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ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

Παραγωγή ασταξανθίνης από μικροφύκη με χρήση μαγνητικού φωτοβιοαντιδραστήρα. Μελέτη σκοπιμότητας.

ΕΠΙΒΛΕΠΩΝ ΚΑΘΗΓΗΤΗΣ: ΕΡΕΥΝΗΤΗΣ Β', ΖΟΥΜΠΟΥΛΑΚΗΣ ΠΑΝΑΓΙΩΤΗΣ

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UNIVERSITY OF THESSALY SCHOOL OF HEALTH SCIENCES DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY



NATIONAL HELLENIC RESEARCH FOUNDATION INSTITUTE OF BIOLOGY, MEDICINAL CHEMISTRY & BIOTECHNOLOGY

INTERISTITUTIONAL PROGRAM OF POSTGRADUATE STUDIES IN BIOENTREPRENEURSHIP



MASTER THESIS

Production of astaxanthin from microalgae by using magnetic photobioreactor. Feasibility study.

SUPERVISOR: RESEARCHER B', ZOUMPOULAKIS PANAGIOTIS

FRAGKI MARIANTHI A.M. 00028 ATHENS, 2019 Η παρούσα διπλωματική εργασία εκπονήθηκε στο πλαίσιο σπουδών για την απόκτηση του Μεταπτυχιακού Διπλώματος Ειδίκευσης στο

ΒΙΟΕΠΙΧΕΙΡΕΙΝ

που απονέμει το Τμήμα Βιοχημείας και Βιοτεχνολογίας του Πανεπιστημίου Θεσσαλίας, σε συνεργασία με την Qualia Pharma S.A.

Εγκρίθηκε τηναπό την τριμελή εξεταστική

επιτροπή:

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EXECUTIVE SUMMARY

The population growth and tendency to a healthier lifestyle increase the demand to the investigation of additional human needs for food and natural products. At the same time, the will to decrease and replace compounds from synthetic sources has led to the emergence for new natural substances to be used as food additives, as ingredients in drugs and nutraceuticals, or in cosmetics. Under these circumstances, microalgae and bioactive compounds from microalgae compose with any doubt an incredible solution pursuant to their high added value in new industrial applications.

Algae are able to manufacture different kinds of biologically active metabolites such as, vitamins, carotenoids, proteins, lipids, PUFAs, pigments, polysaccharides and phenolic compounds. Among them compounds, carotenoids are generally reported with considerable interest at the recent years. Specifically, astaxanthin is a bioactive compound with high nutritional value possessing strong demand as well as important market share. The current market is mainly dominated by the chemical synthesis of astaxanthin due to the high cost of the cultivation, production and harvesting of the natural counter pack, which is reached of 2.200-6.220 €/kg.

Thus, the overall mission of this study constitutes the change in the ordinary cultivation methods of microalgae with utilizing a new magnetic nanotechnology to cultivate them. The goal of this research is to reduce the cost of astaxanthin production up to competitive levels with those of synthetic one. This study outputs will bring to the market an economically, environmentally and socially new procedure at the production of astaxanthin as a raw material and finally the exploitation of this bioactive compound in a broad variety of industrial applications with remarkable outcomes.

Key words: *microalgae, bioactive compounds from algae, carotenoids, astaxanthin, astaxanthin from H. pluvialis, magnetic photobioreactor*

OBJECTIVE

In the case of large scale astaxanthin production, this pigment seems to have been high economic growth potential in the market. The main disadvantage of natural astaxanthin is that the production and exploitation of this bioactive compound is required high costs for infrastructure of culture systems as well as additional methods of processing. The purpose of this study is to provide the reader a new magnetic cultivation and production system of algae and more specifically astaxanthin from *H. pluvialis* with a cost-effective way. The current research gives information on common cultivations systems as compared to the novel magnetic system, the industrial applications of this pigment, the expected market at the emerging period and the competition worldwide. Furthermore, an economic performance during the production process of microalgae for the scaling up of astaxanthin with the suggested system constitute the core of this Master Thesis.

All this information is useful for the reader to understand the idea of Valuemag project and the scope which it was developed, with the final aim of presenting the competitive advantage of the natural astaxanthin for a wide range industrial application and its superiority to competing products.

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1. THE CONCEPT

1.1 Introductory

During the latest years, consumers have been pulling more and more notice to environmental and sustainability issues with the same focus as to life-style matters and their impact on their health. There is a great concern about the origin and safety of products, likewise, manufacturing processes with the environmental consequences. Regarding these concerns, there is a need for investigation of natural products with a great variety of functions, as well as food, nutraceuticals and cosmetics. Also, due to the safety of natural sources, consumers orient towards them as compared with synthetic ones (Pimentel *et al.*, 2018). As a result of this can be strong encouragement for the industrial world to examine new opportunities in novel/ alternative natural **raw materials** with extra attributes and functions that go further than their ordinary applications (e.g., nutrition) (Harnedy and FitzGerald, 2011); (Bixler and Porse, 2011); (Pimentel *et al.*, 2018).

Accomplishing a good enough nutrition may be a growing international concern with the rise in world population. Consequently, cost-effective sources of nutrients which will easy and speedily manufacture large quantities of products of high nutritional value is required. Algae can be a significant source of a various number of strictly necessary nutrients to support human health (García *et al.*, 2017).

1.2 Microalgae

In the past few years, microalgae have attracted increasing attention for manufacturing a wide range of **"high value-added compounds"**, that are helpful in many aspects of our life. Bibliography data demonstrate that microalgae are made up of molecules which will be utilized in several industrial sectors like pharmaceutics, nutraceuticals, food additives and natural cosmetics (Gordon and Juergen, 2007); (Leu *et al.*, 2014); (Molino *et al.*, 2018); (Wang *et al.*, 2015); (Wang *et al.*, 2017). The commercial applications at these markets are characterised by high-quality safe products at competitive prices. Within this context, microalgae represent the starting point to produce natural compounds with a sustainable method with low environmental impact (Pulz and Gross, 2004); (Spolaore *et al.*, 2006).

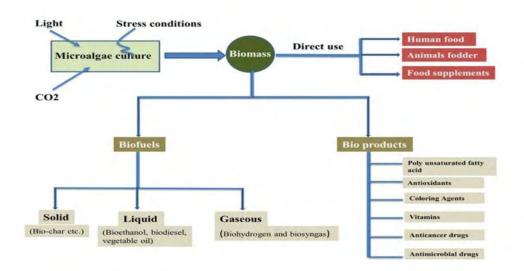
Marine algae, additionally known as seaweed, are photosynthetic eukaryotic organisms that may be found in fresh water and marine environment with tenacious vitality (Wang *et al.*, 2017). Grow in a variety of aquatic habitats, as well as lakes, pounds, rivers, oceans, and even wastewater (Barsanti *et al.*, 2008). Algae are generally classified by size as macroalgae or microalgae and in line with their

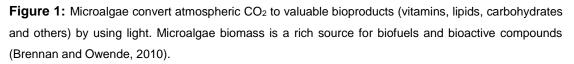
morphological pigmentations as Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) (Khan *et al.*, 2018).

1.3 Bioactive Compounds from microalgae and applications

Microalgae can adapt rapidly in harsh/extreme environmental conditions, tolerating a wide range of conditions (e.g., variable salinity, temperature, nutrients, UV–irradiation) due to a completely different adaptation strategy. Therefore, they will produce a great variety of fascinating secondary metabolites (having putative biological activities) with novel structures and potential to survive in such various diverse and extreme environments, that are typically not found in other organisms. Since microalgae are one among the most commonly studied and used marine resources, bioactivities of the constituent of marine algae have been widely investigated (Mobin and Alan, 2017); (Wang *et al.*, 2017).

Microalgae have a major ability to convert atmospheric CO_2 to useful bioproducts, as well as **polysaccharides**, **lipids**, **pigments**, **proteins**, **vitamins**, **bioactive compounds and antioxidants** (Figure 1) (Khan *et al.*, 2018).





Microalgae can be a rich source of carbon compounds which may be utilised in a wide range of industrial applications (Das *et al*, 2011). Its application for biofuel production and wastewater purification have been extensively reported (Mobin and Alan, 2014); (Mobin and Alan, 2018). Microalgae may be used for other industrial applications like **high-value food and pharmaceutical products**, **nutraceuticals (nutritional ingredients)**, health food for human, food and fodder additives, polysaccharides, cosmeceuticals, bioremediation, antioxidant, dyes, food for cultivation or bioplastic production, wastewater treatment and atmospheric CO₂ mitigation (Mobin and Alan, 2017).

1.4 Microalgae species

Microalgae have over 300,000 species out of that around 30,000 are reported (Mobin and Alan, 2017). In line with their classification, each group contains many species and each of these species have thousands of strains. Only a little number of these selections have been explored for potential beneficial use. Table 1 highlights some major microalgal species/groups, their products and biotechnological applications (Mobin and Alan, 2017).

Species/group	<u>Product</u>	Areas of application
Arthrospira (Spirulina) platensis	Phycocyanin, biomass	Health food, cosmetics
Arthrospira (Spirulina)	Protein, Vitamin B ₁₂	Antioxidant capsule, immune system
Aphanizomenon flos-aquae	Protein, essential fatty acids, β -carotene	Health food, food supplement
Chlorella spp.	Biomass, Carbohydrate extract	Animal nutrition, health drinks, food
		supplement
Chlorella vulgaris	Biomass, Carbohydrate extract	Health food, food supplement, feeds
Dunalienna salina	Carotenoids, β-carotene	Health food, food supplement, feeds
Haematococcus pluvialis	Carotenoids, astaxanthin	Health food, pharmaceuticals, feeds
Odontella aurita	Fatty acids, EPA	Pharmaceuticals, cosmetics, anti-
		inflammatory
Porphyridium cruentum	Polysaccharides	Pharmaceuticals, cosmetics
Isochrysis galbana	Fatty acids	Animal nutrition
Phaedactyhon tricomutum	Lipids, fatty acids	Nutrition, fuel production
Lyngbya majuscule	Immune modulators	Pharmaceuticals, nutrition
Scennedesmas spp.	Protein	Aquaculture, human nutrition
Schizochytrium sp.	DHA and EPA	Food, beverage and supplement
Crypthecodinium cohnii	DHA	Brain development, Infant health and
		nutrition
Nannochloropsis oculata	Biomass	Food for larval and juvenile marine Fish
Nannochloropsis sp	EPA	Food supplement and pharmaceuticals

Table 1: Some major microalgal species, products and areas of application (Mobin and Alan, 2017).

1.5 Currently applications of microalgae and new opportunities

Many species of microalgae are used as source of wealthy nutrient food, feed, and health promoting compounds (Shah *et al.*, 2016). **Presently**, microalgae products are available both in pure form as extracts, tablets, or capsules and as additives to a few food products which are either used as nutrients or food colouring agents with high nutritional value (Khan *et al.*, 2018).

Among the commercially vital compound of microalgae, carotenoids have been presented attractive in industrial world with astaxanthin to play the leading role in recent years. **Astaxanthin from** *Heamatococcus pluvialis* is the richest source of natural astaxanthin that is regarded as "super anti-oxidant" with considerably greater antioxidant capability than the synthetic one, as we will explain at the following sector (Shah *et al.*, 2016). Astaxanthin has vital applications and are extremely promising for potential uses within the nutraceuticals, cosmetics, food, and aquaculture industries (Shah *et al.*, 2016). Astaxanthin will fairly reduce free radicals and oxidative stress and facilitate human body maintains a healthy state (Shah *et al.*, 2016). With significant productiveness and increase in demand, astaxanthin is one among the high-value microalgal products in the future (Panis *et al.*, 2016); (Shah *et al.*, 2016).

1.6 Limitations

Even though microalgae are possible for uses like as biofuel sources and biopharmaceuticals generally, some limitations and challenges remain, that should be overcome to improve the technology from pilot-phase to industrial level. The foremost challenging and major issues are:

- enhancing microalgae growth rate and synthesis of final products
- dewatering algae culture for biomass production (filtration, centrifugation)
- pre-treating biomass

- isolation and purification of bioactive compounds (e.g. rate of recovery, purity, content)

- and finally optimizing manufacturing process in all stages (Khan et al., 2018).

Moreover, costs of infrastructure, cultivation and harvesting are the most crucial matters at the production of astaxanthin. To make astaxanthin extraction from *H. pluvialis* additional competitive than synthetic one and, at the same time, promote its use within the same markets. Consequently, it's necessary to overcome important points associated with the biomass concentration, treatment and degradation reactions that may provide each quality and quantity of the extracted astaxanthin (Mercer and Armenta, 2011).

1.7 Microalgae cultivation systems

The commercial cultivation of microalgae has begun five decades ago (Mobin and Alan, 2017). Algae to grow and multiply, it only needs the support of sunlight and of basic components such as CO_2 and water for the conventional plant photosynthesis to

convert solar energy into chemical energy through CO₂ fixation (Adeniyi *et al.*, 2018). There is a great variety of microalgae cultivation systems that can be placed outdoors or indoors. Cultivation systems range from open shallow raceway ponds to closed photobioreactors, as illustrated in Figure 2 (Enzing *et al.*, 2014).

1. Open Systems/ Natural Cultivation

Natural cultivation methods include pond, lakes, and lagoon, which are classified based on natural water bodies e.g. shallow ponds, circular ponds and raceway ponds, Fig. 2(A).

2. Closed Systems/ Artificial Cultivation

Artificial cultivation methods are majorly photobioreactors which come in different shapes such as flat plate, tubular, and column, Fig. 2(B), (C) and (D) respectively.

3. Indoor Production Systems

Small scale for research and heterotrophic production systems (Enzing et al., 2014).



Figure 2: Algae cultivation methods (A) natural cultivation method, (B) artificial cultivation method (flat plate photobioreactor), (C) artificial cultivation method (tubular photobioreactor), (D) artificial cultivation method (column photobioreactor) (Adeniyi *et al.*, 2018).

1.8 Comparison of the different cultivation systems

Natural cultivation systems are normally cheaper to construct and operate, but the ponds make use of more energy to homogenize the nutrients. However, this cultivation system is more susceptive to environmental conditions such as variability in water, temperature, lighting, and evaporation. On the other hand, artificial cultivation system provides better controls overgrowth parameters and culture conditions, safe and

protected environment against predators, reduced CO₂ losses and higher volumetric productivity (Adeniyi *et al.*, 2018).

All the limitations and challenges affecting the natural cultivation methods have been eliminated in artificial cultivation system is known as photobioreactors, but the development of more economical and efficient closed culturing systems is needed (García *et al.*, 2017). As a result, the economic feasibility is still questionable due to the high operating cost (Adeniyi *et al.*, 2018).

The future challenge of microalgal biotechnology is determined by the improvement of large-scale photobioreactors (PBRs) capable of operating under defined optimal conditions with minimal or without contamination risks (Wang *et al.*, 2012). Also, escalate methodologies need to be improved for managing efficient light provisions, minimum CO₂ losses, and effective mixing and reuse of generated oxygen (Gupta *et al.*, 2015).

1.9 New magnetic cultivation and extraction techniques

According to all previous challenges, the team of the project VALUEMAG (Valuable Products from Algae Using New Magnetic Cultivation and Extraction Techniques) try to obtain a revolutionary solution for microalgae cultivation, harvesting and exploitation of biomass in order to manufacture and purify high-value biomolecules in an affordable and environmentally-friendly way. More specifically to arrive this complicated aim, VALUEMAG, as the main scope, develops an advanced magnetic method for micro-algae cultivation (https://www.valuemag.eu/). Moreover, the exploitation of algae and other aquatic biomass is in focus targeting the production of molecules for pharma, nutraceuticals, food additives and cosmetic applications (https://www.valuemag.eu/).

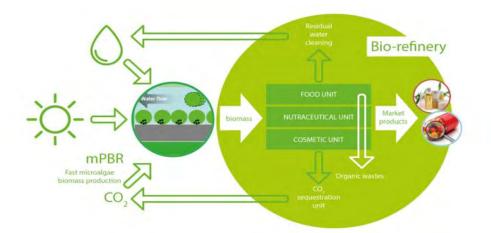


Figure 3: The concept of VALUEMAG project, Schematic representation mPBRs (magnetic PhotoBioReactor) section (<u>https://www.valuemag.eu/</u>).

VALUEMAG will symbolized an integrated complicated system which will involve the transformation of microalgae by introducing Super Paramagnetic Ions (SPANs) and their immobilization in SOMAC (Soft Magnetic Cone) surface in a very closed-controlled system (<u>https://www.valuemag.eu/</u>). This can permit fast microalgae biomass production at an affordable cost. Additionally, SPAN based-methodology can make purification step easy, economic and accurate (<u>https://www.valuemag.eu/</u>). At the identical time, wastewater recycling and CO₂ capturing systems are performed to gain on the environmental benefits that microalgae-based processes and procedures provide (<u>https://www.valuemag.eu/</u>). In brief,

- ✓ an innovative methodology is implemented based on the utilization of magnetic nanoparticles and a soft magnetic steel cone to cultivate algae and to purify the high-value products resulting from their metabolism
- ✓ the advanced and revolutionary technology has been already tested at the small scale providing promising results
- ✓ the project is completely self-sustaining, eco-friendly and can offer socioeconomic benefits for the individuals
- ✓ the project addresses the dramatic reduction of microalgae production costs, permitting this technology to become a competitive durable alternative within the current market (<u>https://www.valuemag.eu/</u>).

1.10 Objective and scope of the present investigation

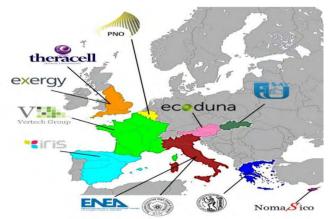
The innovation of this project is to produce high added value products with a costeffective way and enter to the current market with **an economic & competitive innovative technology, environmentally friendly approach, socio-economic and development driving force**. After having conducted research for some bioactive compounds extracted from algae, we selected astaxanthin since it is a compound with high nutritional value existing strong demand as well as important market share. The result of this project is the production of astaxanthin as a pilot extractant and finally the exploitation of this bioproduct for a wide range of industrial/commercial applications. Thus, the overall mission is the change of the cultivation method of microalgae compared with open and closed systems to a new technology which will dramatically reduce the cost of micro-algae cultivation (from 5-12€/kg to <0,3 €/kg of algae) and thus the astaxanthin production cost from harvested micro-algae.

2. THE TEAM

2.1 Participants

VALUEMAG is the result of the collaboration of 11 members from 9 European countries who provide all the necessary background and expertise to achieve the objectives of the project. The VALUEMAG Project combines primary production and processing industries, consumer brands, SMEs, research and technology centers and universities (https://www.valuemag.eu/). More specifically:

- Leading research organizations with extensive experience in the continuous development of next generation technologies that combine top class academic know-how with a long tradition in providing solutions to major European industries (SUN, NTUA, ENEA, IPSAS)
- Market-oriented industrial actors with international experience in the implementation of very large projects and the launching of new products and services (PNO, VTG) and
- Highly specialized high-tech SMEs (NOMASICO, ECODUNA, THERACELL, IRIS, EXERGY). At Table 2, (Appendix A) are given the participants of the program.



THERACELL is part of Qualia group of companies and provides its know-how in the commercialization process of those high-added value products with a history in cosmetics, nutraceuticals and hygienic food. THERACELL is an active SME in developing new pharmaceutical products and is particularly interested and skilled in treating nanoparticles to be inserted in various types of cells, including stem cells. This way, **QUALIA** will lead the task in industrialization of the raw material of astaxanthin and other high-added bioactive compounds from micro-algae in cosmetics and nutraceuticals.

3. THE PRODUCT

3.1 Generally - Valuable compounds extracted from microalgae

Marine life is incredibly rich in manufacturing numerous and unique chemical components, each primary and complex. Because of the tough conditions like high salinity, lack of nutrients, light and space, that make the marine surroundings competitive, organisms adjust to the environment by manufacturing numerous substances and metabolites, a lot of specific secondary metabolites produced by algae (macro and microalgae), that permit them to maintain their survival and defend themselves against external intimidations. Therefore, sea biodiversity presents the chance to investigate these molecules and realize novel and natural bioactive compounds (Pimentel *et al.*, 2018).

Microalgae are extraordinarily prominent producers of the many high-value natural compounds therefore as a result, algae produce a wide vary of important and valuable products for business purposes. The products which will be produced from microalgae are very plentiful and various (Table 3, Appendix A) and have been suggested with large range of functions for health effects, as illustrated (Table 4, Appendix A). They manufacture vitamins, that promote their importance as a nutritional food for people and animals. They produce differing kinds of medicinally necessary polysaccharides. Numerous species manufacture bioactive and commercially vital pigments, carotenoids synthesized by plants, algae and bacteria that play completely different physiological purposes and since of that they provide huge nutraceutical values. These pigments are crucial in therapies for tumorigenesis, neuronal disturbance, and visual diseases. Also, proteins can be extracted from microalgae with rich content such as phycobiliproteins from cyanobacteria and a few red algae. PUFAs (polyunsaturated fatty acids) are currently principally derived from fish oil however because of several concerns (e.g. exploitation of marine reserves in a high level, accumulation of toxic compounds in marine life, peculiar smell, unpleasant sense and flavour, oxidative instability), there's an increasing interest in exploitation microalgae by other source of PUFAs. Several microalgae are producers of various exopolysaccharides (EPS) that are widely known like thickeners and gelling additives with implementation in the food industry. Sterols will be acquired from microalgae, an alternative bioactive compound with interest. In addition, many microalgae contain phenolic compounds in high content that are the same as or maybe more than several common food sources. Finally, microalgae have been presented to be a supply of volatile compounds with antimicrobial activity (Khan et al., 2018); (Islam et al., 2017); (García et al., 2017); (Shah et al., 2016).

Most of the substances which extracted by microalgae have therapeutic effects (Khan *et al.,* 2018). A few of them described below:

- ✓ Anti-diabetic properties
- ✓ Antioxidant activity
- ✓ Anti-inflammatory activity
- ✓ Anti-microbial activity
- ✓ Anti-viral activity
- ✓ Antitumoral activity

After research of all these bioactive compounds from microalgae, we observed that astaxanthin from microalgae to be presented noticeable interest since it is a pigment including potential benefit, global market new entries/opportunities and incorporation of astaxanthin production into biorefining (Shah *et al.*, 2016) with the new suggested magnetic cultivation system and extraction techniques. Moreover, this bio compound is incriminated for a variety of biological activities in human health with strong evidences, as we will see below §3.4, 3.7. As a result, astaxanthin has a wide range of commercial importance providing strong role in food, feed/aquaculture, cosmetics and biopharma.

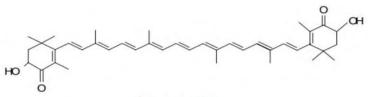
This study focuses on reviewing interesting biochemicals from algae biomass with leading role in the carotenoids. More specifically our product is the **astaxanthin**, **as a raw material from natural sources** (*Haematococcus pluvialis*), that cultivated with a new magnetic cultivation system and extracted with the highest feasible content combining the Valuemag technology. So that concept bringing it very close to the market, with environmentally (water cleaning and CO_2 capturing) and socially (inexpensive production) sustainable issues involved at the production of natural astaxanthin.

3.2 Carotenoids from microalgae

Carotenoids are natural pigments that are in numerous plants and organisms (Grand View Research, 2016). Carotenoids which originated from algae provide necessary bio compounds in different forms that possess significant biological activities with industrial interest. There are over 400 publicly recognised carotenoids however some of them are used commercially, among the most important are the β -carotene, α -carotene, lycopene, astaxanthin, lutein, zeaxanthin, canthaxanthin, fucoxanthin, violaxanthin etc (Table 3, 4 Appendix A). The most common uses of carotenoids are as food colourants and as supplements for human and animal nutrition (Milledge J., 2012).

3.3 Astaxanthin

An important lipophilic **carotenoid pigment** is astaxanthin ($C_{40}H_{52}O_4$, 3,3'-dihydroxy- β , β -carotene-4,4'-dione) belonging to the xanthophylls cluster (Molino *et al.*, 2018). Astaxanthin is a bright red secondary carotenoid from the similar family as lycopene, lutein, and structurally same to β -carotene, synthesize by aquatic and non-aquatic microorganisms like microalgae, plants, yeast, bacteria and attended in many of seafood like salmon, trout, red sea bream, shrimp, lobster and fish eggs (Shah *et al.*, 2016); (Pimentel *et al.*, 2018).



Astaxanthin

Figure 4: Structure of the carotenoid astaxanthin

Astaxanthin molecule consists of three completely unlike configurations: 2 enantiomers (3'R 3R and 3'S 3S) and one meso-form (3R 3'S) with the most widespread natural type is that the 3'S 3S form. Artificial astaxanthin consists of a mixture composed by the three of them that confer higher stability to the substance (Molino *et al.*, 2018). Currently this pigment received from **natural (yeast, bacteria, krill/ shrimp, microalgae, others)** and **synthetic sources (synthesised by petrochemicals)**. Though it's a natural substance, however principally utilized in synthetic type because of lower production costs, larger purity and stability (Nguyen, 2013).

In comparison to other antioxidants and the synthetic form, natural astaxanthin is more powerful than vitamin C, Vitamin E etc. (Figure 5).

ASTAXANTHIN IS:		
6000 times	stronger than Vitamin C	
3000 times	stronger than Resveratol	
800 times	stronger than CoQ10	
550 times	stronger than Green tea catechins	
500 times	stronger than Vitamin E	

Figure 5: Natural astaxanthin in terms of other antioxidants, in trapping energy from singlet oxygen (Nishida *et al*, 2007).

3.4 Health benefits of Astaxanthin

Below are given some scientifically documented health claims/ effects of astaxanthin, see (Appendix A: § Health benefits of Astaxanthin) and Figure 6, (Appendix A: § Health

benefits of Astaxanthin) as was presented from BGG a leader manufacturer of natural astaxanthin.

3.5 Industrial applications of astaxanthin

Astaxanthin has a variety of biotechnological applications for use in the food, feed, cosmetic, aquaculture, nutraceutical, and pharmaceutical industries (García *et al.*, 2017) (Islam *et al.*, 2017); (Khan *et al.*, 2018). Moreover, astaxanthin is extremely engaging for necessary industrial markets due to its fascinating properties, like food grade, colouring and inhibitor agent. More specifically, it's used for different aim, as an example, in aquaculture field as feed additive for salmons, trout's, and crustaceans to produce their characteristic pink/red colour and for fish's growth and nourishment of ornamental birds (Molino *et al.*, 2018).

Astaxanthin is well-known to possess **powerful antioxidant** and **anti-inflammatory activities capable of preventing or decrease protein degradation, macular degeneration, rheumatoid arthritis, cardiovascular diseases, neurodegenerative diseases, like Parkinson's, and cancers** (Table 3, Appendix A) (García *et al.,* 2017); (Islam *et al.,* 2017); (Khan *et al.,* 2018).

Synthetic astaxanthin has a cost of about **1000 \$/kg**, whereas the natural one, is reached of **2,500-7,000 \$/kg**. Most commercially available astaxanthin are synthetic (about 95%-99%) and only a little fraction of astaxanthin (<1%) currently derives from natural origins (Shah *et al.*, 2016). In the last years, due to its precious properties of natural astaxanthin has reached an increasing market trend. A remarkable increase in the market of the astaxanthin is expected to reach more than 700 million euro in 2022 (Research and Markets, 2017). Within the last years, many projects are carried out to clarify the feasibility of astaxanthin extraction from natural sources (Shah *et al.*, 2016). Also, Valuemag have conceived a technique and a method with main scope targeting the dramatic reduction of cost on astaxanthin able to cultivate in a minimum possible cost and further the feasibility of the proposed extraction techniques of this natural compound.

3.6 Haematococcus pluvialis - as a major source of astaxanthin

According bibliography, there are several microalgae strains that are suggested as potential sources to produce astaxanthin, such as *Chlorella sp., Chlorococcum sp.* and *Scenedesmus sp.* (Del Campo *et al.,* 2004); (Ma and Chen, 2001); (Qin *et al.,* 2008), *D. Salina, H. Pluvialis.* However, the accumulation of astaxanthin inside *Haematococcus pluvialis* cells exceeds any other microalgae strain (see Table 5, up to 4% of dry biomass), in response to stressful environmental conditions (Khan *et al.,*

2018), and thus it is the most preferred species for large scale natural astaxanthin production and exploitation (Zhekisheva *et al.*, 2005).

Astaxanthin (%) on the Dry Weight Basis
3.8
3.8
3.6
3.4
2.7
0.2
0.001
0.6
0.02
0.01
0.02
0.01
2.2
0.5
0.5
0.2
0.12
0.015

Table 5: Microorganism sources of astaxanthin (Ambati et al., 2014).

During this field, *H. pluvialis* will be thought of a hopeful source of astaxanthin because of its ability to accumulate high quantities regarding other sources and *H. pluvialis* derived natural astaxanthin corresponds to < 1% of the commercialized amount (Molino *et al.*, 2018). Dried biomass of *Haematococcus pluvialis* has been commercialized as astaxanthin rich source until nowadays.

3.6.1 Haematococcus – Microalgal culture conditions for growth and carotenogenesis

Haematococcus pluvialis (Chlorophyceae, Volvocales) is considered as the best natural source of astaxanthin and the principal commercial organism of this product, under stress conditions (Shah *et al.,* 2016); (Mobin and Alan, 2017). *H. pluvialis* is unicellular freshwater strain of green microalgae with a unique life, which is divided in two stages (Boussiba, 2000).

Photoautotrophic, heterotrophic and mixotrophic (based on growth condition), indoor or open raceway ponds or closed photobioreactors are used for *H. pluvialis* cultivation (Mobin and Alan, 2017). For commercial production and exploitation of astaxanthin, two stages of cultivation strategy are costumed. First step involves growing the algal green biomass and the second stage involves producing astaxanthin by red biomass.

More specifically, the first refers to a green in which the microalgal cells constantly divide and proliferate, composing chlorophyll. The second refers to a red stage in which cell division stops and chlorophyll levels do not fluctuate, consequentially in a continuous increase of astaxanthin content and cellular dry weight (Panis *et al.*, 2016) (see Figure 7).

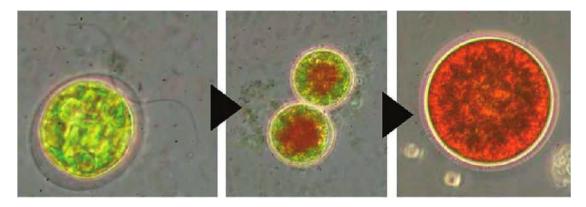


Figure 7: 'Green stage': actively growing *H. pluvialis*, 'Red stage': *H. pluvialis* that have accumulated astaxanthin as a result of nutrient starvation and adverse environmental conditions (Lorenz and Cysewski, 2000); (Kristoffersen *et al*, 2012)

In order *Haematococcus pluvialis* to transit from stage one ('green stage') to stage two ('red stage'), specific stress conditions during cultivation are needed such as light density, high temperature, pH, salt concentration and nutritional stresses (Boussiba and Vonshak, 1991); (He *et al.*, 2007); (Markou & Nerantzis, 2013). The extremely complicated and dynamic composition of cell-wall permits H. pluvialis to survive in hostile surroundings, however on the other hand represents an issue once H. pluvialis biomass must be processed to extract valuable intracellular compounds like astaxanthin (Molino *et al.*, 2018). The cellular composition of H. pluvialis have obvious changes between of these two stages of cultivation (Mobin and Alan, 2017), is shown at Table 6.

Composition content (% of DW)	Green stage	Red stage
Proteins	29-45	17-25
Lipids(% of total)	20-25	32-37
Neutral lipids	59	51.9-53.5
Phospholipids	23.7	20.6-21.1
Glycolipids	11.5	25.7-26.5
Carbohydrates	15-17	36-40
Carotenoids(% of total)	0.5	2-5
Neoxanthin	8.3	n.d
Violaxanthin	12.5	n.d
β-carotene	16.7	1
Lutein	56.3	0.5
Zeaxanthin	6.3	n.d
Astaxanthin (including esters)	n.d	81.2
Adonixanthin	n.d	0.4
Adonirubin	n.d	0.6
Canthaxanthin	n.d	5.1
Echinenone	n.d	0.2
Chlorophylls	1.5-2	0

Table 6: Ceccular composition of H.pluvialis biomass in two cultivation stages, "green" and "red" (Mobin and Alan, 2017).

During this phase, "red phase", due to the colour transition from green to red, H. pluvialis will increase its astaxanthin synthesis from the lower content within the green part (0.5% on dry basis) until the range of 1.5% to 3 on dry basis (Cerón *et al.,* 2007), can accumulate up to 5% DW of astaxanthin.

3.7 Astaxanthin, from *Haematococcus pluvialis*, in human and animal health

Several researchers have been investigating the health effects of microalgae, with positive outcomes regarding some types of cancer, central nervous system, cardiovascular system, inflammation and antioxidant, antibacterial and antiviral activity among many others (Pimentel *et al.*, 2017).

Astaxanthin provide diverse human health benefits with nutraceutical implementation. Enough bibliography data is obtainable with evidences based in vitro and animal models (Shah *et al.,* 2016). The effect followed of astaxanthin from the strain *Haematococcus pluvialis* in animal and human is presented in Table 7, Appendix A.

3.8 Photobioreactor approach for H. pluvialis

Firstly, *H. pluvialis* is that the best well known producer of the carotenoid astaxanthin with worthy value, approximately to \in 4000 per kg as a result this product will completely excuse detrimental cultivation systems which required for this organism (Shah *et al.*, 2016).

Secondly, *H. pluvialis* is responsible for astaxanthin formation and deposition of triglycerides under stress/extreme conditions. It has been shown that these two reactions are closely related for deposition of astaxanthin inside lipid bodies to confer its protective function (Solovchenko, 2015).

Thirdly, *H. pluvialis* is a mixotrophic alga with extremely beneficially development in biorefineries approach. *H. pluvialis* can use carbon sources (CO₂ etc) as a result there is reduction of the cultivation costs and/or acceleration of the cultivation (Wu *et al.*, 2013); (Shah *et al.*, 2016).

These three aspects of *H. pluvialis* make it an extraordinary suitable strain to produce algal and further the exploitation of astaxanthin with the technology of biorefineries producing high value-added final products. According to the previous claims about *H. pluvialis* and since it is possible, at this current study we suggest this microalgae strain to combine with the technology VALUEMAG for production and exploitation of astaxanthin as a raw material.

3.9 Challenges

Nowadays societies are turn to "green" and environmentally friendly solutions as a result natural astaxanthin form *H. pluvialis* constitute to be more attractive than the synthetic form due to structure, source, strength, efficacy, superiority and functions, wide range of application in several sectors, security and safety as a natural compound (Shah *et al.*, 2016).

There are four main areas where further progresses are needed. Three key areas of focus should be:

1. cost and efficiency of the cultivation, harvesting, infrastructure system

- ✓ Firstly, due to the complicated life cycle of *H. pluvialis*, it is noteworthy to maximize the algal cell production and efficacy in the "green stage" of the culture to maximize astaxanthin yield from the "red stage".
- ✓ Lower amount of consuming water and lower probability of contamination.
- ✓ Reutilization of carbon dioxide and nutrient resources at the production of valuable products could help decrease cultivation costs (Shah *et al.,* 2016).
- 2. good practice of cultivation and controlling predators
- **3. isolation, purification, quantification of astaxanthin** with economical friendly solutions and generally recognised as safe (GRAS) (Shah *et al.,* 2016).

4. and exploitation of the abundant bioactive compounds, that can be extracted from microalgae.

Improvements at these issues can overcome the main drawback, the cost of production of astaxanthin by *H. Pluvialis* (Shah *et al.*, 2016). At the end, the main challenge of this whole process is to add value to final products (Pimentel *et al.*, 2017). This can be reached in several different ways, namely, by **1.** exploring and development new natural raw materials that are not only rich in functional and bioactive compounds but also provide additional properties that the current ones **2.** utilization these molecules in a viable way **3.** processing them through green procedures and eco-friendly processes, with low or neither environmental impact and **4.** dissemination of novel solutions and distributing products that come from consumers demand and expectations (Pimentel *et al.*, 2017). Technology of Valuemag has developed to face all these challenges.

3.10 Current status of microalgae at the market

The total chemical composition of microalgae prepares them as valuable biosustainable ingredient for a wide range of applications, with more interest at the sector of industries in which the ingredients used in the formulations; whether as active substances, excipients, or additives. All of these are elements of high added value and differentiation of a final product (Pimentel *et al.*, 2018).

At the market of food additives and supplements, microalgae are dominated. The forms which are often used for food additives are either as **dry biomass powder** or **as extracts** by bioactive compounds like PUFA's and carotenoids. At the other hand, food supplements are manufacture like **powders** and **in formulations as oils, tablets** (Molino *et al.,* 2018).

3.10.1 Regulations issues

Food Safety Regulations in the European Community (EC 178/2002) and the Novel Food Regulation (EC 258/97) are regulated the microalgae products, production and selling, which intended for use in food and feed sector. Also, food and feed products based in microalgae, which are destined for selling, are regulated by the Federal Food, Drug and Cosmetic Act (FD&C) and by the Dietary Supplement Health and Education Act (Molino *et al.*, 2018).

3.10.2 Final cost

The composition of the microalgae and the percentage of their bioactive compounds (EPA/ DHA, astaxanthin, lutein etc) play a crucial role for microalgae final products cost. Moreover, the type of cultivations (open or closed systems) is another important

issue that have an impact at the cost of production and after to the final product (Molino *et al.*, 2018).

3.11 Current status of astaxanthin in the market

Below are listed in the Table 8, the main microalgae products of astaxanthin which currently approved in Europe and by the U.S. Food and Drug Administration (FDA) with their applications sectors, characteristics, with regulations and prices:

Table 8: Astaxanthin biomass and products applied in food, feed, and food supplements, authorized by EU regulations and the FDA (Li *et al.*, 2011); (García *et al.*, 2018); (Norman *et al.*, 2018).

Products Name	Application	Characteristics	Regulations	Price
H. pluvialis meal	Color additive for fish feed (salmonids)	Dry and comminuted solid biomass containing not less than 1.5% astaxanthin	CFR-TITLE 21-FDA	US\$489/kg
Astaxanthin	Color additive for fish feed	Physical state, solid, 0.05 percent solution in chloroform Astaxanthin content minimum 96%	CFR-TITLE 21-FDA	€99/kg (astaxanthin content: 0.5%)
Astaxanthin-rich oleoresin from H. pluvialis	Novel food and food supplement	Astaxanthin is extracted by CO2-SFE or diluted ethyl acetate using olive oil, sunflower oil or medium chain triglycerides.	Regulation (EC) 2017/2470 Directive 2002/46/CE	€499/kg (astaxanthin content: 5%)
		Protein 0.3–4.4%		
		Carbohydrates 0–52.8%		
		Ash 0.0–4.2%		
		Fiber <1%		
		Fat 42.2–99%		
		Total astaxanthin 2.9–11.1%		
		All-trans astaxanthin 79.9–91.5%		
		9-cis astaxanthin 0.3–17.3%		
		13-cis astaxanthin 0.2–7.0%		
		Beta-carotene 0.01–0.3%		
		Lutein 0–1.8%		
		Canthaxanthin 0–1.30%		

The most common forms of astaxanthin as a raw material, bulk of astaxanthin, are represented at the following (Figure 8) with their possible uses and industrial applications.



Oil Extract For softgel capsules, cosmetic applications



Powder For hard capsules, tablet



Watersoluble For beverages, liquid nutraceutical supplements.



Beadlet CWS beadlets for instant powdered drinks



For softgel capsules and food applications



Softgel Capsules Ready-made soft caspules

Figure 8: Forms of astaxanthin for industrial applications. **Oil extract**, for softgels capsules, cosmetics applications. **Powder**, for hard capsules, tablet. **Water-soluble**, for beverages, liquid nutraceutical supplements. **Beadlet**, for dietary supplements in powder sachets. **Biomass**, for hard capsules, tablet. **Softgel Capsules**, ready-made soft capsules (<u>http://www.fujichemical.co.jp/english/</u>)

3.12 Nowadays applications of H. Pluvialis

- Dietary supplements containing Haematococcus astaxanthin approved for use in human in the US, the EU, Japan, Korea and many other areas in the world. Also, food supplements have been shown to be safe for humans' consumptions and have been widely used for over 15 years as dietary supplements without the negative side effects of their supplementation (Yang *et al.*, 2013). As component for food supplements, species like *H. pluvialis* utilize in the nutraceutical field because of the high concentration of vitamins and antioxidant properties (Molino *et al.*, 2018).
- Aquaculture/ animal feed, for the application of H. pluvialis in nowadays. Primary proteins, vitamins, carotenoids and omega-3 fatty acids which can be received from this strain in a worthy content are responsible for uses at the aquaculture sector. Currently, the Food and Drug Administration has already approved the use of H. pluvialis in the red phase in the animal feed market and especially in aquaculture as a coloring additive for the feeding of shrimps and salmonids (Udayan *et al.*, 2017); (Shah *et al.*, 2016). Dry biomass or as astaxanthin extracts, dry and committed material within the form of oleoresin (rich in astaxanthin) extracted using the CO2-SFE technique, are the types/ forms that utilized at present (Molino *et al.*, 2018).
- In the cosmetics field, as colouring agents and for UV protection with the high

content of carotenoids. In addition, *H. pluvialis* with an extremely good production of lipids, will be used to produce for skin like softness (Molino *et al.,* 2018).

3.13 Results from Valuemag

Regrettably, the market for astaxanthin dominated by synthetic forms. For the purpose to promote the natural form of astaxanthin from microalgae strain *H. pluvialis*, there has been applied green and safe extraction techniques using generally recognized as safe (GRAS) solvents like ethanol and acetone or extraction method with CO₂-SFE (Molino *et al.*, 2018).

Several studies at bibliography have presented experiments on supercritical CO₂ extraction for the maximum recovery of astaxanthin from *Haematococcus pluvialis*. Results of these different studies are showed that the recovery efficiency of astaxanthin was determined at 84%, 92% and 97% with modified optimal conditions for the temperature and pressure or the presence of co-solvents and concentration of them respectively (Panis *et al.*, 2016).

Results of the proposed magnetic photobioreactor of Valuemag as shown, the **maximum recovery of astaxanthin (~94%)** was found without co-solvent at 550 bar and 50°C. However, a comparable result was achieved **with co-solvent (~92%)** at 550 bar and 65°C. Also, with co-solvent, **the highest purity (~18%)** was achieved with an extraction time of 40 min at 80°C and 400 bars. **Without co-solvent, the highest purity (~34%)** was found with an extraction time of 80 min at the same pressure and temperature (Molino *et al.,* 2018).

4. THE MARKET

4.1 Total market of algae products – The size

In the last years, there is a growing concern of customers on 'food with nutritional value' or 'healthful' products. In accordance with this fact, there is an increasing rate at the demand for these products which are promote better health, are increase longevity and are protect against the beginning of chronic diseases. Consequently, the utilization of microalgal biomass/raw material or its derived bioactive compounds have become a novel approach for the research and development food products with nutritional value (Gouveia *et al.,* 2008); (Garcia *et al.,* 2017).

According to the most recent obtainable statistics from FAO (Food and Agriculture Organization), approximately 23.8 million tons of macroalgae (\$6.4 billion) and other algae are harvested annually. The main producing countries are China (54%) and Indonesia (27%), followed by the Philippines (7.4%), Republic of Korea (4.3%), Japan (1.85%), and Malaysia (1.39%) (FAO, 2012). In Asian countries, macroalgae are traditionally used as source of food, for medicated functions, or as fertilizers. Also, they have uses as raw material in animal feed (Pimentel *et al.*, 2017). Generally, according to international trends and habits, there is an increasing demand for edible algae and algae-based products (Wells *et al.*, 2017).

Searching the current market size of microalgae-based products, dried whole algae Spirulina have the largest share, more than **12000 tons of** *Spirulina* biomass are produced each year (at about 30 US\$/kg) mainly in China, India and Taiwan (Garcia *et al.*, 2017). Worldwide, *Chlorella* producers cultivate an approximate number of **5000 tons** each year. Following, *D. salina* (about **3000 tons** for carotene), *A. flosaquae* (about **1500 tons** for food), *H. pluvialis* (about **700 tons** for astaxanthin), *C. cohnii* (**500 tons** of DHA) and *Shizochytrium* (**20 tons** of DHA) (Garcia *et al.*, 2017).

As per Transparency Market Research (2018), '*the global nutraceutical market* (including functional food and beverage ingredients, dietary supplements, personal care and pharmaceuticals), which was valued at US\$ 182.60 Bn in 2015, will rise to US\$ 278.96 Bn by 2021, exhibiting a CAGR of 7.3% for this period ''. As a result, the research and development of new functional food and beverages with added ingredients will be faced a great interest in the coming years (Bagchi and Nair, 2017).

There are also hopeful market opportunities for some nutraceuticals, with carotenoids to play a crucial role. Therefore, the global market for carotenoids is projected to reach

US\$ 1.7 Bn by 2022 (https://www.strategyr.com/Marketresearch/Carotenoids Market Trends.asp). Global market for both synthetic and natural source astaxanthin in aquaculture, nutraceuticals, cosmetics, food and beverages is projected to reach 670 tons valued at US\$ 1.1 Bn by 2020 (Garcia *et al.*, 2017).

Below at Chart 1, 2 (Appendix B) represented the expected increase in the global algae products market, based on some market reports.

4.1.1. Trends of this market

Below are given the key driving factors which will be affect the algae products market:

• Multiple industry coverage/ Growing demand for algae products from the nutraceuticals and pharmaceutical industries

Microalgae have gathered much attention as important source of bioactive molecules due to wide range of applications and higher productivity when compared to plants and microbes (Khan *et al.*, 2017). So, the demand for biological compounds is growing dramatically because of their unique features such as secure utilization, antioxidant properties and durability under stress conditions like variable light, pH, and temperature conditions. Also, the increasing utilization of products from microalgae, as algae meal in aquaculture, as ingredients to dietary supplements, nutraceuticals and cosmetics are driving the growth of algae products market.

 Increase in consumer awareness concerning the health benefits of algaebased products

Food & beverage products which originate from algae are gaining the favour of consumers due to health benefits of these products. The health benefits provided by these products refer to brain health, eye health, skin health and UV protection, cardiovascular support etc.

• Alternate food source and food ingredient

Increasing consumer choice for alternative protein sources has also drive to the demand for algae-based food ingredients in ordinary food and health drinks. Additionally, the vegetarian tendency in North America and Europe is anticipated to led the demand for algae products in food, leading to the development of this market (Markets and Markets, 2018).

• The need to shift from fossil fuel resources to renewable energy sources.

Rise concerns about carbon-based emissions is causing an increase in use of biofuels derived from algal sources, consequently driving in raise in the global algae market. Worldwide, many governments are encouraging production of biofuels derived from algae. Thus, the associated policies put forth by such bodies are causing the global algal market to grow at a fundamental rate. In the next few years, this market is expected to attend a potent expansion, thanks to a rising awareness to use biofuels derived from algae (Transparency Market Research, 2018)

• Furthermore, as the adoption level of algae products by consumers is increasing,

it helps companies innovate and develop product lines for algae products, which has further contributed to the growth of the algae products market (Markets and Markets, 2018).

4.1.2. Market segments

 Table 9: Segmentation of the global algae products market (Markets and Markets, 2018)

	-Food & beverages
	-Nutraceuticals & dietary supplements
On the basis of Application	-Feed
On the basis of Application	-Personal care products
	-Pharmaceuticals
	-Others (pet food, fortified food, and infant food)
	-Lipids
	-Carrageenan
On the basis of Type	-Carotenoids
	-Algal protein
	-Alginate
	-Others (algal flour, dried algae, and agar)
	-Brown algae
	-Blue/green algae
On the basis of Source	-Green algae
	-Red algae
	-Others (yellow-brown algae and golden algae)
On the basis of Form	-Solid
	-Liquid
	-North America
On the basis of Region	-Europe
	-Asia Pacific
	-RoW (South America, Middle East & Africa)

The global algae products market is segmented based on:

• **Application** (nutraceuticals, food and feed supplement, pharmaceuticals, paints & coatings, pollution control and others) (Credence Research, 2018).

According the report Credence Research (2018), among these applications, nutraceuticals accounted the global algae products market in 2016 with the major market share (Figure 9). Algae products with uses as nutraceuticals containing the dietary supplements, food supplements and non-food supplements such as tablets, capsules, etc (Credence Research, 2018). Food & feed supplements formed the

following part, the second highest market share of global algae products market. Algae contain proteins that can be used in various foods and feed application, for example like as protein source for fish meal in aquaculture. Therefore, this sector is expected to create high demand for algae products in the market (Credence Research, 2018); (Research and Market, 2017). Other applications of algae products are also expected to boost the market during the following period from 2016 to 2023 (paints & colorants, pollution control, and pharmaceuticals) (Credence Research, 2018).

By applications, "aquafeed occupied the largest market share in 2015, accounted for about 40%, and will continue to maintain in the coming period due to the growing demand for fish" (Gos Reports, 2016).

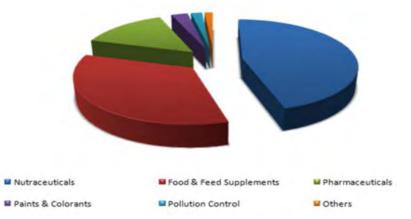


Figure 9: Global Algae Products Market Revenue, By Application, 2015 (\$ Mn) (Credence Research, 2018)

Also, other sources claim that based on application, the food & beverage segment accounted with the highest market share, followed by the nutraceutical and dietary supplement segment, in 2017 (Markets and Markets, 2018).

 Product type (hydrocolloids, carotenoids, omega-3 PUFA, spirulina, chlorella, and others),

The algae products market, depending on their type, is divided into segments like lipids, carrageenan, carotenoids, algae protein, alginates and others (that include dried algae, algal flour, and agar) (Markets and Markets, 2018). Lipids figured the largest market share in 2017. This can be attributed to algae as alternative sources to produce lipids in high content and fulfil the growing demand from the vegan population (Markets and Markets, 2018).

o Source

Based on source, brown algae-derived products figured a larger market share in 2017, followed by blue-green algae. Brown algae contributing to the growth of this market

because seaweed have the required properties for nutraceutical applications like antihypothyroid, anti-rheumatic, antibacterial, anticoagulant and antioxidant. Other products that can be extracted from brown algae are carotenoids and lutein, thus rendering it an extremely used algae type (Markets and Markets, 2018).

o Form

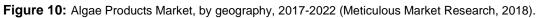
Based on form the algae products market has been segmented as liquid or solid form (Markets and Markets, 2018).

o Region

Geographically, the market is segmented into five major geographies, namely North America, Europe, Asia-Pacific, Latin America, and Middle East & Africa (Markets and Markets, 2018).

North America are mentioned with the largest quota of the algae products market in 2017 (Figure 10) (Markets and Markets, 2018). The large share of North America is principally attributed to the growing demand of nutraceutical market. Essential factors of this demand are rendered in rising of aging population, increasing healthcare awareness, and higher incidence of allergies and intolerance (Markets and Markets, 2018). North America is also anticipated to push high demand for algae products soon due to the existing algae research industries in the region which are increasingly being developed (Research and Markets, 2017)





Europe accounted to be the largest consumer of algae products worldwide (Research and Markets, 2017) due to the increasing demand for algae from the pharmaceutical and nutraceuticals industries in this region. During the following period, additional regions such as Asia Pacific, Latin America and Middle East and Africa are also expected to provide growth of global algae products market (Research and Markets, 2017)

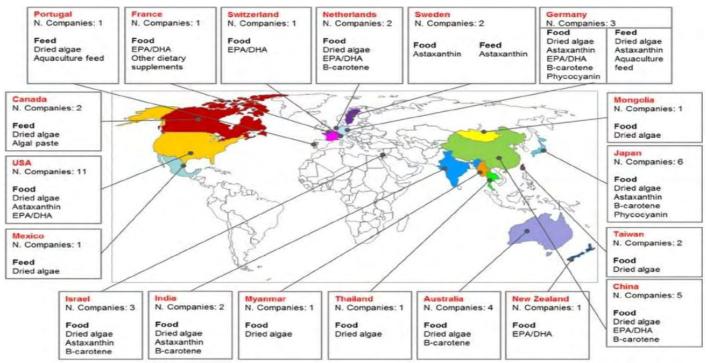


Figure 11: Global distribution of private companies producing commercial food and feed products derived from micro-algae (Vigani *et al.*, 2015).

Leading market participants in the industry over the past 3 years (2014-2017) see Appendix B, § Leading market participants in the industry of algae.

4.2 Total market of carotenoids/ Astaxanthin – The size

Carotenoids are organic pigments that are in several plants and organisms (Grand View Research, 2016). Global carotenoids market demand was 1.150,4 tons in 2015 with increasing demand for human nutrition and skin care cosmetics in emerging countries such as China, India, South Korea, Brazil, Singapore, and Thailand. Increasing the sector of Research and Development of valuable natural carotenoids is anticipated to create new growth opportunities for this market and industrial acceptance (Grand View Research, 2016).

Astaxanthin is utilized on a large scale due to its functional attributes, easy availability and industrial exploitation in almost all the sectors (Algae Industry Magazine, 2016). As animal feed components/ ingredients, human antioxidants and for the colouring of shrimps, salmon, and trout, astaxanthin is predicted important growth over the following period (Grand View Research, 2016) (Fig. 12).

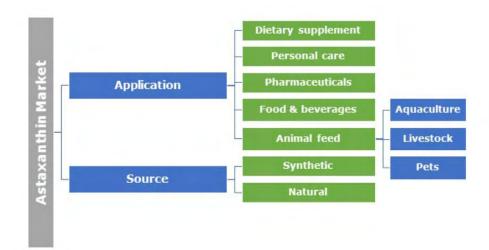


Figure 12: Astaxanthin Market, by application and by source (Global Market Insights, 2018).

"The global astaxanthin market size was more than 250 kilo tons in 2014, and is expected to reach 340 tons by 2020, and is likely to grow at a CAGR of over 7% from 2016 to 2023" (Algae Industry Magazine, 2016). Below, based on some reports about global carotenoids and global astaxanthin market, the following foreseen results are presented (Chart 3,4,5,6,7 Appendix B).

4.2.1 Trends of this market

4.2.1.1 Carotenoids

The market of carotenoids is driven by factors such as:

• increasing health consciousness among the customers/ and increasing consumer preference for natural products over synthetic products.

Dietary supplements are anticipated to reach significant acceptance of the consumers due to rising health consciousness and adapting a different lifestyle. Carotenoids are key additives which found application in human health supplements because of their benefits in immunity enhancement and richness in vitamins (Grand View Research, 2016). Some of the prominent trends that the market is witnessing include lucrative opportunities through development of high-value natural carotenoids and rising consumer interest towards naturally sourced products (Research and Markets, 2017).

• rising end-use applications of carotenoids

-The growth of the carotenoid market is attributed to their increasing applications in pharmaceuticals, cosmetics, food, and animal feed (Markets and Markets, 2016). These products act as antioxidants within the body supporting eye, skin, cardiovascular health and cancer prevention. Potent development of pharmaceutical and cosmetic industry in emerging countries such as China, India, and South Korea will generate additional opportunities at this market in the future. Furthermore,

GlaxoSmithKline, Pfizer, Sanofi and Novo Nordisk will drive the demand because they are consisted of some of larger pharmaceutical companies with strong presence in the industry area (Grand View Research, 2016).

- The demand of carotenoids in the animal feed sector is expected to introduce increase due to the industrialization of pork, poultry, and aquaculture business and the growing focus on the quality and appearance of meat and fish products (Algae Industry Magazine, 2016)

- Moreover, an extra factor is the population growth driving the demand for these applications. The growing middle-class population, educational development, and the rising disposable incomes have driven to increasing consumer awareness concerning the health benefits of dietary supplements (Grand View Research, 2016)

• The rising demand for functional and processed food owing to busy lifestyles is expected to positively impact market growth.

4.2.1.2 Astaxanthin - Growth Drivers

The astaxanthin market has been gaining importance due to:

- High demand due to its antioxidant properties and potential industrial applications are key agents affirming development of this market.
- Other prominent factors are estimated to promote growth is the rise in healthconscious population and alteration demand for natural food colouring agents without adverse effects by synthetical sources. People in developed countries are more focused on consuming food products derived from natural sources (Grand View Research, 2017)
- Increasing consumer requirements on personal care and cosmetic products along with rising awareness concerning nutraceuticals will drive the astaxanthin industry growth. Also, propelling demand for cosmetics with anti-ageing properties as other products by the reason of developing life expectancy (Global Market Insights, 2018).
- In addition, astaxanthin is gaining popularity and acceptability in numerous wellness spa service providers and cosmetics manufacturers owing to its skinfriendly and anti-oxidant properties (Global Market Insights, 2018)
- Regulatory policies over the use of synthetical compounds is also expected to fuel the growth of global astaxanthin market in the near future (Future Market Insights, 2019)
- Furthermore, the approval of astaxanthin by the U.S. FDA as generally recognized as safe (GRAS) for dietary supplements and approval from various

European food agencies has played crucial role in the growth of astaxanthin market (Shah *et al*, 2016)

 In addition, lack of raw material and inefficient advanced technologies are limited the market (Research and Markets, 2017). There is important limitation in this industry is raw material resource by the reason that as main raw material of natural astaxanthin, microalga (*Haematococcus pluvialis*) is easily interference by pests and diseases (<u>https://thestatetime.com/</u>).

4.2.2 Market segments

4.2.2.1 Carotenoids

The carotenoids market has been segmented, based:

 on type/ product, as Astaxanthin, Beta-carotene, Lutein, Lycopene, Canthaxanthin, Zeaxanthin, and Others (annatto, capsanthin, fucoxanthin, and trans-β-apo-8'-carotenal) (Markets and Markets, 2016).

Astaxanthin had been represented the largest market share in 2015, followed by betacarotene and lutein (<u>http://www.algaeindustrymagazine.com/</u>). The Astaxanthin segment dominated the carotenoids market, in terms of value, in 2016 (Markets and Markets, 2016).

• **on application,** the carotenoids market has been segmented into feed, food, supplements, cosmetics, and pharmaceuticals (Markets and Markets, 2016).

"Animal feed segment led the market with the largest share in 2015 and accounted for 41% of the overall revenue share" (Grand View Research, 2016) (Fig. 13).

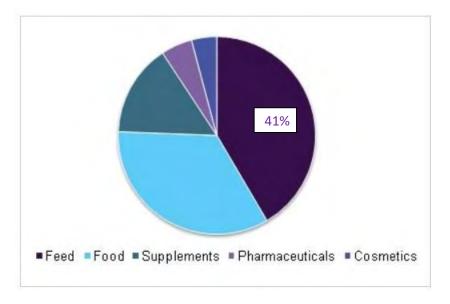


Figure 13: Global carotenoids market volume, by application, 2015 (%) (Grand View Research, 2016).

"The feed segment is estimated to account for the largest share in the carotenoids market in 2016" (Markets and Markets, 2016).

Based on application, the carotenoids market is led by the feed segment, followed by the supplements and food segments. Bodies of animals are not able to produce carotenoids as a result these nutrients are added to animal feed providing immunity against diseases (Grand View Research, 2016). Along with adding nutrients to the feed, carotenoids also increase the palatability of feed (http://www.algaeindustrymagazine.com/). All the above will drive growth at the market of these essential nutrients at the near future.

o by source

By source, market is segregated by synthetic and natural. "Synthetic sources dominated the overall industry valued at USD 903.6 million in 2015 and natural sources are expected to witness substantial revenue growth at a CAGR of 6.1% from 2016 to 2025" (Grand View Research, 2016)

\circ by region

The global carotenoids market is segmented region-wise, these regions include North America, Europe, Asia-Pacific, Latin America, and the Rest of the World (RoW) (Markets and Markets, 2016).

Caused by increase of demand for supplements and animal feed in Europe had been calculated to occupy the largest market share at the carotenoids globally, in terms of value, in 2016 and is anticipated to witness steady revenue growth at a CAGR of 3.8% from 2016 to 2025 (Grand View Research, 2016) (Figure 14). According to Markets and Markets, 2016 the leading markets for carotenoids in Europe are Germany, France, Italy, The Netherlands, and Spain. The leader's players at the cosmetic industry such as L'Oréal, Unilever, Beiersdorf, and Henkel is predicted to play a crucial role at the growth of carotenoids market in this region (Grand View Research, 2016).

North America had accounted the next one largest market share in 2015 and is anticipated to grow at the highest CAGR from 2016 to 2021 (Grand View Research, 2016). In addition, the strict norms are connected to the use of synthetic carotenoids laid down by the EU have driven to the natural form of carotenoids market (Markets and Markets, 2016).

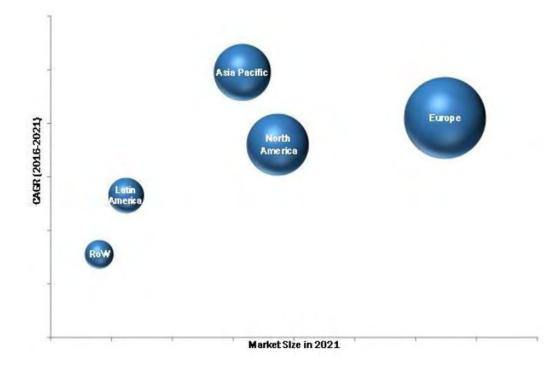


Figure 14: Carotenoids Market Size, By Region, 2021 (USD Million) (Markets and Markets, 2016). By Region: North America –22%, Europe – 56%, Asia-Pacific– 19%, RoW – 3% (Markets and Markets, 2016)

In the Asia-Pacific region is predicted to grow from several multinational manufacturers the carotenoids market. Moreover, extensive R&D initiatives have been undertaken in this region for exploring the applications of carotenoids (Markets and Markets, 2016).

Asia Pacific is expected to show high profits on account of growing usage and exploitation of carotenoids additives in feed, supplement, food, pharmaceutical and cosmetic applications. The sector is anticipated to be driven by technological advancements, industrial growth in a range of applications, economic growth and low costs of production with efficient way in countries such as China, Japan, and India (Grand View Research, 2016).

Key players recognised in the carotenoids market, see Appendix B § Key players in the carotenoids market.

4.2.2.2 Astaxanthin

By Source	-Natural (microalgae, yeast, krill/shrimp)				
	-Synthetic				
	-Dried algae meal or Biomass				
By Product	-Oil				
	-Softgel				
	-Liquid				
	-Others				
	-Nutraceuticals				
By Application	-Cosmetics				

 Table 10: Segmentation of the global astaxanthin market (Markets and Markets, 2018)

	-Aquaculture and animal feed			
	-Others			
	-North America (U.S., Canada)			
By Region	-Europe (UK, Germany)			
	-Asia Pacific (Japan, China, India)			
	-Central & South America (Brazil, Mexico)			
	-Middle East & Africa (South Africa)			

Global Astaxanthin Market can be segmented on the basis of:

o Source, as natural (microalgae, yeast, krill/shrimp) or synthetic.

Synthetic source will gain a major share in the market, with over 55% demand rate by the end of 2024 (Global Market Insights, 2018). Natural sources are appreciated to witness exponential CAGR of over 24.5% over the following period, 2018-2025 (Grand View Research, 2017).

The fundamental growth at astaxanthin from natural sources is attributed to its its health benefits for human and animals along with the great preference over the synthetic counterpart. Some of aquaculture companies are also supplying natural forms, even at a higher price (Global Market Insights, 2018). The primary market of synthetic astaxanthin from petroleum is used as animal feed while in contrast the natural form is majorly utilized for human uses and consumption. Nevertheless, the demand for natural astaxanthin in animal nutrition has been noticing significant increase in recent years due to a number of safety issues and concerns which associated with the consumption of animal meat treated with petroleum-based astaxanthin (Grand View Research, 2017).

In addition, astaxanthin from natural sources provides better pigmentation for some species of fishes instead of synthetic. All these factors will show changes at this market in its proliferating sales, further strengthening penetration at this area (Global Market Insights, 2018).

 Product, as dried algae meal or biomass, oil, softgel, liquid and others (Grand View Research, 2017).

Dried algae are expected to dominate the market principally as animal feed due to not extra procedures and low cost of the production. This sector had held a revenue share of 25.9% of the overall market in 2016 (Grand View Research, 2017).

The segment of soft gels is projected to witness CAGR of over 19.5% over the next period. Serious agents that can be ascribed to this development are increased use of soft gels in nutraceutical due to ease of administration and easy availability in a variety of doses according to the needs (Grand View Research, 2017).

Liquid astaxanthin is also increasingly looking for use as a food coloration factor because of its easily miscible nature and low negative effects (Grand View Research, 2017).

\circ Application

Global Astaxanthin Market can be further segmented based on application (Transparency Market Research, 2018). Based on application can be segmented into dietary supplements, food & beverages, cosmetics, aquaculture and animal feed (Future Market Insights, 2019).

The major market share of astaxanthin is find applications in aquaculture and in animal feed industry (Transparency Market Research, 2018). Increasing government support for promoting aquaculture is further reinforcing the product demand as a colorant pigment (Global Market Insights, 2018). Also, astaxanthin market from animal feed will dominate the industry, in possession of share of the global demand over 40% (Global Market Insights, 2018). In the U.S., the segment held a 39.6% share in 2016 (Grand View Research, 2017) (Figure 15).

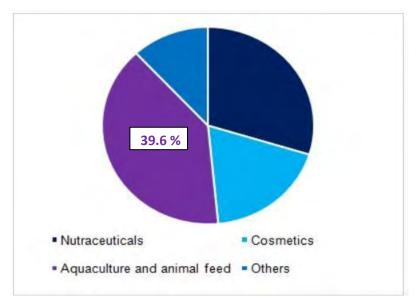


Figure 15: Global astaxanthin market, by application, 2016 (%) (Grand View Research, 2017).

The application of astaxanthin in the dietary supplements industry accounted for the second-largest share in 2016 (Grand View Research, 2016). The numerous health benefits, such as cardiovascular and neurological health, provided by the antioxidant functions of astaxanthin, coupled with the increasing demand for nutraceuticals, are the crucial agents contributing to the exponential growth of dietary supplements segment (PRNewswire, 2017).

A desk research¹, at Dietary Supplement Label Database (DSLD), was conducted for the purpose of this thesis, focusing on the dietary supplements which contain astaxanthin and other synergistic substances. Results indicated that:

- 68 products which contain 'astaxanthin' in the product name, with the following health claims: Supports joint and tendon health -Supports skin health during UV and sun exposure -Supports eye health -Supports anti-aging through cellular health -Supports healthy immune function -Supports cardiovascular health -Supports the body in recovery from exercise
- 598 products which contain 'astaxanthin' anywhere on the label, and some of them with omega-3 fatty acids from krill oil and virgin salmon oil. These dietary supplements function synergistic with other substances and have the following product names like as: Carotenoid Complex, OmegaXanthin Blend, Manganese, Super Antioxidant Blend, Krill Oil Blend, Astaxanthin Phospholipid Proprietary Blend/ natural Astaxanthin, Wild Alaskan Salmon Oil and Haematococcus pluvialis Algae extract.

Astaxanthin is also used in several nutraceuticals products, due to its properties as a result this segment is calculated to witness the most profitable growth over the next period between 2018-2025 (Transparency Market, 2018); (Grand View Research, 2017).

In food and beverages, demand of astaxanthin is used to cover some specific proteins from natural sources and forms (Transparency Market, 2018). Moreover, the approvals granted by international food agencies, including Canadian Food Inspection Agency and European Food Safety Agency will support and promote the industry growth (Global Market Insights, 2018).

Astaxanthin also finds its application in cosmetics & personal care products (Transparency Market, 2018). From bibliography astaxanthin has been reported for its high antioxidant and skin-friendly properties as a result it finds applications in numerous cosmeceutical manufacturers and wellness spa owners are using it in new products and associated therapies. Last but not least, increasing geriatric population, prosperity of cosmetic industry, and the alteration to natural cosmeceuticals are factors appreciate to expedite growth of this market segment (Grand View Research, 2017).

¹ The Dietary Supplement Label Database (DSLD) includes full label derived information from dietary supplement products marketed in the U.S. with a Web-based user interface that provides ready access to label information. Key words that are used: astaxanthin (product name, ingredient name, brand name, manufacturer)

Market Sectors	Market Size (as of 2009) (Million USD)	Potential Market (2020) (Million USD)	
Animal feed coloring agents	300	800	
Antioxidant neutraceuticals	30	300	
Pharmaceuticals	Emerging	500	
Cosmetics	Emerging	30	

Figure 16: Presentation on industrial training (plant Biotechnology – Algae) (Google, accessed 16/5/2018)

o Region

By Region - North America-25%, Europe-50%, and Asia-Pacific- 25% (Transparency Market Research, 2018).

Geographically, global astaxanthin market can be divided into the following regions: North America, Eastern Europe, Asia Pacific, Japan, Latin America, Western Europe and Middle East and Africa (Future Market Insights, 2019). Globally, among all of these previous regions, North America is leader in the commercial production of astaxanthin, followed by Asia Pacific and both of them are anticipated to be the most potential markets for Astaxanthin manufactures (Grand View Research, 2017). Other countries which will contributes at the total production of astaxanthin globally is India, Japan and Israel (Transparency Market Research, 2018)

The Asia-Pacific region was the largest regional market for astaxanthin in 2016 including China, India and Australia-New Zealand (PRNewswire, 2017). There are several factors that will play vital role in the high astaxanthin growth of value over the next period like:

- growth in pharmaceutical industry, especially in developing countries such as China and India (Coherent Market Insights, 2018). Attendance of key manufacturers like BGG and InnoBio in China, is a noteworthy agent for the potential growth at this region (Grand View Research, 2017). According to IBEF, "US\$ 55 billion will be generated by the Indian pharmaceuticals industry by 2026" (Coherent Market Insights, 2018).

- increasing awareness about natural astaxanthin and a flourishing cosmeceutical industry from natural products (Grand View Research, 2017). Countries such as India, Singapore, and Malaysia are key regions in Asia Pacific for active wellness tourism

and leisure activities. Demand natural oils and cosmetics in spa and wellness centres due to low concerns are some other key factors asserting growth from natural products (Grand View Research, 2017).

-demand for animal feed application across the region (Global Market Insights, 2018).

North America held a substantial market share of 46.5% in 2016 (Grand View Research, 2017). The North America market will expose increase because of extremely large demand from the dietary supplement industry coupled with growing awareness about health benefits (Global Market Insights, 2018). Last but not least, presence of organizations such as Natural Algae Astaxanthin Association (NAXA) is evaluated to the potential growth the regional market share (Grand View Research, 2017).

The market in Europe is the key market for Astaxanthin due to the varied health benefits and its demand for uses in animal feed (Markets and Markets, 2016).

Astaxanthin market is oligopolistic in nature and is dominated by several **key players**, see Appendix B, § Key players of the market of astaxanthin.

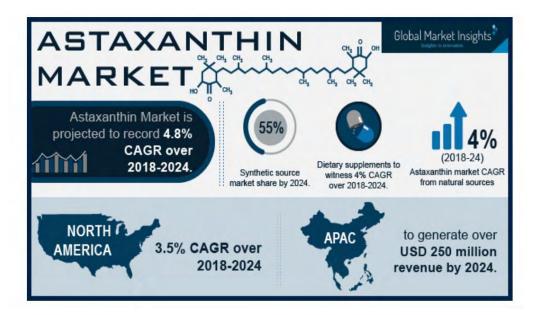


Figure 17: A schematic of astaxanthin market by the report Global Market Insights, 2018 (Global Market Insights, 2018)

4.3 Production of natural astaxanthin

From 2011 to 2016, "global natural astaxanthin production increased from 43279 Kg to 73717 Kg, with an average increase rate of 11.24%". China, Europe, North America and Asia are some of the global production regions of natural astaxanthin (PR Newswire, 2017).

In 2015, "Europe produced about 9.20% of global total natural astaxanthin. North America produced 46959 Kg, accounting for 67.12% in 2015" (PR Newswire, 2017). The largest volume of Haematococcus pluvialis is appreciated to have consumed from the global nutraceutical industry in 2017 accounting for 54.8% and reaching 190 metric tons by 2024 (PR Newswire, 2018). Despite this, "the emerging market of Food & Beverages for H. pluvialis microalgae astaxanthin consumption will witness the fastest CAGR between the next period, 2017 and 2024" (Industry Experts, 2018); (Research and Markets, 2018)

Nowadays, the estimated market value of astaxanthin depending on products' purity varies from $2200-6620 \notin$ kg to about $13.240 \notin$ kg pigment from *H. pluvialis* in some cases, while the production cost is estimated at about 880 \notin per kg of astaxanthin from *H. pluvialis* (Shah *et al.*, 2016)

4.4 Target Market

VALUEMAG both seeks and welcomes opportunities to collaborate with industry and research groups. Key areas of interest include providing ingredients/ raw materials for human and animal health and nutrition, finished products for aquaculture and agriculture, and technology for environmental remediation.

Firstly, target audience is distributors and further:

- Astaxanthin manufacturers
- Food ingredients manufacturers
- Astaxanthin importers and exporters
- Astaxanthin traders, distributors, and suppliers
- Astaxanthin raw material suppliers
- Astaxanthin end-product manufacturers

• Associations and industry bodies such as the Food and Drug Administration (FDA), the European Food Safety Authority (EFSA), Natural Algae Astaxanthin Association, and other food & safety associations.

5. THE COMPETITION

5.1 Direct Competitors

Although downstream demand for natural astaxanthin is too much, there are only several major suppliers in this industry.

- The major players covered in Global Natural Astaxanthin Market report -Cyanotech, Fuji, Parry Nutraceuticals, Algatechnologies, Biogenic, Jingzhou Natural Astaxanthin, Igene, Yunnan Alphy Biotech, ADM, BGG, Piveg.
- Main Product Types covered in Natural Astaxanthin industry Astaxanthin Oleoresin, Astaxanthin Powder.
- Applications covered in Natural Astaxanthin industry Nutraceuticals, Cosmetics, Food & Beverages, Feed (Research Reports Inc. 2018).

Algatech, Cyanotech and Fuji are the three leading astaxanthin producers have combined forces and established the Natural Astaxanthin Association **(NAXA)** for the purpose of imparting awareness regarding the differences between the natural and synthetic forms of astaxanthin (Industry Experts, 2015); (<u>https://astaxanthin.org/</u>). Below are given the new entrants in the production of astaxanthin from *H. pluvialis*.

Table 11: New Entrants in the H. pluvialis Astaxanthin Sector (Industry Experts, 2015).

Company	Country
Algae Health (Slainte Alga Teoranta)	Ireland
Algae to Omega Holdings Inc.	United States
Algaetech International Sdn Bhd	Malaysia
ALGALO Industries Company Ltd.	Israel
Andexs Biotechnology SRL	Peru
Astaxa GmbH	Germany
Avoca, Inc.	United States
Synthetic Genomics Inc.	United States
Garden State bioEnterprises, LLC (GS bioE)	United States
Guangdong Hairong Environmental Protection Technology Co., Ltd	China
Heliae Development LLC and SINCERE Corporation	Japan
Maui Tropical Algae Farm, LLC (MTAF)	United States
MBD Energy Limited	Australia
MicroA AS	Norway
Tianjin Norland Biotech Co. Ltd	China

Below at Table 12, Appendix C is given an extensive research of the leading commercial companies and their *H. pluvialis* - derived astaxanthin with the culture

system which they use, the official company name, the country, the brand name at the market, the forms of bulking and the application area.

In addition, for each one of the direct competitors, are presented information about their entity, the market segments they are active in, their product/services and business model. Regarding entities, are mentioned the maturity and type of their business, their business model. Regarding market segments, are mentioned their market position (leader, large, niche, small) (Appendix D, § *Company Profiles*).

After the investigation of the other competitors and as shown in Table 9, the culture systems of astaxanthin which are exist on the market are mainly focused mainly on the common/ordinary cultivation systems. It has been noticed that neither company has so far exploited the effect of magnetic cultivation which Valuemag proposed.

In addition, it was observed that on global market, there are various market players with leader position, experience and dynamic presence in the field of algae production; and especially at the production of astaxanthin from *H. pluvialis*. These players are likely to increase competition.

Owning to its unique way of cultivation and the innovations of the project, Valuemag permit optimal cultivation, enhance biomass productivity and dramatically lower costs of biomass production. So, that bringing natural astaxanthin from *H. pluvialis* very close to the market, with environmentally and socially sustainable issues.

5.2 Indirect competitors

Below are given the alternative natural sources, from which can be produced astaxanthin, and the top market players of them.

Commercial Astaxanthin Production	Sources	Company Name	Country
	Yeast (Xanthophyllomyces dendrorhous*/Phaffia	Igene Biotechnology, Inc.	USA
	rhodozyma)	Archer Daniels Midland (ADM)	USA
Astaxanthin Production from Natural Sources	Bacteria (Paracoccus carotinifaciens and	JX Nippon Oil & Energy Corporation	Japan
	Lactobacillus sp.)	Guangzhou UDA Biotech Development Co., Ltd	China
	Shrimp Shells	BlueOcean NutraSciences Inc	USA

Table 13: Astaxanthin Production from natural sources and leading commercial companies

5.2.1 Microalgae source of Astaxanthin, compared with other natural sources

At this time, the primary source of astaxanthin and carotenoids are synthetic forms produced by chemical means. There are also natural sources available from processed krill, crawfish and a yeast called *Phaffia* (https://www.cyanotech.com/). At these other natural sources contain relatively low astaxanthin concentration, from 0.3mg/100g. in crawfish and lobster to 800mg/100g. in *Phaffia Yeast*, as we can at Table 14. The competitive advantage and the benefits of using an algae source of astaxanthin is that the other natural forms like the krill, crawfish and *Phaffia* sources contain low concentrations of astaxanthin (Ambati *et al.*, 2014).

Source	Average Astaxanthin Content
Lobster, crawfish	0.3mg/ 100g.
Salmon	3mg/ 100g.
Krill	4mg/ 100g.
Phaffia Yeast	800mg/ 100g.
Heamatococcus Pluvialis Microlagae	6,000mg / 100g.

Table 14: Sources of natural astaxanthin with the average content (<u>http://www.fujichemical.co.jp/english/)</u>

Also, feeds may require so much addition of these products for effective pigmentation that it adds unwanted bulk and ash, decreases palatability, and alters the nutrient balance of the diet (<u>https://www.cyanotech.com/</u>). The astaxanthin derived from *Haematococcus* algae is the most concentrated source of natural astaxanthin that is available (Shah *et al.*, 2016).

5.2.2 Natural Astaxanthin, compared with Synthetic

The companies that are active in synthetic astaxanthin are: BASF SE (Germany), Divis Laboratories Limited (India), Royal DSM NV (The Netherlands), Zhejiang Medicine Co., Ltd. (China), Zhejiang NHU Co., Ltd. (China) (Research and Markets, 2018) (Industry Experts, 2018) (Table 15).

Commercial Astaxanthin Production	Sources	Company Name	Country
		DSM (Roche) Synthesis Process	Netherlands
Synthetic Astaxanthin Production	Synthetic sources	BASF Synthesis Process	Germany
		Zhejiang NHU Synthesis Process	China

Table 15: Synthetic Astaxanthin production and leading commercial companies

Synthetic astaxanthin has 20 times lower antioxidant capacity than its natural counterpart and to date has not been approved for human consumption (Lorenz and Cysewski, 2000); (Koller *et al.*, 2014). Additionally, about the safety of using synthetic astaxanthin for direct human consumption there are concerns and uncertainties due to both different stereochemistry and possible carryover of synthesis intermediates (Shah *et al.*, 2016).

According to market research of Grand View Research at 2017, "the market for synthetically produced astaxanthin is calculated to be impacted with negative way by the increasing demand for the natural astaxanthin counterpack". Moreover, astaxanthin which is originated from chemical synthesis is also expected to decrease the market share of this segment due to the requirement of high capital and labour. As a result, these reasons make natural astaxanthin a preferred choice for high-end markets with competitive advantage from synthetic one.

5.2.3 Other Indirect competitors

In addition, there are other indirect competitors of astaxanthin on the market that have to do with the potential uses of this compound. For example, there are other substances that are used as colourant in food industries or in aquaculture. Also based on astaxanthin antioxidant, anti-inflammatory and anti-aging functions there are other bioactive compounds that can be incorporated into nutritional formulations, dietary supplements, nutraceuticals and cosmetics. All of the above factors may be escalated the competition of astaxanthin.

5.3 SWOT ANALYSIS

SWOT analysis is a framework used to estimate a company's or product (raw material) competitive position and to improve strategic planning. Recognizing core strengths, weaknesses, opportunities and threats based on fact analysis, new prospects and fresh ideas. SWOT analysis evaluates internal and external agents, as well as current and future potential (https://www.investopedia.com/). See, Appendix C.



6. THE BUSINESS MODEL

The cultivation and production of microalgae will take place either in Cyprus or in Greece with the new magnetic cultivation system and with a genuine ecological crop. The company will be responsible to undertake the Quality Control department and will proceed with the acquisition of all relevant quality certificates. Our products will meet the most rigorous global quality and safety standards and will have all the appropriate regulatory approvals (self-affirmed GRAS and produced in a certified facility, Non-GMO project verified, USDA Organic).

Finally, our main concern will be to find distributors in North America, Europe and Asia to sell the astaxanthin as raw material, in bulking. At the first two years Qualia is aimed to find distributors in Europe. Afterwards, at the 3rd year in North America and 4rd and 5rd year at the Rest of the World, as we explain at the following sector (§7. Funding's & Revenues, *market share*).

Group of Companies of Qualia has a broad and comprehensive coverage of all areas of the modern Health Care Sector and build collaborations to foster innovation with Research Institutes and Universities. Qualia Pharma was founded in 2009, focusing exclusively in the Health Care sector and providing a broad range of added value products in Pharmaceuticals, Food Supplements, Medical Devices, Cell therapies and Genetics (<u>http://www.qualiapharma.com/</u>).

Given the presence at the sectors of pharmaceuticals and supplements Qualia has already suppliers at these sectors formulations, so that they will be the potential distributors at the beginning dissemination of astaxanthin. These suppliers from different European countries will be the firstly contact for the sale of astaxanthin as a raw material with a range of industrial applications.

7. THE SCHEDULE

The schedule of this work plan has been split up into six (6) work packages. A short description of each work package follows below:

- ✤ WP1- Magnetic algae preparation
- ✤ WP2 Magnetic cultivation of Algae
- WP3 Animal Food, Nutraceutical & Cosmetics from Algae
- WP4 –Operation and Assessment
- WP5 Exploitation and Business Development
- WP6 Project Management & Dissemination

Chart 8, (Appendix C: Gantt chart) is a Gantt chart showing the project 's timeline. Management and disseminations actions are grouped within WP6 while exploitation activities are considered within WP5. The WP1, WP2, WP3, WP4 deal with interconnected scientific and technological activities in order to demonstrate the system.

The completion of the project is calculated at the end of March of 2020. Then Qualia will find distributors and will start the dissemination and selling of astaxanthin to their future potential distributors globally. According to this state we begin our assumptions at our estimations of funding's and revenues from the 2020, see § Funding's and Revenues.

8.1 Market estimation

As we referred at the sector of Business Model, Qualia S.A. main concern will be to find distributors to sell the astaxanthin as raw material, in bulking. In order to calculated estimated earnings, we are depended on some assumptions.

Firstly, owing to high price of natural astaxanthin, global consumption regions are mainly distributed in Europe, North America and China. In 2017, North America consumed about 25724 Kg. Followed by Europe, with a consumption amount of 15032 Kg (Market Watch, 2019). These data give us the base facts to forecast the market for the first five years of the new raw material product presence. The business model refers to 3 segments markets which is Europe, North America and the Rest of the World.

Also, according to Research Reports Inc. (2018), from 2013 to 2017, global natural astaxanthin production increased with an average increase rate of 11.23%, we suppose that we will have the same average increase rate in consumption of global natural astaxanthin. Based on these figurative, we are relied to calculate the market as presented in the following table.

Region (kg)	2020	2021	2022	2023	2024
Europe	20.685	23.007	25.590	28.463	31.659
North America	35.398	39.373	43.794	48.712	54.182
Rest of the World	20.217	22.487	25.012	27.820	30.944
Total	76.300	84.867	94.396	104.995	116.785

Table 17: Market of natural astaxanthin in kilos by region

8.2 Market Share

Based on our competitive advantage which has to do with the final cost of astaxanthin as a raw material, we expect to gain a fairly market share of distributors, particular from 5% - 40% depending on the market as presented in the following table. We suppose that in the first three years there will be an upward increase in market share because we will be more competitive with our price and we expect to reach double rising of the market share between 2022 and 2023, then a more conservative increase of 5% per year.

At the first two years we are aimed to find distributors in Europe. Afterwards, at the 3rd year in North America and 4rd and 5rd year at the Rest of the World. In addition, we

are developed at 2 scenarios Business Plan (Normal Case, and Worst case) in order to analyse all possibilities and asses the viability of this concept.

Normal Case Scenario ²	2020	2021	2022	2023	2024
Europe	5%	15%	30%	35%	40%
North America			5%	15%	30%
Rest of the World				5%	15%

 Table 18: Normal Scenario - Market share of natural astaxanthin with the technology Valuemag

Table 19: Worst Scenario - Market share of natural astaxanthin with the technology Valuemag

Worst Case Scenario ³	2020	2021	2022	2023	2024
Europe	3%	5%	7%	10%	12%
North America			3%	5%	7%
Rest of the World				3%	5%

8.3 Pricing

There is a wide range in the pricing of natural astaxanthin and it depends of the cultivation method and the extraction techniques. Due to the absence of data about the current market prices of natural astaxanthin per kilos across countries either the rate of reduction of astaxanthin price, we depend on different **content/composition of astaxanthin (1%, 5%, 10% and 97%)** and their price that exist at the current market globally, see Table 19. Our technology offering an efficient and a cost-effective way of the production and selling of astaxanthin as a result on this case of a fast market penetration strategy, the price should be lower from the current one by common cultivation techniques (such as open ponds, horizontal tubular photobioreactors and flat panel photobioreactors).

In this case, based on the assumption that the innovative cultivation method proposed by VALUEMAG consortium will allow to further reduce the cost (~30%) of biomass production with respect the tubular or flat panel photobioreactors, as shown following. A floor price can be set by content of astaxanthin meeting the above criteria, as shown in the next table.

² Normal Case Scenario: Astaxanthin as a raw material producing from innovative Valuemag magnetic technique achieved the acceptance from the distributors.

³ Worst Case Scenario: penetration of other distributors with a cost-effective way and with highest purity of astaxanthin or we failed to find distributors and let them know our innovative technology

Current Market Prices		Proposed Price with Valuemag Technology		
Content % of Astaxanthin	Price/kg (€)	Content % of Astaxanthin	Price/kg (€)	
1%	90	1%	63	
5%	675	5%	472,5	
10%	1.125	10%	787,5	
97%	2.560	97%	1.792	

Table 20: Current market price of astaxanthin and expected price with proposed magnetic cultivation.

8.4 Profit and Loss forecast

Based on the expected market share of distributors in the normal and worst scenario it is calculated with the number of consumptions in kilos in each region and are multiplied by the proposed price with Valuemag technology. Also, we analyse two options of them, in a low content (5%) and a high (97%) of astaxanthin.

The following is the projected Profit and Loss table.

Revenues (Euro) = Consumption/ Demand X Market Share X Proposed Price

	Normal Case Scenario					
Market (powder specification 5%)	Year 1	Year 2	Year 3	Year 4	Year 5	
Europe	488.683	1.630.621	3.627.382	4.707.068	5.983.551	
North America	0	0	1.034.633	3.452.463	7.680.298	
Rest of the World	0	0	0	657.247	2.193.156	
Total	488.683	1.630.621	4.662.015	8.816.778	15.857.005	
		Worst Case Sco	enario			
Market (powder specification 5%)	Year 1	Year 2	Year 3	Year 4	Year 5	
Europe	293.209	543.54	846.389	1.344.876	1.795.065	
North America	0	0	620.779	1.150.821	1.792.069	
Rest of the World	0	0	0	394.348	731.052	
Total	293.209	543.54	1.467.168	2.890.045	4.318.186	

Table 21: astaxanthin with content 5%, normal scenario and worst scenario

	Normal Case Scenario				
Market (powder specification 97%)	Year 1	Year 2	Year 3	Year 4	Year 5
Europe	1.853.376	6.184.281	13.757.184	17.851.993	22.693.171
North America	0	0	3.923.942	13.093.785	29.128.243
Rest of the World	0	0	0	2.492.672	8.317.747
Total	1.853.376	6.184.281	17.681.126	33.438.450	60.139.161
Worst Case Scenario					
Market (powder specification 97%)	Year 1	Year 2	Year 3	Year 4	Year 5
Europe	1.112.025	2.061.427	3.210.009	5.100.569	6.807.951
North America	0	0	2.354.365	4.364.595	6.796.590
Rest of the World	0	0	0	1.495.603	2.772.582
Total	1.112.025	2.061.427	5.564.374	10.960.767	16.377.123

Table 22: astaxanthin with content 97%, normal scenario and worst scenario

The following is the projected Profit and Loss table containing the total production costs and total operating costs including also increased promotional and administration cost, see Table 23 Appendix D: Profit and Loss forecast. Cost of Goods which represents the cost of raw material, packaging and manufacturing can be further decrease as the quantities produced are higher.

It is remarkable that the astaxanthin as raw material is profitable since the first year after launch, see Table 24, 25, 26, 27, 28 Appendix D: Profit and Loss forecast. A similar P&L analysis was performed for the Normal and Worst scenario for the two options in composition of astaxanthin, low (5%) and high (97%). Even in the worst-case scenario the business remains profitable.

9. CONCLUSION - DISCUSSION

One of the major drawbacks in microalgae production systems is the high costs of cultivation, harvesting and infrastructure. Moreover, the exploitation and dissemination of abundant bioactive secondary metabolites that can be extracted from microalgae should be faced. In order to overcome these obstacles for the viability of microalgae, additional advances are necessary to develop economic and efficient microalgae production systems for the feasible exploitation of their bioactive molecules.

The present study was aimed at presenting the innovative Valuemag project. This project was based on these facts and in the gap, which exists in the algae production and cultivation systems at the current market. In more details, VALUEMAG project scope to provide revolutionary solutions for microalgae production and harvesting as well as scaling up exploitation of algae valuable extracted compounds. Production, objectives are achieved cultivation and harvesting by using magnetic nanotechnologies as a result these innovations permit optimum cultivation, enhance biomass productivity and dramatically lower costs of biomass production. Biomass and extracted bioactive compounds from microalgae are directly utilized by VALUEMAG multi-facilities bio-refinery for the production molecules in a wide range of industrial applications like pharmaceutical, nutraceuticals, food additives and cosmetics (https://www.valuemag.eu/).

After research of some bioactive compounds that can be extracted from microalgae, carotenoids and more specifically astaxanthin present considerable interest in comparison with the other extracted compounds. Astaxanthin, a xanthophyll carotenoid, is a secondary metabolite synthesized by a number of natural sources (bacteria, microalgae, yeasts and others) or synthetic sources and is represent a valuable compound with wide range of industrial applications. There are several microalgae strains that are suggested as potential sources to produce astaxanthin. Among them *H. pluvialis* can accumulate the maximum content of this antioxidant compound in stressfully environmental conditions.

Since it is a compound with high nutritional value, strong demand at the global market, great potential in the market and high market value (2200-6200 (kg)), in depth investigation of *H. pluvialis* efficient culture systems and production of added value final products are highly required for further development of this sector. Therefore, Valuemag design to overcome all of these barriers. Its purpose is to develop a natural raw material of astaxanthin with an efficient way; and with the feasible recovery and purity of this pigment from the strain *Haematococcus pluvialis*. Due to the innovative technology that proposed and that astaxanthin will offer a variety of applications in

pharma, cosmetics, foods and beverages, it was considered to be a fairly competitive raw material compared to the existing ones. That is why it was necessary to assess what its prospect will be in the global market and a feasibility study was done.

Firstly, a market analysis was carried out on the global algae market further at global carotenoids market and finally at global natural astaxanthin market. It was found to be highly developed with many prospects based on studies showing an increase in CAGR% over the coming years. However, following an investigation into existing competition, it has been found that due to the disadvantages of existing cultivation and exploitation methods of algae, the magnetic cultivation system of Valuemag is able to fill the gap in the market globally. In addition, when it comes to target market, distribution channels and promotion, some suggested actions have been mentioned.

Subsequently, the cost of production of the proposed method was calculated, including staff expenses, operating costs, techniques and raw material costs. Certain assumptions were made to it is possible to calculate the necessary funding's and the expected inflows and outflows that the company will expect in each year. Taking into account all elements of the financial plan, it is estimated that the company will present profitability from the first year of production either in the normal scenario (astaxanthin achieved the acceptance from the distributors) either at the worst scenario (penetration of others distributors or failure to find distributors and dissemination of the innovative magnetic technology). As the project has not yet been finished, we are not be able absolute sure about these final estimations at funding's and revenues.

In conclusion, the incredible potential of microalgae could make significant possibilities in the industrial world in the forthcoming years. Natural astaxanthin production which derived by the strain of *Haematococcus pluvialis* is an appealing option from a business point of view with competitive advantage in comparison to the synthetic astaxanthin with the proposed magnetic cultivation system of Valuemag. At the end, eventual domination of natural astaxanthin from the synthetic one will offer expansion in the applications in the pharmaceutical, nutraceuticals, food additives and cosmetics sector as well.

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- http://www.fujichemical.co.jp/english/
- + https://www.valuemag.eu/about/
- https://astaxanthin.org/
- https://www.investopedia.com/
- + https://www.cyanotech.com/
- http://www.parrynutraceuticals.com/
- https://examine.com/
- https://www.algatech.com/
- <u>https://heliaeglobal.com/news/</u>
- <u>http://www.atacamabionatural.com/</u>

APPENDICES

Appendix A: Tables

Table 2: List of participants (https://www.valuemag.eu/)

	National Technical University Of Athens Greece	PNO	PNO Innovation The Netherlands www.pnoconsultants.com
Università degli Stati della Campania Luigi Varwitelli	Università degli Studi della Campania "L. Vanvitelli" Italy www.universita.it	Ficial	Iris – advanced Engineering Spain www.iris-eng.com
EREE Dester verste state	ENEA – Agenzia nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile Italy www.eneait	Exergy Engineering that aspires	EXERGY LTD. United Kingdom www.exergy.uk.com
NomaSico	NomaSico Cyprus www.nomasico.com	VERTECH	Vertech Group France
theracell	Theracell Greece www.theracell.eu	eceduna	Ecoduna Austria www.ecoduna.com
E	Institute of Phisics, Slovak Academy of Science Slovakia www.fusav.sk		

Table 3: Microalgae products (García et al., 2017).

<u>Products</u>	ucts (García et al., 2017). <u>Microalgae</u>	
Food	Chlorella, Spirulina,Odontella auriata, Tetraselmis chuii Aphanizomenon flosaquae, Nostoc, A. sacrum, Spirogyra, Oedogonium	
Feed	Chlorella, Spirulina, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema, Thalassiosira	
Vitamins & carotenoids		
Vitamin B12	Chlorella, Spirulina	
Vitamin E	Porphyridum cruentum	
β-carotene	D. salina, Haematococcus pluvialis, Synechococcus, Nanocloropsis gaditana	
α-carotene	Chlorella	
Astaxanthin	D. salina, H. pluvialis, Chlorella	
Lutein	Murellopsis sp., Chlorella, Scenedesmus almeriensis, Auxenochlorella protothecoides	
Zeaxanthin	D. salina, Chlorella, Synechococcus, N. gaditana	
Canthaxanthin	Scenedesmus komarekii, D. salina, Chlorella	
Fucoxanthin	Phaeodactylum tricornutum	
Phytoene	D. salina	
Phytofluene	D. salina	
Violaxanthin	Chlorella, Synechococcus, N. gaditana	
Antheraxanthin	Chlorella	
Echinenone	Botrycoccus braunii	
Cryptoxanthin	D. salina	
Phycobiliproteins		
Phycocyanin	Spirulina	
Phycoerythrin	Porphyridium	
Allophycocyanin	Spirulina	
Chlorophyll		
Chlorophyll A	A. flosaquae	
PUFAs		
Eicosatetraenoic acid (EPA)	Phaeodactylum tricornutum, Monodus subterraneus, P. cruentum, Chaetoceros calcitrans, Nannochloropsis,Schizochytrium	
Docosahexaenoic acid (DHA)	Crypthecodinium cohnii, Isochrysis galbana, Pavlova salina, Schrizochytrium	
Linoleic acid	D. salina	
y-linolenic acid	Spirulina	
Oleic acid	D. salina, Spirulina	
Lauric acid	Spirulina	

Porphiridium spp.
Nostoc flagelliforme
D. salina, Dunaliella tertiolecta
Chlorella, Nostoc, Anabaena, Tolypothrix, Chlamydomonas
D. salina
D. salina
D. salina
D. salina
Synechocystis sp.
Spirulina
Chlorella, chlamydomonas
Nostoc, Ulkenia
Chlorella

Table 4: Selected microalgae-derived bioactive compounds: their sources and function (Islam et al., 2017).

Bioactive compounds		Microalga source	<u>Function</u>
Pigments	Lutein	Muriellopssis sp. Scenedesmus almeriensis Chlorella sp.	Prevention of cancer, protection from macular degeneration and cognitive impairment
	Astaxanthin	Chlorella zofigiensis Dunaliella salina Haematococcus pluvialis	Antioxidant and anti-inflammatory properties, effective against cancer, protein degradation, Parkinson's disease, reduced vision, rheumatoid arthritis
	β-carotene	Nannochloropsis Gaditana Dumaliella salina Haematococcus pluvialis	Prevention of breast cancer and macular degeneration
PUFAs	EPA	Phaeodactylum Tricornutum Nannochloropsis sp. Monodus subterraneus	Neural development, prevention of cardiovascular disease, lessen and protect against COPD, asthma, rheumatoid arthritis, atherosclerosis, Crohn's disease and cystic fibrosis.
	DHA	Cryptheocodinium cohnii Pavlova salina Isochrysis galbana	Facilitate child and infant development
	Linolenic acid	Dunaliella salina Spirulina platensis	

Proteins	Phycobiliproteins, Hormone-like bioactive peptides, 2- 20, and essential amino acids, etc.	Haematococcus pluvialis Chlamydomonas Reinharditi Chlorella sp. Spirulina sp. Scenedesmus sp. Dunaliella sp. Oscillatoria sp. Chlamydomonas sp.	Anticancer, immunomodulation, Hepatoprotective, anti-inflammatory, Antioxidant, antimicrobial properties, protection of DNA Production of recombinant proteins, e.g., for type 1 diabetes detection, etc.
Vitamins	Cobalamin (vitamin B12) Tocopherols (vitamin E) Thiamin (vitamin B1) Nicotinic acid (vitamin B3) Ascorbic acid (vitamin C) Biotin (vitamin B7)	Dunaliella tertiolecta Chlorella sp. Sprirulina sp. Dunaliella tertiolecta Porphyridium cruentum Tetraselmis suecica Tetraselmis suecica Tetraselmis suecica Chlorella sp.	Antioxidant activities, role in body function, immunity, digestive system, etc.
Polysaccharides	Sulfated polysaccharides Insoluble fiber and others	Porphyridium sp. Spirulina platensis Phaeodactylum sp. Chlorella stigmatophora Gyrodinium impudicum	Antiviral, antioxidant, antitumor, anti-inflammatory, antihyperlipidemic and anticoagulant activities
Phenolic compounds	Heptadecane, Tetradecane, etc.	Nitzschia laevis Nostoc Ellipsosporum Nostoc Piscinale Chlorella Protothecoides Synechococcus sp. Chlorella Vulgaris Anabaena cylindrica Tolypothrix tenuis Chlorella pyrenoidosa Crypthecodinium cohnii Chlamydomonas nivalis	Anticancer, antidiabetic action, lessen the risks of cardiovascular and neurodegenerative diseases, protection from biotic and abiotic stress
Phytosterols	Brassica sterol, sitosterol, stigmasterol, etc.	Dunaliella tertilecta Dunaliella salina	Cholesterol-lowering and neuromodulator activities, flights nervous system anomalies, autoimmune encephalomyelitis, amyotrophic lateral sclerosis

Table 7: Human and animal health benefits of *H. pluvialis*-derived astaxanthin on various physiological systems (Shah *et al.,* 2016).

<u>Physiological</u> <u>System</u>	<u>Subject</u>	Effect followed	Main outcome
Anti-oxidation	Rabbits	Thioredoxin reductase; Paraoxonase acticity	Enhanced; No effect
	Rats	Hepatoprotective and antioxidant activity	Improved
	Rats	Antioxidant enzymes, catalase, superoxide dismutase, peroxidase, and lipid peroxidation in plasma and liver	Increased
	Men (bilateral cataract)	Antioxidative effects through changes in superoxide scavenging activity, and hydroperoxides production in aqueous humor	Enhanced; Suppressed
Eye function	18 healthy men	Deep vision	Improved
	10 healthy men	Eye function	Improved
	40 asthenopia patients	Eye accommodation power	Improved

	49 healthy men	Uncorrected far visual acuity	Improved
	87 men (visual display terminal	Eye accommodation amplitude (the adjustment in the lens of the eye that allows it to focus);	Improved
	workers)	Eye soreness, dryness, tiredness, and blurred vision	Reduced
Skin	Healthy female or male	Skin wrinkle, corneocyte layer, epidermis, and dermis	Improved
	46 healthy women	Skin elasticity and moisture	Improved
Immune response	14 healthy women	Oxidative stress and inflammation markers; Immune response	Reduced; Improved
Inflammation	Rats	Gastrointestinal health	Improved
Gastric ulcer	H. pylori-infected mice	Bacterial load Gastric inflamation	Reduced
	Rats	Gastir ulcer markers	Reduced
	44 patients with functional dyspepsia	Inflammatory markers; gastrointestinal discomfort	No effect; No effect
Cardiovascular	20 adult men	Blood flow time	Improved
system	Men	Blood plasma levels	Reduced
Muscle	16 non-trained	Lactic acid accumulation after run	Reduced
endurance	men		
	19 non-trained men	Respiratory and sympathetic nervous system activities	Improved
	20 non-trained men	Strength/explosiveness tes; strength/endurance test	No effect; Improved
	20 resistance- trained men	Markers of skeletal muscle injury	No effect
Cancer	Rats	Growth of colon cancer cells	Inhibited
Central nervous	Healthy mice	Memory	Improved
system	10 healthy men (50-69 years)	Response time and accuracy of several tasks	Improved
	Middle aged/elderly men	Cog Health battery scores (Nuropsychological memory test)	Improved
	and women		
Male fertility	and women 20 sub-fertile men	Semen quality, pregnancy rate	Improved
Male fertility		Semen quality, pregnancy rate Sperm velocity; oxidation markers; Pregnancy rate	Improved Improved; Reduced Improved
Male fertility	20 sub-fertile men 30 sub-fertile men	Sperm velocity; oxidation markers; Pregnancy rate	Improved; Reduced Improved
Male fertility Metabolic	20 sub-fertile men	Sperm velocity; oxidation markers;	Improved; Reduced

Appendix A: Health benefits of Astaxanthin

4 1. Longevity

Rationale

Appears to stimulate stem cell proliferation at a very low concentration relevant to oral supplementation

1.2 Non-Mammalian Interventions

Astaxanthin may be able to promote cellular longevity secondary to boosting antioxidant defences

4 2. Cardiovascular Health

2.1 Blood pressure

Astaxanthin is thought to promote blood flow in part secondary to its antioxidant properties

2.2 Red blood cells

6mg astaxanthin or higher appears to reduce membrane oxidation in red blood cells

- **4** 3. Interactions with Glucose Metabolism
- 4. Obesity and Fat Mass
- 4.1 Adipokines

Increase adiponectin as a result the reductions in triglycerides and increase in HDL-C

- **4** 5. Skeletal Muscle and Physical Performance
- 4 6. Inflammation and Immunology
- **4** 7. Interactions with Oxidation
- 7.1 Antioxidant Enzymes

Can increase Nrf2 activity and antioxidant enzymes in retinal cells secondary to activating the PI3K/Akt pathway

7.2 Lipid Peroxidation

Studies in overweight persons have noted significant improvements in general oxidation and the superoxide dismutase enzyme

- **4** 8. Interactions with Hormone
- **4** 9. Interactions with Organ Systems
- 9.1 Eyes

Appears to have general antioxidant properties in eye tissue 2. May increase ocular blood flow without any significant changes in ocular blood pressure measurements

9.2 Lungs

Smokers appear to have less overall exposure to astaxanthin following oral supplementation

• 10.Interactions with Aesthetics

• 10.1 Skin

Topical application or oral supplementation of astaxanthin appears to improve elasticity and symptoms of skin aging

- 11. Sexuality and Pregnancy
- 11.1 Menopausal Symptoms

48% reduction in symptoms of menopausal symptoms (https://examine.com/)

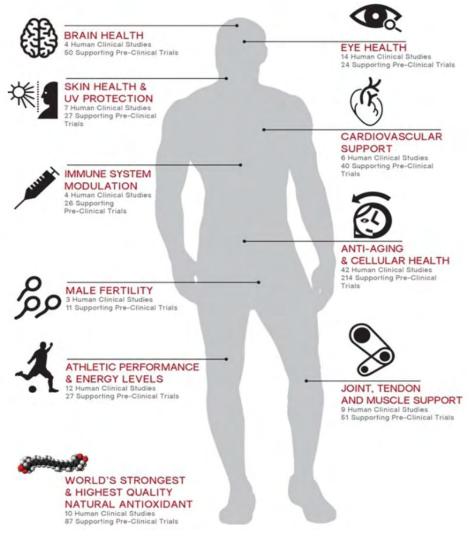


Figure 6: Health benefits of Astaxanthin with Human Clinical Studies and Supporting Pre-Clinical Trials which represented from company Beijing Gingko Group Biological Technology Co. Ltd (BGG) (<u>https://bggworld.com/</u>)

Appendix B: Charts

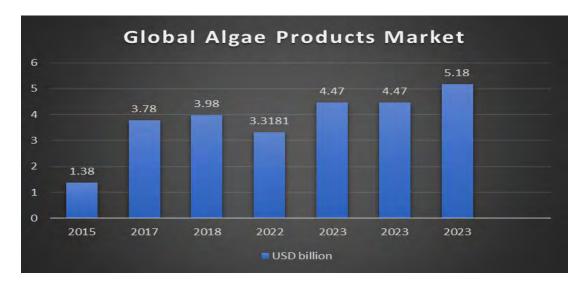


Chart 1: Represent the expected increase in the **global algae products market** (USD billion) according to the following reports in specific periods. Reports: Grand View Research (2017), Markets and Markets (2018) a, Markets and Markets (2018) b, Research and Markets (2018), Credence Research (2017), Research and Markets (2017), Markets and Markets (2018).





From Chart 1 we can draw the result that global algae products market will be flat from 2015 to 2018. Afterward until 2022 it will suffer from a slightly decrease and on 2023 and forward, we are going to have a boom with a noticeable increase. For the both of the charts, the outcomes show business fluctuations due to that the presented results have been collected from various market research.

⁴ GAGR %: Compound Annual Growth Rate

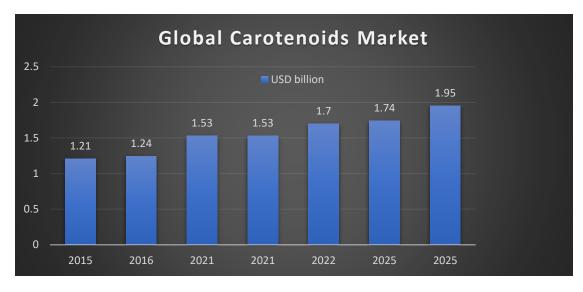


Chart 3: Represent the expected increase in the **global carotenoids market** (USD billion) according to the following reports in specific periods. Reports: Grand View Research (2016), Markets and Markets (2016), Research and Markets (2016), Markets and Markets (2016), Global Industry Analysts, Inc, Research and Markets (2017) a, Research and Markets (2017) b.

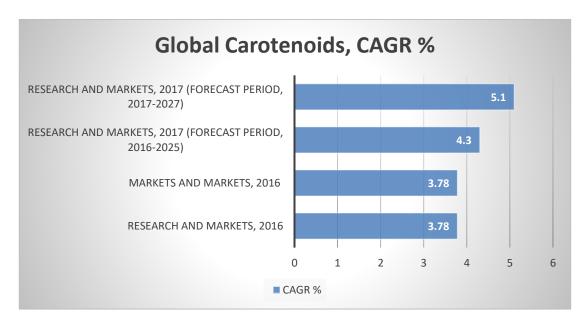


Chart 4: Represent the expected increase in the **global carotenoids market (GAGR %)**⁵ according to the following reports in specific periods. Reports and forecast periods: Research and Markets 2017 {(2016-2025), (2017)2027)}, Markets and Markets 2016 (2016-2021), Research and Markets 2016 (2016-2021).

According to various market studies and research we notice that the global market of carotenoids, will be remain in a stably rate with a slight increase from 2015 to 2025 and with annual compound growth rate 4.2%.

⁵ GAGR %: Compound Annual Growth Rate

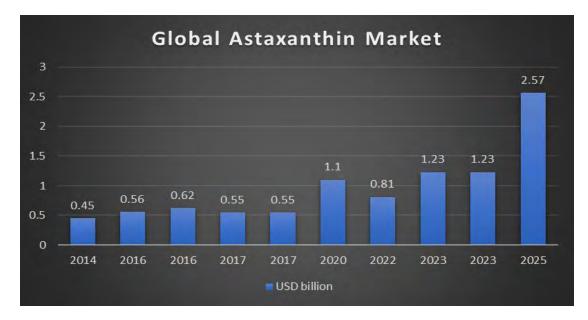


Chart 5: Represent the expected increase in the **global astaxanthin market** (USD billion) according to the following reports in specific periods. Reports: Research and Markets (2015)/ Industry Experts (2015), Grand View Research (2017), Research and Markets (2017), Global Market Insights (2018), PRNewswire (2017), Research and Markets (2015)/ Industry Experts (2015), PRNewswire (2017), Research and Markets (2015), Version (2017), Research and Markets (2017), Grand View Research (2017), Research and Markets (2015), Grand View Research (2017), Research and Markets (2015), Grand View Research (2017), Research and Markets (2017), Grand View Research (2017), Research and Markets (2017), Research and Markets (2017), Research and Markets (2017), Research and Markets (2017), Research (2017), Research and Markets (2017), Research (201

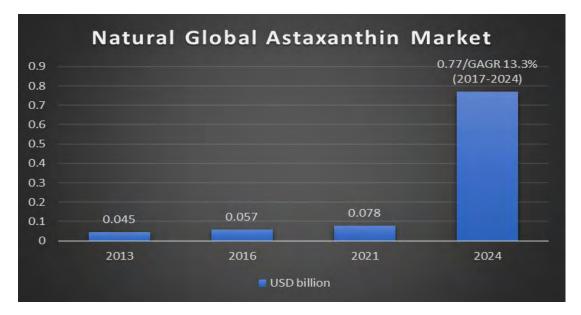


Chart 6: Represent the expected increase in the **natural global astaxanthin market** (USD billion) according to the following reports in specific periods. Reports: Market Reports World (2017)/ Market Update (2018), Research and Markets (2018).

According to Chart 5, the global astaxanthin market have differentiation of the total size which is expected to reach from 2014 to 2023. At 2025 is predicted to reach the highest size, approximately double increase of the total market size since the forecast period 2017- 2025 from 2015 – 2018 (Veracious Statistics Research, 2018). Concerning to natural global Astaxanthin Market there is a remarkable increase from 2013 to 2024 with Annual Growth Rate reaching approximately 7.5%. Also, the outcomes show

business fluctuations due to that the presented results have been collected from various market research.

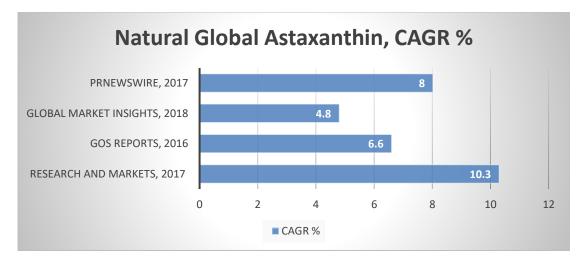


Chart 7: Represent the expected increase in the global **algae astaxanthin market** (GAGR %)⁶ according to the following reports in specific periods. Reports and forecast periods: Prnewswire, (2017){(2017-2022)}, Global Market Insights (2018){(2018-2024)}, Gos Reports, (2016){(2016-2020)}, Research and Markets (2017){(2016-2023)}

⁶ GAGR %: Compound Annual Growth Rate

Appendix B: Leading market participants in the industry of algae

The key players operating in the global algae products market are DIC Corporation (Japan), Cellana Inc. (U.S.), Taiwan Chlorella Manufacturing Company (China), Algaetech International Sdn Bhd (Malaysia), Cyanotech Corporation (U.S.), Blue BioTech Int. GmbH (Germany), Algatechologies Itd. (Israel), Pond Technologies Inc. (Canada), E.I.D. - Parry (India) Limited (India), Tianjin Norland Biotech co., Itd., (China), AlgaeCan Biotech Ltd. (Canada), Corbion NV (The Netherlands), Qponics Limited (Australia), Royal DSM (The Netherlands), Heliae Development, LLC (U.S.), and Sun Chlorella Corporation (Japan (Research and Markets, 2018)

Appendix B: Key players in the carotenoids market

include BASF SE (Germany), Royal DSM N.V. (The Netherlands), Chr. Hansen A/S (Denmark), FMC Corporation (U.S.), Kemin Industries, Inc. (U.S.), and Cyanotech Corporation (U.S.) (<u>http://www.algaeindustrymagazine.com/</u>). Other companies that have a strong presence in the carotenoids market contain D.D. Williamson & Co., Inc. (U.S.) Allied Biotech Corporation (Taiwan), ExcelVite Sdn. Bhd. (Malaysia), and Döhler Group (Germany) (Markets and Markets, 2016).

Appendix B: Key players of the market of astaxanthin

Some major players of this market are Algatechnologies; Cyanotech Corporation; Fuji Chemical Industry Co., Ltd.; BlueOcean NutraSciences, Inc.; MicroA AS; Fenchem; EID-Parry (India) Ltd.; Beijing Gingko Group (BGG); Heliae Development, LLC; IGENE Biotechnology, Inc.; and JX Nippon Oil and Energy Corporation. They are highly focused on new product development, mergers & acquisitions, and expansion to untargeted geographies in order to enhance their market position.

Appendix C: Competition

Table 12: Leading commercial companies and their *H. pluvialis*-deriver astaxanthin.

Culture System	Company Name	Country/Location	Brand Name/ Marketed product	Forms	Product particulars/ Application area	B2B	B2C
1. Closed PBRs			BioAstin [®] (extract)	Super Critical Extract 5% (SCE5) Oil	Astaxanthin extract packaged in soft gel, beadlets; dietary supplement		
Combined with Raceway Ponds	Cyanotech Corporation	USA	Naturose™ (powder)	Spray dried and formulated into a fine dark red powder	Algae meal; pigmentation source for ornamental fish and animals	V	V
	Parry Nutraceuticals Ltd.	India	Zanthin®		Astaxanthin oleoresins, beadlets, and soft gel	-	٧
	Valensa International (subsidiary of Parry Nutraceuticals Ltd.)	USA	Zanthin®		Astaxanthin extract, soft gel, beadlets	-	٧
	Jingzhou Natural Astaxanthin	China	NaturAsta™	Astaxathin powder (1.5-3.0%)	Dry algal biomass; Food and Feed Grade	V	
	Inc.	Cinita	Ναταί Αστα	(5-10%) Natural Astaxanthin oleoresin	astaxanthin soft gel; Nutraceutical, Cosmetics and Food products	v	
			Supreme Asta Oil™	Oleoresin, 5% and 10% standardized content of Astaxanthin complex	oleoresin for food, beverage dietary supplements and cosmetic products		
	Atacama Bio Natural Products S.A.	Chile	Supreme Asta powder™	Beadlets, free flowing powder, 2% and 2,5% standardized content of Astaxanthin complex	for applications in tablets and hard capsules	٧	-
				red meal oil, 1.5%	feed supplement/ feed grade		
	Heliae Development LLC	USA	TruAzta™	10% astaxanthin oleoresin	nutraceutical supplements, food products, cosmetics, and aquaculture	٧	٧
				Haematococcus Cracked Dried Powder	Algae biomass (feed supplement)		
	Algaetech International SDN BHD	Malaysia	Astaxanthin Premia-Ex	Astaxanthin Oleoresin (2.4% – 5.0%)	oleoresin (Nutraceutical industry, Cosmetics Industry and Food products),	٧	٧
				Astaxanthin Capsule (2.5% – 5.0% Oleoresin)	soft gel		
	Mera Pharmaceuticals Inc.	USA	AstaFactor [®] (algae meal extract)	_	Astaxanthin packaged as soft gel; dietary supplement, human nutraceutical	V	-
			AquaXan®		pigment for shrimp and salmon feed		

Culture System	Company Name	Country/Location	Brand Name/ Marketed product	Forms	Product particulars/ Application area	B2B	B2C
				5%, 10%, 20% oleoresin	in soft gel capsules and oil based products such as topical creams and emulsions		
			AstaPure™	2.5% Astaxanthin beadlets 2% Astaxanthin vegeterian beadlets	for tablets and hard-shell capsules	-	
2. Closed Tubular Photo-bioreactors	Algatechnologies Ltd.	Israel	Israel 4m	4mg, 12mg Astaxanthin Vegeterian Softgels	ready softgels supplied in bulk	٧	-
				2% Astaxanthin CWS powder	for liquid supplements, sachet, stick pack, gummples and more	_	
			AstaPure™ ARAVA	4mg Bulk Astaxanthin Whole Algae Tablets	Dry algal biomass, astaxanthin beadlets, and oleoresin	-	
				5%, 10%, 20% astaxanthin oleoresin	Softgel capsules; cosmetics		
				Astaxanthin Water Dirspesible Powder 1%, 2%	hard capsules; drinks, sachets		
	Beijing Gingko Group Biological Technology Co. Ltd	China	AstaZine®	Astaxanthin Water Dirspesible Emulsion 1%	for drinks and cosmetics	. v	V
	(BGG) / Algae Health Sciences, Inc.,			Astaxanthin fish gelatin Beadlets 2%, 2.5%	tablets, hard capsules		·
				Astaxanthin Veggie Beadlets 3%	tablets, hard capsules		
				Astaxanthin Spray Dried Powder 4%, 5%	tablets, hard capsules and softgel capsules		
				2% Astaxanthin Powder, 2% CWS powder water soluble	Powder		
	Yunnan Alphy Biotech Co., Ltd.	China	Alphy™	2.5%, 3%, 3.5% Natural astaxanthin biomass	Biomass	٧	
				5%, 10% astaxanthin oleoresin	Oleoresin	-	

Culture System	Company Name	Country/Location	Brand Name/ Marketed product	Forms	Product particulars/ Application area	B2B	B2C
				Oil Extract	For softgel capsules, cosmetic applications		
			Powder For hard capsules, tal		For hard capsules, tablets		
3. Closed Tank/Cylinder Photo-	Fuji Chemical Industry Co.,	Japan, Sweden, USA	AstaREAL [®] (Supercritical CO2	Watersoluble	For beverages, liquid nutraceuticals supplements	V	v
bioreactors	Ltd (AstaReal Co., Ltd)		extracted oil)/ AstaTROL®	Beadlets	For dietary supplements in powder sachets		
			Biomass		For hard capsules, tablets		
				Softgel Capsules	Ready-made soft capsules		
	Algae to Omega Holdings, Inc.	USA	AstaMatrix®		Dietary Supplement	-	٧
4. Closed Plastic Bag Photo-bioreactors	Supreme Health New Zealand Ltd.	New Zealand	AstaNZ™/ ASTAS™		Supplements, Cosmetics; Age Defined Skincare, Capsules; Edurance and Recovery	-	٧
5. Closed Flat Panel Photo-bioreactors	Fraunhofer IGB & Subitec GmbH (independent research unit)	Germany	human dietary supplement containing Astacarox/ feed supplement for horses /feed supplement for animals		*provide the scientific know-how to produce large quantities of Haematococcus algae biomass with a high astaxanthin content.	-	-
			AstaXine® AstaCaroxe® (crashed powder)	Natural Astaxanthin Oleoresin (Haematococcus Extract)	Dietary supplement containing algae crushed, and dried algae meal		
6. Indoor/ enclosed PBR	BioReal (Sweden) AB (subsidiary of Fuji Chemical)	Sweden	AstaEquus®	Natural Astaxanthin Softgel	Astaxanthin extract feed supplement for horses	٧	٧
			Novaasta® (powder)	Haematococcus Algae Biomass (Cracked)	Astaxanthin extract feed supplement for animals		
				Natural Astaxanthin Oleoresin (Haematococcus Extract)			
	Kunming Biogenic Co. Ltd.	China	AstaBio®	Natural Astaxanthin Softgel	Algal biomass, oleoresins, beadlets, and soft gels of Astaxanthin	٧	-
				Haematococcus Algae Biomass (Cracked)			
				1.5%, 2%, 2.5%, 3%, 3.5%, 4% Astaxanthin Powder	Food and Feed		
n (Wefirst Biotechnology Co.	China	A nto Einne TM	Astaxanthin oleoresin (3%, 5%, 7%, 10%)	Food supplements (ideal for manufacting softgel) and cosmetics	J	
n.s.	Ltd.	China	AstaFirst™	Astaxanthin soft gels	Functional beverages and cosmetic applications	V	-
				enacapsulated astaxanthin (1% and 2%)/ OEM	hard and soft capsules		
n.a.	Stazen Inc.	USA	Stazen®		Dietary supplement containing algae crushed and dried algae meal	-	٧

Appendix C: Swot Analysis

	Strengths	Weaknesses
Internal	 Significant decrease of the cost of natural astaxanthin production Ability to produce high quality and valuable raw material using in cosmetics, food and nutraceuticals Sustainable ways of water cleaning and CO₂ capture Production-cultivation and harvesting objectives are achieved by using magnetic nanotechnologies, an innovation with competitive advantage Astaxanthin, with powerful antioxidant properties, proved to have benefits on various health conditions including inflammation, diabetics, cardiovascular, vision and CNS conditions. Astaxanthin has a wide range of applications in the food, feed, cosmetic, aquaculture, nutraceutical, and pharmaceutical industries <i>Haematococcus pluvialis</i>, a freshwater microalga, was found out containing the highest level of astaxanthin 	 The need of a new production infrastructure The first project applying this concept, despite of the number of publications and papers already available Requires specialized and trained staff
	Opportunities	Threats
External	 Easy penetration in the astaxanthin production market The need of clean water in many areas of the planet, together with the necessity for CO₂ footprint reduction A new technology of astaxanthin cultivation will be provided to European industries along with a new integrated biorefinery concept Use of the technology for other bioactive compounds Expected increase of demand for natural astaxanthin to produce high quality pharmaceutical molecules, nutraceuticals, food additives and cosmetics, open new markets in different directions Exploitation and research & development for new utilizations or incorporated in already existing lines of products 	 Economic crisis that affects Greece as well as other European countries and globally Distributors may affect final price High competition level from synthetic astaxanthin High competition from top players/ leaders in the bulking of astaxanthin with long-term presence in the global market Probability imitation of VALUEMAG method or new producing technology from other technology competitors and penetration to the market*

* Worldwide, research and demonstration programmes are being carried out to develop the technology needed to expand algae biomass production and exploitation. In the following, we mention some of the most relevant projects concerning the VALUEMAG project (Table 16):

Table 16: Worldwide projects, new technologies of production and exploitation of algae.

Project Title	Area
Biofuel from Algae Technologies	BIOFAT
Sustainable Fuels from Marine Biomass Project -	BIOMARA
Bio-waste and Algae Knowledge for the Production of 2nd Generation Biofuels	BIOWALK4BIOFUELS
Energetic Algae	ENALGAE
Utilisation of Micro-algae for Wastewater Treatment with Energy Purposes-	ENERBIOALGAE
Marine Algae as Biomass for Biofuels	MABFUEL
Biotechnological Exploitation of Marine Products and By Products	BIOTECMAR
Control of Light Use Efficiency in Plants and Algae from Light to Harvest	HARVEST
Towards a Better Sunlight to Biomass Conversion Efficiency in Micro-algae	SUNBIOPATH
Enabling European SMEs to Remediate Wastes, Reduce GHG Emissions and Produce Biofuels via Micro- algae Cultivation	BIOALGAESORB
Development of an algal platform for production of building blocks for the chemical industry and their further conversion to products	SPLASH
A sustainable chain for continuous biofuel production using micro- algae as a production platform, thereby making 2nd generation biofuels competitive alternatives to fossil fuels.	Fuel4Me
Demonstration of integrated and sustainable enclosed raceway and photobioreactor micro-algae cultivation with biodiesel production and validation.	INTESUSAL
Pilot scale algal research centre (including lipid production) Relevant to Biology & Biotechnology, Economic assessment	ALGAEPARC
Novel algae-based solution for CO2 capture and biomass production	ALGADISK
Genetic Improvement of Algae for Value Added Products	GIAVAP
Sustainable Production of Biologically Active Molecules of Marine Based Origin	BAMMBO
Oil production with algae	Emerald Oils

Appendix C: Gannt Chart

	YEAR 1		YEAR 2					YEAR 3				
WPS	2 4 6 8 10 12	14 16	5 18	20	22	24	26	28	30	32	34	36
WP1. Magnetic algae preparation										İ		
Task 1.1. System for the magnetic nanoparticle development												
Task 1.2. Uptake system of magnetic nanoparticles by algal cells												
Task 1.3. Determination of parameters of uptake of magnetic nanoparticles from algal cells												
WP2. Magnetic algae cultivation system												
Task 2.1. Green-house development												
Task 2.2. Development of SOMAC												
Task 2.3. Development of the algal cell de-watering system												
Task 2.4. Selection of the most suitable methods for CO2 capture and water recovering												
Task 2.5. System integration												
Task 2.6. System functionalization and initial operation												
WP3. Animal Food, Nutraceutical & Cosmetics from Algae												
Task 3.1. Characterization of microlagae for bio-products												
Task 3.2. Evaluated pre and post harvest of microlagae extract against plant pathogenic fungi												
Task 3.3. CO2-SF experimental test for microlagae and optimization tests												
Task 3.4. CO2 SF experimental test for microalgae on pilot scale												
Task 3.5. Selective Magnetic Separation of natural compounds												
Task 3.6. Process modelling and design												
Task 3.7. Risk analysis and Safety control												
WP4: Operation and Assesment												
Task 4.1. Operation												
Task 4.2. Modelling and simulation of the overall process										Í		
Task 4.3. Technical and economic assesment												
Task 4.4. Energy systems analysis												
Task 4.5. LCA assesment												
Task 4.6. Assesment of regulatory and policy framework												
WP5: Exploitation and Business Development												
Task 5.1. Market and socio-economic impact analysis												
Task 5.2. Stakeholders Analysis and Exploitation Plan												
Task 5.3. Market Penetration												
Task 5.4. Develop Business Plan												
WP6: Project Management & Results Dissemination												
Task 6.1. Project coordination												
Task 6.2. Financial and administrative management												
Task 6.3. Dissemination plan												
Task 6.4. Project website and Media relations												
Task 6.5. Communications marketing material & Newletters												
Task 6.6. Events Participation & Social Media												
Task 6.7. Open Workshop												

Chart 8: Cantt Chart

Appendix D: Profit and Loss forecast

Table 23: Equipment and operating expenses for the production astaxanthin with the technology of Valuemag for the first five years.

Equipment Co	Equipment Costs and Operating Expenses							
Annual Expenses (€)	Costs (€)							
	Year 1	Year 2	Year 3	Year 4	Year 5			
Staff expenses	18000	18.000	19.800	21.780	23.958			
Operational Costs								
Energy/ Cultivating water	7.000	7.000	7.000	7.000	7.000			
Cleaning	5.000	5.000	5.000	5.000	5.000			
Machinery maintenance	10.000	10.000	12.000	12.000	12.000			
Promotion and Marketing	10.000	10.000	13.000	13.000	16.900			
Packaging Costs	10.000	10000	12.000	12.000	14.400			
Other unpredicted expenses	5.000	5.000	5.000	5.000	5.000			
Storage	2.000	2.000	3.000	3.000	4.000			
Rent and building expenses	10000	10000	10000	10000	10000			
Total	80.000	80.000	86.800	88.780	98.258			
Pre-treatment, Extraction, Drying and								
Quantification, Purification, Separation	75.000	82.500	90.750	99.825	109.807			

Table 24: P&L and Basic ratios - Normal Scenario (low content of Astaxanthin)

	Year 1	Year 2	Year 3	Year 4	Year 5
Sales	488.683	1.630.621	4.662.015 8.816.778		15.857.005
Cost of Goods (~15%)	73.302	244.593	699.302	1.322.516	2.378.550
GP (~ 85%)	415.381	1.386.028	3.962.713	7.494.262	13.478.455
OPEX	260.381	1.223.528	3.785.163	7.305.657	13.270.390
EBITA (~ 30%)	182.266	856.469	2.649.614	5.113.959	9.289.273
Taxes (~ 25%)	45.566	214.117	662.403	1.278.490	2.322.318
Net Profit	136.700	642.352	1.987.211	3.835.469	6.966.955

Table 25: P&L and Basic ratios - Worst Scenario (low content of Astaxanthin)

	Year 1	Year 2	Year 3	Year 4	Year 5
Sales	293.209	543.54	1.467.168	2.890.045	4.318.186
Cost of Goods (~15%)	43.981	81.531	220.075	447.006	647.727
GP (~85%)	249.228	462.009	1.247.093	2.443.039	3.670.459
ΟΡΕΧ	94.228	464.509	1.069.543	2.254.434	3.462.394

EBITA (~30%)	65.959	325.156	748.680	1.578.103	2.423.675
Taxes (~25%)	16.489	81.289	187.170	394.525	605.918
Net Profit	49.470	243.867	561.510	1.183.577	1.817.757

Table 26: P&L and Basic ratios - Normal Scenario (high content of Astaxanthin)

	Year 1	Year 2	Year 3	Year 4	Year 5
Sales	1.853.376	6.184.281	17.681.126	33.438.450	60.139.161
Cost of Goods (~15%)	278.006	927.642	2.652.168	5.015.767	9.020.874
GP (~ 85%)	1.575.370	5.256.639	15.028.958	28.422.683	51.118.287
OPEX	1.420.370	5.094.139	14.851.408	28.234.078	50.910.222
EBITA (~ 30%)	994.259	3.565.897	10.395.985	19.763.854	35.637.155
Taxes (~ 25%)	248.564	891.474	2.598.996	4.940.963	8.909.288
Net Profit	745.694	2.674.422	7.796.988	14.732.890	26.727.866

Table 27: P&L and Basic ratios – Worst Scenario (high content of Astaxanthin)

	Year 1	Year 2	Year 3	Year 4	Year 5
Sales	1.112.025	2.061.427	5.564.374	10.960.767	16.377.123
Cost of Goods					
(~15%)	166.803	309.241	834.656	1.644.115	2.456.568
GP (~85%)	945.222	1.752.186	4.729.718	9.316.652	13.920.555
OPEX	790.222	1.589.686	4.552.168	9.128.047	13.712.490
EBITA (~30%)	553.155	1.112.780	3.186.517	6.389.632	9.598.743
Taxes (~25%)	138.288	278.195	796.629	1.597.408	2.399.685
Net Profit	414.866	834.584	2.389.887	4.792.223	7.199.057

Table 28: Sales & EBITA for the 2 Scenarios,

SALES (€)	Year 1	Year 2	Year 3	Year 4	Year 5
Normal	136.700	642.352	1.987.211	3.835.469	6.966.955
Worst	49.470	243.867	561.510	1.183.577	1.817.757
EBITA (€)	Year 1	Year 2	Year 3	Year 4	Year 5
Normal	745.694	2.674.422	7.796.988	14.732.890	26.727.866
Worst	414.866	834.584	2.389.887	4.792.223	7.199.057

Appendix E: Company Profiles COMPANY PROFILES⁷

Cyanotech (<u>https://www.cyanotech.com/</u>)

Company Overview

As a world leader in the production of high value natural products derived from microalgae Cyanotech Corporation has been guided by the principle of providing high quality microalgae products for health and nutrition in a sustainable, reliable, and environmentally sensitive operation. Cyanotech Corporation, a world leader in microalgae technology for over 30 years, produces BioAstin® Hawaiian Astaxanthin® and Hawaiian Spirulina Pacifica®.

They are third party certified to GMP (Good Manufacturing Practices) for dietary supplements, reinforcing their commitment to quality in their products, quality in their relationships (with their customers, suppliers, employees and the communities they live in), and quality of the environment in which they work. Their mission that guides their company is to "Fulfill the Promise of Whole Health Through Hawaiian Microalgae."

All Cyanotech products are produced from microalgae grown at our 96-acre facility in Kona, Hawaii using patented and proprietary technology and are Generally Recognized as Safe (GRAS) for use in food products. Cyanotech sells its products direct to consumers at retail locations in the United States and online and distributes to dietary supplement, nutraceutical and cosmeceutical manufacturers and marketers. The Company is regulated by the FDA.

- 1. Cyanotech Corporation Earns Non-GMO Project Verification for BULK SCE BioAstin Natural Astaxanthin Products
- 2. Cyanotech Corporation Earns Halal Certification for BioAstin. Bulk raw material, BioAstin® SCE 5 and vegan BioAstin soft gels, have received halal certification from the Islamic Food and Nutrition Council of America (IFANCA).

Method of cultivation of microalgae

Cyanotech's patented Ocean Chill DryingTM method allows the algae to dry in a chamber containing less than 1 percent oxygen in less than 7 seconds. This preserves the integrity of the antioxidant content. Furthermore, Cyanotech is one of the few companies in the world with the technology to accurately measure Natural Astaxathin levels. Cyanotech's methods have been accepted by the United States, Canadian, and Japanese governments. In 1999, BioAstin® was the first source of astaxanthin for human nutrition to be reviewed by the U.S. Food and Drug Administration (FDA). In fact, it passed review by the FDA and is now allowed to be sold in the US as a human dietary supplement.

Product and End-User/ Application

BioAstin® Hawaiian Astaxanthin® is a potent natural antioxidant that provides support for recovery after exercise, joint, skin, and eye health. BioAstin is derived from natural microalgae, *Haematococcus pluvialis*, that is cultivated, grown, and processed on the Kona coast of the Big Island of Hawaii.

⁷ All information has been collected from the official site and pages of every company separately.

BioAstin® is sold primarily as a packaged consumer product through Nutrex Hawaii to natural product distributors, retailers and online channels, or directly to consumers. It is also sold in liquid lipid form as a raw ingredient to dietary supplement manufacturers, health food formulators and cosmetic manufacturers. BioAstin® gelcaps are sold in bulk quantities to international distributors.

Parry Nutraceuticals Ltd. (EID Parry) (<u>http://www.parrynutraceuticals.com/</u>)

Company Overview

Parry the world leader in micro algal technology. Certified for major International Food and Safety Standards, Parry Nutraceuticals products have charted constant growth in all its markets worldwide. Parry Nutraceuticals pioneered micro-algae cultivation in the market of dietary supplements and has been instrumental in bringing to the market algae-based products like Spirulina, Astaxanthin and Natural Mixed Carotenoids. Parry Nutraceuticals is the only company in the world to cultivate all three microalgae - Spirulina, Astaxanthin and Chlorella for human health. Headquartered in Chennai, India, they are a division of E.I.D. Parry (I) Ltd. and a part of the USD 4.3 billion Murugappa Group.

Parry Nutraceuticals' products have become an integral part of several major brands in more than 38 countries, the main markets being in North America, Europe, South East Asia and the Far East for many years now.

Method of cultivation of microalgae

Parry employs a unique, proprietary two-part process using all-natural Deep Extract® (high pressure) supercritical CO₂ to create the highest quality extraction. They also utilize our O₂B® Peroxidation Blocker stabilization technology to ensure long-term stability and efficacy for the consumer. This process uses no co-solvents or ethoxyquin obtaining the highest amount of active ingredients.

Valensa, subsidiary of EID Parry (India) Ltd is the only producer of Astaxanthin in the United States. Valensa only uses extra virgin olive oil to standardize Astaxanthin. Valensa International is the first company to have finished form Astaxanthin material approved for use by regulatory agencies in both the United States (NDI – New Dietary Ingredient) and Europe (Novel Foods).

Product and End-User/ Application

Their astaxanthin begins with the microalgae *Haematococcus pluvialis* which is grown in a remote, pristine Chilean environment. The microalgae are extracted at their organic certified facility in Eustis, Florida using their proprietary Deep Extract® supercritical CO₂ extraction process. Zanthin® Natural Astaxanthin is very clean and free from solvent residues. Zanthin® Natural Astaxanthin is also very stable. Valensa uses, all-natural O₂B® peroxidation blocker to prevent degradation of astaxanthin during storage, encapsulation and packaging. O₂B® peroxidation blocker provides Zanthin® Natural Astaxanthin with a superior four years of shelf life. These features combine to make Valensa's Zanthin® the best astaxanthin on the market. Product offered in **Oleoresin, Beadlets or Softgels**.

Jingzhou Natural Astaxanthin Inc.

Company Overview

Jingzhou Natural Astaxanthin Inc is a leading enterprise producing natural Astaxanthin by culturing *Heamotococcus pluvialis* in China, Asia. They were founded in 2003 to develop and

commercialize microalgae-derived products for the aquatic feed & human food industry. Today, their company hold $32000 \,\mathrm{m}^2$ culture area for *Heamotococcus pluvialis*.

Product and End-User/ Application

They can supply high-quality food grade and feed grade natural **astaxathin powder (1.5 - 3.0%)** about 1.5 Ton/month, And **5-10% Natural Astaxanthin oleoresin** content & Natural Astaxanthin softgel.

NaturAstaTM Food Grade Haematococcus pluvialis Powder which is Natural esterified Astaxanthin complex in the form of *Haematococcus pluvialis* algae biomass, raw material for the extraction of a concentrated Astaxanthin complex. The Astaxanthin concentration of their algal powder is average 2.0 - 2.3% (2000-2300PPM).

NaturAsta[™] 5% *astaxanthin oleoresin* is a natural astaxanthin complex extracted from Haematococcus pluvialis algal biomass by Supercritical CO2 extraction technology. It is a dark red viscous oleoresin, for use in Nutraceutical, Cosmetics and Food products.

Atacama Bio Natural Products S.A. (http://www.atacamabionatural.com/)

Company Overview

Atacama Bio Natural Products S.A. is a private, Chilean, biotechnology corporation that cultures algae to extract healthy ingredients for human well-being. They are industrial producers of natural astaxanthin from their cultures of *Haematococcus pluvialis* microalgae and they can supply their company with industrial volumes on a permanent and regular basis. They are interested in developing long-term business relationships with their clients for mutual benefits.

The value of Atacama: "Just few places in the world provide the ideal conditions for low cost production of natural Astaxanthin from *H. pluvialis* microalgae".

A 250 acres facility in a high light intensity region with pure water from the Andes mountains makes Atacama Bio a prime producer that can greatly expand its production capacity to meet demand. Company initiates the year 2003 and after a long work of research has developed a robust, state-of-the-art, proprietary culture technology of closed and open photobioreactors.

Their Process

Pure water from the Andes Mountain range, after a careful process to ensure sterilization is used for the preparation of the growing media. NonGenetically Modified microalgae is inoculated in the aseptic culture medium of the photobioreactors.

As microorganism contamination is the principal risk in this industry, they use for culture growth different types of enclosed photobioreactors that are kept very clean. They use HEPA filtered air and reverse osmosis and ozone treated water as well as food grade nutrients.

The last stage of production is done in open pond photobioreactors where *Haematococcus pluvialis* grows in a way that resembles their natural environment. Harvested microalgae is ground and vacuum dried at low temperature, preserving the original characteristics and obtaining a powder of very high bioavailability that is mixed with vegetable oil.

Product and End-User/ Application

Their prime product is natural, 3S, 3'S Astaxanthin stereoisomer from *Haematococcus pluvialis*, NAXA verified Astaxanthin. It has several uses for human consumption and animal feed purposes. Their main products are oleoresin extracted by supercritical CO₂ and dried microalgae biomass.

Food Grade

Oleoresin

Extracted from the milled and dried biomass of *H. pluvialis* by means of CO₂ supercritical extraction process. For applications in food, beverage, dietary supplements and cosmetics products, it is supplied in **5% and 10% standardized content of Astaxanthin complex**.

Beadlets

Free flowing powder, **2% and 2,5% standardized content of Astaxanthin** complex for applications in tablets and hard capsules.

Feed Grade

As a natural ingredient to manufacture feed for fish, crustaceans and hens.

They also are experienced culturing sp. Spirulina and Dunaliella salina.

Heliae Development LLC (https://heliaeglobal.com/news/)

Company Overview

Heliae is an applied life sciences and technology company. A critical component of Heliae's core mission is to create sustainable products and solutions that enhance soil, plant, animal, and human health. Their focus is on researching and developing microalgae and other cuttingedge biological platforms for commercial scale production of useful products. Heliae both seeks and welcomes opportunities to collaborate with industry and research groups.

Their partnerships range for the development and operation of new microalgae production facilities, such as their Alvita joint venture with Sincere Corporation of Japan.

In 2008, Heliae was founded on the premise that algae can provide significant value to society if commercialized with the appropriate technology. This long view has allowed Heliae to build a base of technology that provides a distinct competitive advantage in the industry and has positioned the company as the leader in providing algae technology solutions to the industry and algae products to consumers.

The collaboration began in late 2014 when Heliae partnered with Sincere Corporation to design a microalgae production facility to produce natural astaxanthin, from microalgae for the Japanese market. Heliae provided the technological and biological expertise along with project management leadership in launching Alvita.

Method of cultivation of microalgae

Heliae has experience in commercial scale production of products from a variety of biological platforms using novel and conventional phototrophic, mixotrophic and heterotrophic growth systems. Heliae currently employs all three at its research and demonstration production facilities located near Phoenix, Arizona, USA.

The location of the Saga City facility, near the incineration plant, offers Alvita the opportunity to recycle CO2 and use low grade waste heat for optimal temperature control of the reactors.

Heliae and Alvita utilize a fully enclosed phototrophic raceway production system seeded by a proprietary seed growth system. This harmonization of technology and infrastructure allows natural sunlight to induce astaxanthin production without exposure to contamination found with other approaches to microalgae production. This approach mimics nature while protecting product quality and purity.

The test facility consists of six open pond reactors housed inside a locally built greenhouse. Along with the reactors, a fully functioning laboratory was shipped for analysis of culture growth parameters. As of early May 2015, they have carried out seven successful production runs in the test reactors with their Japanese partners.

Product and End-User/ Application

Heliae is constantly developing new commercial products for distribution in a variety of fields. Currently they sell a high quality astaxanthin ingredient and PhycoTerra®, an agricultural product that has demonstrated promising efficacy in initial field trials. Heliae supplies its human supplement grade astaxanthin to leading retail outlets through a preferred supply agreement with a commercial supplement formulation partner. With ongoing and regular increases in production, Heliae can supply the same high quality astaxanthin to other customers for applications including animal nutrition, aquaculture, and cosmetics. They also engage in contract research and manufacture; collaborative research; and licensing.

Algatechnologies Ltd. (https://www.algatech.com/)

Company Overview

Algatechnologies is a rapidly growing biotechnology company, specializing in the commercial cultivation of microalgae. Founded in 1998, Algatech is a world leader in the production and supply of AstaPure®, a premium natural astaxanthin - one of the world's most powerful antioxidants sourced from the microalga *Haematococcus pluvialis*.

Located in Israel's Arava desert – with its stable climate and high light intensity – Algatechnologies is ideally positioned to cultivate algae. The company produces astaxanthin in an innovative process, and markets its AstaPure® brand to the nutrition, food & beverages and cosmetics industries.

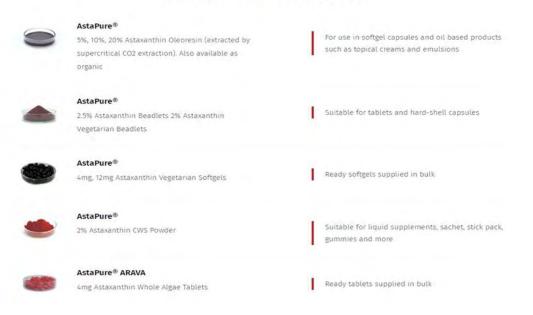
Algatechnologies enjoys a global presence through a well-established marketing network of selected distributors and agents. The company's sales organization serves more than 30 countries, via an extensive network of exclusive distributors and by direct sales to multi-national customers. The company provides logistics, warehousing and lab support services, offering customers reliable supply and prompt response.

Algatechnologies' objectives are: 1. To provide the highest quality of natural astaxanthin, backed by experienced technical and marketing support. 2. To continue developing, producing and commercializing advanced algal-based products to the fastest growing markets by utilizing proprietary algal cultivation and processing technologies.

Product and End-User/ Application

AstaPure® astaxanthin is derived from a microalga called Haematococcus pluvialis.

ASTAPURE[®] LINE OF PRODUCTS



Beijing Ginko Group (BGG) Biological Technology Co. Ltd. (<u>https://bggworld.com/</u>)

Company Overview

BGG is a global leader in the development and purification of high quality active natural plant ingredients for the dietary supplement, pharmaceutical, personal care, and food and beverage industries. Founded in 1995, BGG employs a staff of approximately 400, has 6 production sites, international branches in North America, Japan, China and Switzerland and manages sales in more than 70 countries. BGG selects raw materials, which are primarily from wild-crafted, organic, and from non-GMO sources, whenever possible. From these they produce health products and nutritional solutions that aid and enhance people's well-being and quality of life.

BGG is a leader in Astaxanthin production and technology worldwide. BGG has aimed to impart the absolute highest quality algae-based ingredients at an appreciative price. It features products which are unmatched in terms of purity, potency and overall quality. Their product range of BGG is consisted of the world's first Certified Organic *Haematococcus pluvialis* microalgae powder. The company has been predicted to be the world's largest producer of Natural Astaxanthin by the year 2020. Nutraceutical ingredients from algae are promptly evolving into an important segment of the supplement industry (Industry Arc, 2018).

For instance, the year 2014 witnessed China-based Beijing Gingko Group Biotechnology Co Ltd (BGG) inaugurating a 150-acre *Haematococcus pluvialis* production base in Shilin County, making the facility the largest in Asia-Pacific, with an annual *H. pluvialis* algae freeze-dried powder production capacity of 100 tons. The company has also disclosed intentions for further increasing this capacity to 300 tons per year over the coming years.

Product and End-User/ Application

They supply dry biomass (powder) of *Haematococcus pluvialis* with different astaxanthin content in bulk, which can be used for food and feed as well as further refinement. Key products include: AstaZine®, Natural Astaxanthin from *Haemattococus Pluvialis* algae farmed in world's First Organically Certified Astaxanthin Farm.

AstaZine® is a line of natural astaxanthin products from *Haematococcus pluvialis* microalgae produced *in a fully-closed, 100%-glass tube* eco-friendly photobioreactor system.

AstaZine®:

- · Contains 97% pure astaxanthin in the carotenoid fraction and just 3% of other carotenoids
- Organically Certified astaxanthin product (certified by Ecocert®)
- Covered by two NDIs with US FDA at 12mg and 24mg per day
- Supported with 239 health claims submitted to US FDA without objection

• Astaxanthin with two European Novel Foods approvals for both supercritical CO2 and ethanol extracts.

- Self-affirmed GRAS
- · Registered for sale in most of the world's most regulated markets

	Astaxanthin content	() tablets	hard capsules	soffGel capsules	↓ drinks	sachels	Cosmetics
Astaxanthin Oleoresin	5%. 10%. 20%			Х			х
Astaxanthin Water Dispersible Powder	1%, 2%		x		×	x	
Astaxanthin Water Dispersible Emulsion	1%				Х		x
Astaxanthin fish gelatin Beadlets	2%, 2,5%	x	X			-	
Astaxanthin veggie Beadlets	3%	Х	x			1	
Astaxanthin Spray Dried Powder	4%.5%	x	X	x			

Algae Health Sciences, Inc., a subsidiary of BGG, focuses on the production and marketing of efficacious ingredients from natural algae sources. Algae Health Science's flagship product, AstaZine® Natural Astaxanthin, is extracted from the world's first Certified Organic *Haematococcus pluvialis* microalgae.

Algae Health Sciences, Inc., is a proud member of NAXA, the Natural Algae Astaxanthin Association. Algae Health Sciences and BGG support the NAXA Verification Program (NAVP) to address the growing problem of adulteration within the dietary supplement industry and assure finished product manufacturers and consumers that the astaxanthin they are purchasing is natural astaxanthin derived from *Haematococcus pluvialis*.

Method of cultivation of microalgae

Their Certified Organic *Haematococcus Pluvialis* microalgae powder and other fine Astaxanthin products are the result of years of dedicated research and a huge investment in the world's premier Natural Astaxanthin facility. AstaZine® is produced through cultivation of the microalgae *Haematococcus pluvialis* in high technology 100% glass-tube photobioreactors. AstaZine® is cultivated using clean water from the Himalaya Mountains which still undergoes reverse osmosis before being used to grow our microalgae cultures; this ensures maximum purity and safety. Algae Health's team of scientists and algae production experts has toiled for years to attain the utmost level of technical abilities in the microalgae field.

Yunnan Alphy Biotech Co., Ltd.

Company Overview

Yunnan Alphy Biotech Co.,Ltd. is a high-technology company engaged in engineering largecell culture of Haematococcus pluvialis for the production of natural astaxanthin. The company was founded May 10, 2007, with registered capital of ten million yuan. The registered business scopes include; Biotechnology research and development and technology transfer, Aquaculture of aquatic organisms, and production and marketing of related products.

Product and End-User/ Application

They specialize in the production of natural astaxanthin from green microalgae, Heamatococcus pluvialis and extraction of astaxanthin oleoresin via supercritical CO2.

Products

- Heamatococcus pluvialis biomass/ H.p powder
- Astaxanthin oil/ astaxanthin olereosin
- Astaxanthin CWS powder
- OEM&ODM



Fuji Chemical Industry Co. Ltd. (AstaReal Co Tld) (http://www.fujichemical.co.jp/english/)

Company Overview

The Fuji Chemical Industries Group began its work with astaxanthin, renowned for its benefits in the prevention of disease, as early as 1994. Since then, they have diversified their astaxanthin business from raw materials to the marketing of finished products. They have also been actively involved in seminars and symposiums on astaxanthin and have accumulated a wealth of research evidence in collaboration with universities and research organizations. The Fuji Chemical Industries Group will continue in its role as a leading innovator of astaxanthin in order to make a positive contribution to the evolution of preventive health care.

They currently market their astaxanthin-based AstaREAL® ACT dietary supplement and ASTARISM® skincare base beauty essence to more than 1200 health care facilities all over Japan and are expanding our efforts with the aid of experts in disease prevention & medicine.

Product and End-User/ Application

AstaReal are pioneers in microalgae cultivation. In the early 1990s, we developed a highly advanced and one-of-a-kind technology using specially designed photobioreactors to cultivate the microalgae *Haematococcus pluvialis (HP)*.

Their first indoor production facility was established in Gustavsberg, Sweden, where we became the first company in the world to commercially produce natural astaxanthin from

microalgae in the early 1990s. The facility is ISO, HACCP certified, and our production is both Halal and Kosher certified.

Their commitment to ensuring the supply of superior natural astaxanthin products is further underpinned by the opening of a new manufacturing facility in 2014. Their newest facility is in Moses Lake Washington, USA, and uses the same innovative photobioreactor system developed in Sweden. As a result, the AstaReal Group's production capacity has tripled, allowing them to consistently meet the ever-increasing global demand for superior, natural astaxanthin.

The AstaReal Group together with their parent company Fuji Chemical Industries Co., Ltd. are world leaders in research based natural astaxanthin health products. The Group continues to lead the way in the production and innovation of natural astaxanthin.

AstaReal astaxanthin is offered in a variety of bulk forms to suit your final product.



Oil Extract For softgel capsules, cosmetic applications



Powder For hard capsules, tablet



Watersoluble For beverages, liquid nutraceutical supplements.



Beadlet

for dietary supplements in powder sachets



Biomass For hard capsules, tablet



Softgel Capsules Ready-made soft caspules