA/EPA rich oils are the results of intensive collection/isolation/screening procedures. The most successful candidates shared the following properties (Barclay *et al.*, 2010):

- high growth rates;
- high proportion of DHA/EPA as percentage of total lipids in elevated temperature fermentation (30 °C);
- growth unaffected by low salinity conditions.

All commercial strains have undergone extensive toxicological investigation as part of their conditions for regulatory approval. A useful review of the safety and toxicological aspects of producing single cell oils has been written by Zeller (2006).

DSM Nutritional Products use the micro-algae *Crypthecodinium cohnii* (see Fig. 1) to make their DHASCO^{TM} oil for the infant formula market. This oil has a DHA content of 40–45% w/w, and virtually no EPA. DSM also produce an algal oil for the food, beverage and supplement industries called Life's DHA^{TM} oil. This is made using micro-algae *Schizochytrium* (see Fig. 2) and is standardised at 35% or 40% DHA, and also contain low levels of EPA (<2%). DSM uses a different strain

Fig. 3. Photomicrograph of the micro-algae *Phaeodactylum tricornutum*, courtesy of Kirk Apt, DSM Nutritional Products.

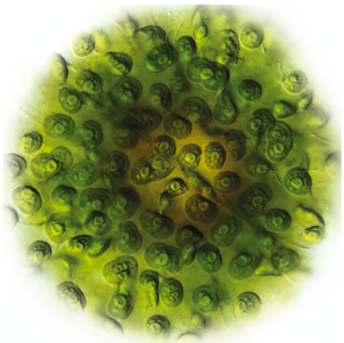

Fig. 4. Photomicrograph of the micro-algae *Nannochloropsis*, courtesy of Aurora Algae Inc.

of *Schizochytrium* to produce an algal oil with a DHA:EPA ratio of ca 2:1 to match high quality fish oils. This product is called Life's Omega^{TM}, containing a minimum of 40% total DHA and EPA, and is specified individually as having minimum levels of 24% DHA and 12% EPA.

Until recently, the production of EPA rich algal oil has been restricted to laboratory scale. According to Milledge (2012), EPA oils production from the micro-algae *Phaeodactylum tricornutum* (see Fig. 3), *Nannochloropsis* and the diatom, *Nitzchia* have been described in the literature.

Aurora Algae cultivates proprietary strains of *Nannochloropsis* micro-algae in large seawater open ponds, in geographic locations with high solar radiation. Aurora Algae uses proprietary techniques to select for strains with high oil yield, high proportion of EPA, and substantially no DHA. Aurora Algae has commercialised a high EPA content (>65%) oil under the product name A2 EPA Pure^{TM}, targeted for use in the supplements and pharmaceuticals sector (Fig. 4).

3 Algal oil production

Yield optimisation of algal oil (and specifically the proportion of DHA & EPA) is key to increasing its share of the

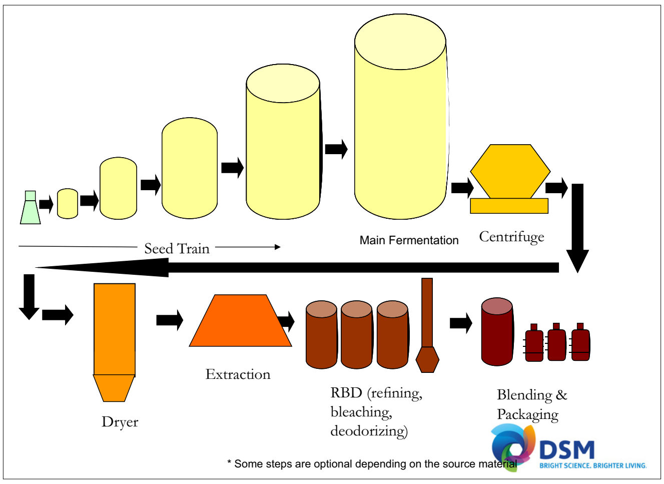

Fig. 5. Production of DHA rich algal oil for use in infant formula.

omega 3 market. There have been a wide range of developments in both upstream and down processing which have been reviewed in detail by Armenta and Mercer, 2013 and Barclay *et al.* 2010. The following text on this matter is based largely on their observations, but the reader is referred to the original papers if they require more detail. Figure 5 is a schematic diagram outlining the production of DHA rich algal oil for use in infant formula.

3.1 Upstream processing

3.1.1 Carbon source

The carbon source ingredients are the most expensive in the fermentation media. Micro-algae differ widely in their preferred source and concentration, hence considerable development time must be spent with a particular organism to ensure the most economic lipid yield. Generally, the concentration of glucose in the fermentation media is in the range 5–40 g/L (Armenta and Mercer, 2013). The usual source of glucose in commercial production is glucose syrup derived from the enzymic conversion of starch from cereals or potatoes.

Glycerol is an interesting potential alternative to glucose. The production of biodiesel results in large quantities of raw glycerol as a byproduct. However, this form of glycerol will generally contain traces of soaps methanol and other impurities that will inhibit micro-algal growth.

Both glucose and glycerol are converted by the micro-algae first to pyruvic acid, then to acetyl CoA (via the citric acid cycle) which serves as the basic source molecule for lipid production.

Other waste streams from the food processing industry that are rich in monosaccharides are also a potentially economic viable carbon source, but also share the problem of growth inhibiting impurities.

3.1.2 Nitrogen source

Nitrogen containing ingredients for the fermentations are the second most expensive. The most common sources are

Dossier

degraded proteins such as yeast extracts and soya peptones. Whilst inorganic nitrogen sources (*e.g.* ammonia) can be used, they lack the trace minerals and other nutrients (*i.e.* vitamins) which are often important to micro-algal growth, hence these would need to be added. The nitrogen source is important in the first phase of algal fermentation – biomass development, where it is used in amino acid synthesis. Only once the nitrogen supply is exhausted in the fermentation matter, do the micro-algae start producing fatty acids from the carbon source.

3.1.3 Culture mode

Algal oil fermentation processes are predominantly in two stages. In the first phase, the micro-algae make use of the excess of nutrients to develop biomass. In the second phase, the lack of nitrogen means the energy previously reserved for DNA/RNA and other protein synthesis can be switched to production of fatty acids. The fermentation process is usually a basic fed batch process, but there is still the opportunity for the development of an efficient semi-continuous or even continuous process.

3.1.4 Dissolved oxygen

The prevailing wisdom is that the fermentation media should contain a high dissolved oxygen (DO) to boost the formation of double bonds in the final omega-3 fatty acids, but in practice this remains debatable. The importance of DO to the fermentation varies widely between different micro-algae. However, whilst DO control is of key importance in the initial phase of biomass accumulation, it is not necessarily required in the second phase of lipid accumulation.

3.1.5 pH

The control of pH is less important than controlling the carbon and nitrogen levels in the biomass. However, it is advantageous to maintain the pH as close as possible to the optimal required by the particular micro-algae concerned, otherwise the organism has to waste energy attempting to restore the optimal pH.

3.1.6 Temperature

Temperature is important in terms of both biomass and fatty acid accumulation. Usually, higher fermentation temperatures favour increased cell growth whilst lower temperatures favour production of fatty acids.

3.1.7 Salinity

The level of salt in the biomass for optimum fermentation varies widely between different micro-algae. Salt is usually an important factor for growth. Usually for marine micro-algae, the salinity levels are adjusted to mimic those found in the sea. Thaustochytrids, despite being marine organisms, can be grown in a low salt environment. This is a considerable advantage for commercial production, because high salt levels in the fermenter cause serious corrosion problems.

3.1.8 Light

The light requirements of different micro-algae differ widely. In autotrophs, the production of EPA and DHA occurs predominantly in photo-synthetic membranes, hence light is required for their production – this can make commercial fermentation complicated and limit the scale of production. However, it has proved possible to select heterotrophic micro-algal strains that can grow without any light in normal, sealed fermenters.

3.2 Downstream processing

Starting with a pure, axenic culture of a micro-algae, growing conditions are optimised to enable maximum lipid production. In some cases, the biomass is dried (by spray or drum drying). The next stage can involve disruption of the algal cells to release the oil from the cells.

3.2.1 Oil extraction

The extraction stage of micro-algal oil production is fraught with difficulty because the protection offered to the LC-PUFA from the natural anti-oxidants present within the micro-algae are not effective once the cell is ruptured. Hence it is very important to minimise exposure to air at this stage. A variety of extraction methods can be used, though all involve rupturing of the cell wall with some form of milling (often assisted with enzymes, sonification etc) followed by extraction with solvent. Classically, hexane, as used in most vegetable oil production, was used as solvent, but more recently solvent-less extraction processes have been developed. A further alternative is the use of super critical fluid extraction (usually with carbon dioxide). However, the most cost effective procedure remains extraction with the solvent hexane, which can be accompanied by mechanical pressing. In this case, careful checks are made to ensure that no residual solvent remain in the oil. The spent, de-oiled biomass can generally be used for animal feed. The crude oil is then subject to standard food oil refining techniques. Waste or oxidised oil can be diverted to biofuel production.

Extraction of algal oil for the production of DHA and EPA is difficult because as soon as the algal cell walls are ruptured, these LC-PUFA's are exposed to potential oxidation. Once these highly unsaturated fatty acids have reacted with oxidised radicals, an unstoppable chain reaction begins which leads to the production of rancid, highly odourous oil which is unsuitable for human consumption. Hence, so far as possible, all sources of materials that can initiate the oxidation process (*e.g.* copper, ferrous metal) should be eliminated for the extraction and oil storage areas. The crude algal oil is kept cool, usually under a nitrogen blanket, ready for refining.

3.2.2 Purification: refining, bleaching and de-odourising algal oils

Crude vegetable oils, including algal oils, require refining to improve colour, clarity, odour and remove any particulate material and chemical contaminants. There are wide range of impurities in the crude oil that can be removed by the refining process, including: free fatty acids, phosphatides (*i.e.* lecithin), pigments (*i.e.* carotenoids, chlorophyll), trace metals, sterols (*i.e.* cholesterol), waxes, mono acyl and diacyl glycerides (MAG's and DAG's), waxes, oxidation products and trace contaminants. Free fatty acids (FFA's) are usually removed by chemical refining where the FFA's are neutralised with caustic soda then easily removed as a soap. Physical refining is less suitable for algal oils because of the high temperatures involved. The neutralisation step is usually proceeded by a degumming process where water is added to remove phosphatides, sterols etc. Removal of the soap is achieved by washing the oil with water followed by physical separation of the aqueous phase and drying under vacuum (which also helps remove any dissolved oxygen).

The next stage is bleaching, where typically absorbent clay or activated carbon is added to remove colour pigments, oxidation products and trace metals. The bleaching aid is then removed by filtration. Additional recovery processes may be used to recover any residue in the spent filter aid before it is sent for landfill. The bleached oil is then de-waxed to improve its clarity. De-waxing (also known as winterisation) removes the high melting waxes (predominantly triglycerides rich in saturated fatty acids) which otherwise will give the oil a cloudy appearance. This process involves chilling the oil to allow the wax crystals to develop and grow. The wax crystals are then removed by filtration and/or centrifugation. The winterised oil is then sent to the deodouriser which can be thought of as a steam cleaner! High pressure steam is added to the oil under high vacuum to remove any remaining oil components that contribute to taste, odour and colour including any produced in the refining process up to this stage. The oil is then cooled. The resulting product is refined, algal oil!

4 Regulatory approval of algal oil in the European Union

DSM's algal oil DHASCO® has been approved for use in infant formula in the European union (EU) under Commission directive 2006/141/EC. This regulation states that when added, 1% of the total fat content should consist of n-3 LC-PUFA's and 2% of the fat content should be n-6 LC-PUFA's of which 1% is arachidonic acid (ARA). DHASCO® is also considered as a food in the EU (*e.g.*, not a novel food) based on its "significant degree of use" prior to 1997.

DSM's Life's DHA™-S algal oil is approved for use as a novel food ingredient in specific food categories and dietary supplements (OJL 144/13, 12.6.2003; OJL 278/56, 23.10.2009). This algal oil must be labeled "DHA rich oil from the microalga Schizochytrium sp" under these regulations.

In July 2012 DSM's DHA and EPA-rich algal oil (now commercialised a Life's Omega™) was authorised for use under the European Novel Food Regulation (EC) 258/97[1].

Commercial Development

The story of the development of DHA, and more recently DHA and EPA, algal oils has been told elsewhere, please see the following accounts (Barclay, 2010; Winwood, 2013).

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[1] See: http://ec.europa.eu/food/food/biotechnology/novelfood/dha_o_authorisation_letter_06072012_en.pdf