

Astaxanthin from the Microalga *Haematococcus* – a superb natural antioxidant for human health

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Algatechnologies (1998) Ltd is an up and coming Israeli company and a global leader in solar-energy-based photobioreactor technology, devoted to exploiting the commercial potential of natural bioactives from microalgae.

Our first product, natural *Astaxanthin*, is a valuable bioactive ingredient for dietary supplements, nutraceuticals and cosmeceuticals.

Astaxanthin also enjoys a broad range of applications in the food industry and in the fish and animal feed arena.

Astaxanthin is produced by a patented biocontrolled growing process of the microalga *Haematococcus pluvialis* A1.



Astaxanthin – The “Jewel Antioxidant” among carotenoids

Carotenoids are lipid-soluble pigments, which participate as accessory pigments in the light-absorption process of photosynthetic organisms. To date, over 700 natural carotenoids have been identified. They are responsible for the orange and red colors in plants and algae, and for the wide range of blue, purple and reddish colors in aquatic animals. Only phytoplankton, algae, plants and certain bacteria and fungi synthesize carotenoids. Animals, including humans, must consume carotenoids as part of their diet and rely on this external supply. *Astaxanthin*, a member of the carotenoid family, is a dark-red pigment which is the main carotenoid found in the marine world of algae and aquatic animals. *Astaxanthin* is present in many types of seafood, including salmon, trout, red sea bream, shrimp and lobster, as well as in birds such as flamingo and quail. This pigment is commercially produced from the microalga *Haematococcus pluvialis*, the richest known natural source for *Astaxanthin*.

Recent scientific findings indicate that *Astaxanthin* is a powerful antioxidant and can serve as a potent free-radical scavenger. Moreover, *Astaxanthin* has been found to provide many essential biological functions, including protection against lipid-membrane peroxidation of essential polyunsaturated fatty acids and proteins, DNA damage and UV light effects; it also plays an important role in immunological defense.

Oxygen is necessary for the metabolic production of energy in our bodies. Mitochondria, through the electron-transport chain, use oxygen to oxidize certain molecules and generate energy in the form of ATP. During this process, oxygen is reduced to water, producing several oxygen-derived free radicals or reactive oxygen species (ROS) which play an important role

in various diseases. Normally, oxygen free radicals are neutralized by natural antioxidants such as vitamin E, or enzymes such as superoxide dismutase (SOD). However, ROS become a problem when either a decrease in their removal or their over-production occurs, resulting in oxidative stress. This stress, and the resultant damage, have been implicated in many diseases, and a wealth of preventive drugs and treatments are currently being studied.

Astaxanthin's powerful anti-oxidant activity has been demonstrated in numerous studies showing the detrimental effects of free-radical-induced oxidative stress^{2,4} and *Astaxanthin*'s potential to target many important health conditions.

There is increasing testimonial evidence that *Astaxanthin* may be effective in enhancing general well-being, improving the quality of life and enhancing the immune system. Recent studies have shown enhanced immune response and decreased DNA damage in human subjects following *Astaxanthin* administration⁵. *Astaxanthin* is capable of crossing the blood-brain barrier in mammals⁶, a unique and important property in the realm of antioxidants. This characteristic allows *Astaxanthin* to extend its superior antioxidant activity to the central nervous system, which, being rich in unsaturated fatty acids is highly susceptible to oxidative damage by ROS⁷.

The efficacy of *Astaxanthin* in limiting the damage produced by ROS-induced oxidative stress and improving health parameters in the tissues and the body was demonstrated in a series of in-vitro experiments, in pre-clinical studies and in human models. The following is a list of diseases and conditions for which *Astaxanthin* has been shown to have beneficial effects, as described in numerous medical articles, patents and excellent reviews^{8,9} over the last 10 years:

- Age-Related Macular Degeneration; the leading cause of blindness in the aging population
- Alzheimer's and Parkinson's Diseases: two of the most important neurodegenerative diseases
- Cholesterol Disease: ameliorates the effects of LDL, the “bad” cholesterol
- Inflammatory, chronic viral and autoimmune diseases
- Dyspepsia
- Semen fertility improvement
- Muscle function
- Sunburn from UV light
- Normalization of cardiac rhythm
- Anti-hypertension agent
- Stress management
- Benign Prostatic Hyperplasia (BPH)
- Stroke: repairs damage caused by lack of oxygen.

A demand for natural *Astaxanthin* is now emerging in the fast-growing, multi-billion dollar nutraceutical market; in particular, increasing evidence suggests that *Astaxanthin* is a much more powerful antioxidant than vitamins C and E, or than other carotenoids such as beta-carotene, lycopene, lutein and zeaxanthin, among others.

The enhanced activity of *Astaxanthin* may stem from its molecular structure. *Astaxanthin* belongs to the xanthophyll group of carotenoids, or the oxygenated carotenoids (see other members of the group in Fig. 1). The hydroxyl and keto functional

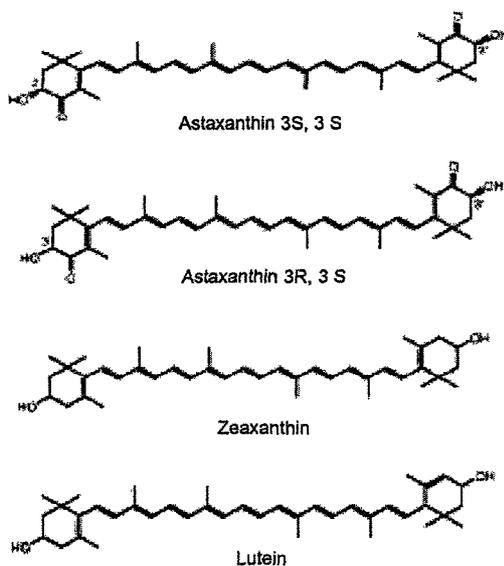


Fig 1: Members of the xanthophyll family

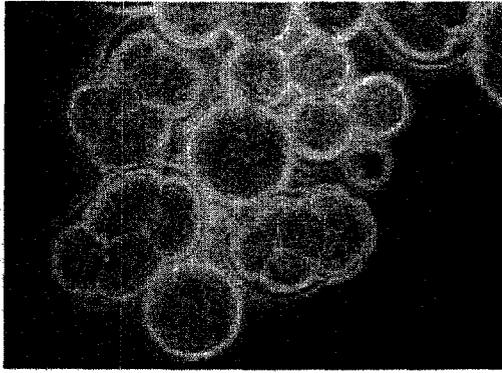


Fig 2: Green cells of *Haematococcus pluvialis* culture

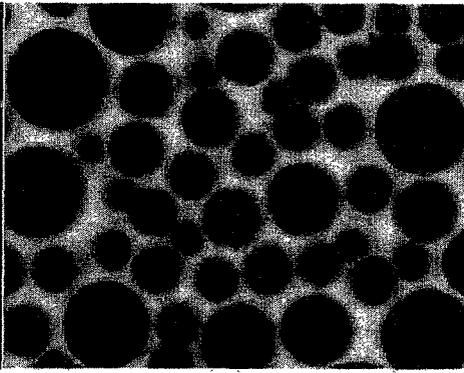


Fig 2: Red cells of *Haematococcus pluvialis* culture

Natural vs. Synthetic Astaxanthin

The chemical difference between natural and synthetic *Astaxanthin* lies in the stereo-chemical orientation of the molecules in space (those different molecules are called "enantiomers").

Astaxanthin exists in three main enantiomeric forms, termed 3S-3'S, 3R-3'S, and 3R-3'R, depending on the spatial orientation of the hydroxyl (OH) groups in chiral carbon

groups (see Fig. 1) present in the ending ionone ring of *Astaxanthin* may be responsible for its uniquely powerful antioxidant activity and for its ability to span the membrane bilayers as a direct result of its more polar configuration relative to other carotenoids^{3,10-14}. Carotenoids with polar end groups like *Astaxanthin* span the lipid membrane bilayer with their end groups located near the hydrophobic-hydrophilic interface, where free-radical attack first occurs.

Haematococcus pluvialis is believed to accumulate the highest levels of *Astaxanthin* in nature. Commercially grown *Haematococcus pluvialis* can accumulate more than 40g of *Astaxanthin* per kilo of dry biomass (see Table 1).

Table 1: Natural sources of *Astaxanthin*

<i>Astaxanthin</i> natural sources	<i>Astaxanthin</i> concentration (ppm)
Salmonids	~50
Plankton	~100
Krill	~120
Arctic shrimp	~1200
Phaffia yeast	~8000
<i>Haematococcus pluvialis</i>	~40,000

The primary use of synthetic *Astaxanthin* today is as an animal feed additive to impart coloration to salmonids (salmon and trout), as well as to red sea bream and tai. In natural habitats, salmonids obtain their coloration from natural food sources, including algae and crustaceans. However in fish farms, the absence of natural pigmentation sources results in salmonids with off-white coloration, imparting an artificial and unattractive look for consumers and making the fish difficult to market.

Today, essentially all commercial *Astaxanthin* for aquaculture is produced synthetically from petrochemical sources, with an annual turnover of over \$200 million, and a selling price of ~\$2000 per kilo of pure *Astaxanthin*.

Other developing applications for synthetic *Astaxanthin* include poultry and egg production.

In recent years, there has been a growing trend toward using natural ingredients in all forms of food nutrients, resulting from increasing concerns for consumer safety and regulatory issues over the introduction of synthetic chemicals into the human food

chain. This is also true for the nutraceutical and cosmeceutical markets.

Good examples of commercially important naturally derived carotenoids are beta-carotene, lycopene, lutein and zeaxanthin, commercial carotenoids with antioxidant properties which have become popular ingredients in many vitamin and mineral supplements. Beta-carotene and lycopene can be produced both synthetically (from petrochemicals) and naturally. A decade ago, natural beta-carotene accounted for a tiny percentage of the total world market. Since then, that market has increased several-fold and today, natural beta-carotene accounts for 15 to 20% of world demand¹⁵. Virtually all nutraceutical producers use natural rather than synthetic carotenoids, and pay premium prices as much as five times that of the synthetic product.

The demand for natural *Astaxanthin* is now emerging in the multi-billion dollar nutraceutical market, and increasingly, medical researchers believe that *Astaxanthin* may have significant pharmaceutical applications. While only a negligible part of today's market, the demand for such applications is expected to grow significantly in the near term as a result of numerous medical studies performed during the last 5 years in the area of *Astaxanthin* applications.

This review supports the conviction that a daily dose of 4 to 6 mg of *Astaxanthin* is of tremendous importance for health management, by protecting body tissues from the oxidative stress caused by free radicals, among others.

Astaxanthin producers have conducted several studies in recent years to demonstrate the safety of natural *Astaxanthin* derived from *Haematococcus*¹⁶⁻¹⁸. A randomized, double-blind, placebo-controlled, 8-week trial designed to determine the safety of *Astaxanthin* in 35 healthy adults was published recently¹⁹. Results revealed that healthy adults can safely consume 6mg of *Astaxanthin* per day from *Haematococcus pluvialis* algal extract.

Based on recent findings, we believe that a daily dose of *Astaxanthin* will have an important influence in preventing a broad array of diseases. Moreover, small daily doses of *Astaxanthin* may prevent or delay the onset of some diseases, thus saving society significant sums of money.

number 3 (see Fig.1). Quite simply stated, chirality and stereo differentiation are crucial factors in biological activity because in nature, at a molecular level, asymmetry dominates biological processes, such as enzymatic and most immunological reactions. Chirality is not a prerequisite for bioactivity but in bioactive molecules where one or more chiral centers are present, great differences are usually observed in the activities of the different enantiomers. This is a general phenomenon that applies to many bioactive substances, such as drugs, flavours, fragrances and food additives.

A recent study showed that farmed salmon, like most of the salmon sold in supermarkets, can be easily distinguished from wild salmon in its *Astaxanthin* isomers, because farmed salmon are fed synthetic *Astaxanthin*²⁰. The pigment in wild salmon is found overwhelmingly in the 3S-3'S enantiomeric form, the same form as that found in *Haematococcus*. Synthetic *Astaxanthin* from petrochemical sources contains a mixture of all the enantiomers of *Astaxanthin*, as a direct result of its chemical synthesis, primarily (~50%) the 3R-3'S enantiomer (the meso form). Indeed, in an elegant human study, Østerlie and co-workers⁷⁴⁻⁷⁶ found that humans selectively absorb the different isomers and their relative concentrations were found to differ in various organs. It is important to note that nearly all studies showing *Astaxanthin*'s health-beneficial effects in humans were performed on the stereoisomer found in *Haematococcus*, 3S-3'S. Although the other stereoisomers may not be harmful, no significant biological effect has been established.

Moreover, natural *Astaxanthin* exists in algae and fish as mono- and di-esters of fatty acids, while synthetic *Astaxanthin* is produced and sold for salmon farming as free hydroxy *Astaxanthin*. In nutraceutical applications as well, scientists have proven that one of the main advantages of natural *Astaxanthin* esters is that the esterified form is inherently more stable than the free form, providing for a significantly longer shelf-life without being oxidized. Several recent studies clearly showed the positive effect of *Astaxanthin* esters mixed with fat formulations on the oral bioavailability of *Astaxanthin* in humans^{21,22}.

The production of natural *Astaxanthin* by *Haematococcus pluvialis*

The microalga *Haematococcus pluvialis* synthesizes and accumulates *Astaxanthin* to relatively high levels. The commercial

production process is based on two distinct cultivation stages. The first is called the "Green Stage," which starts indoors with a single-cell colony of the microalga, and continues outdoors in solar-powered photobioreactors. The aim of this stage is to produce plenty of viable, unstressed "green" algal cells by normal cell-division process (see Fig. 2). The "Green Stage" provides optimal growth conditions in order to achieve maximal biomass production rate. The second cultivation stage is the "Red Stage" (see Fig. 2), in which the algal cells synthesize and accumulate the pigment *Astaxanthin*.

This stage starts by subjecting the cells to severe stress conditions, mainly high radiation intensity and changes in growth media. As a result, the *Haematococcus* cells start to form cysts by producing thick cell walls, and to synthesize and accumulate *Astaxanthin* in its esterified form. Cultivating the algal culture in closed systems allows an environmentally

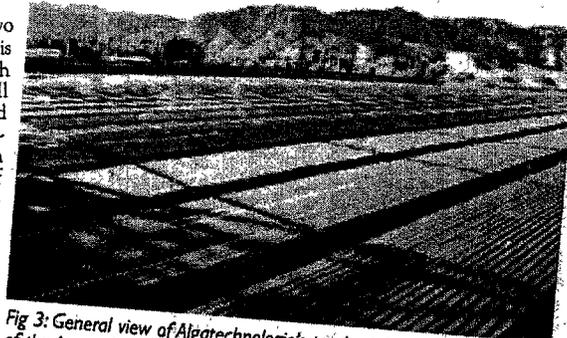


Fig 3: General view of Algatechnologie's production plant in the heart of the Arava desert in Israel

controlled process with less biological and chemical contamination. Following the "Red process", the level of *Astaxanthin* in the "red cells" may reach up to ~4% of their dry weight. The *Astaxanthin* content of the "red cells" is correlated to the severity of the stress conditions, mainly to the light flux through the culture. In due time, the "red culture" is pumped to the down-processing

area, where the cells are cracked (to render the pigment bioavailable), dried, and vacuum-packed. *Haematococcus* oleoresin is produced in an additional step, using the CO₂ Supercritical Fluid Extraction process. Increasingly, both consumers and regulatory agencies are requiring extracts that contain no residual solvents. U.S. Nutra of Eustis, FL, has the technology to extract *Haematococcus* with CO₂ and without any co-solvents.

Very few companies commercially produce *Astaxanthin* from *Haematococcus pluvialis*. The Hawaiian companies Cyanotech Corporation and Mera Pharmaceuticals cultivate the algae using an open pond system for the "Red Stage." The Japanese company Fuji Chemicals operates an indoor facility in Sweden and its "dome-shaped" bioreactors in Hawaii. The Israeli company Algatechnologies uses tubular solar-powered photobioreactors for both the "Green and Red stages" in closed, strictly controlled systems (Figs 3 and 4).

Table 2: Selected patents on Astaxanthin health and nutrition applications

Patent number	Company	Patent Title
EP0786990	US-NUTRACEUTICALS	Use of Astaxanthin for retarding and ameliorating central nervous system and eye damage
EP 1217996	ASTACAROTENE	Use of Astaxanthin for treatment of autoimmune diseases, arthritis and inflammatory diseases
US6475547	ASTACAROTENE	Immunochemical milk production and use thereof
WO0023064	ASTACAROTENE	Treatment of Dyspepsia
US6410602	ASTACAROTENE	Method for increasing the production and improving the quality of cream
US6135015	ASTACAROTENE	Method of the prophylactic treatment of Measles
US6262316	ASTACAROTENE	Oral preparation for hepatohepatic and therapeutic treatment of Hepatitis B infection
US6245818	ASTACAROTENE	Method for improvement of brain function, function of peripheral nerves and disorders of nerves
US6054491	ASTACAROTENE	Use of Astaxanthin in the production of breeding and production animals
US5744502	ASTACAROTENE	Method for increasing the production of breeding and production animals in aquaculture industry
US6133025	CYANOTECH	Method for producing and separating astaxanthin by UV light
US6344070	CYANOTECH	Method for producing and separating astaxanthin, astaxanthin, and astaxanthin
US6238855	CYANOTECH	Method of retarding and ameliorating central nervous system
EP 1283038	SUNTORY LTD	Compositions containing astaxanthin
WO0203556	YAKURAHIROSHICE	Medicine composition having astaxanthin as an active ingredient
WO03003848	AANESSEN BERT ANNIE	Use of astaxanthin in the production of nutraceuticals
WO0709053	BULLCHEN INC. CO.	Acidic solution for eye consulting treatment
FR20000451572	PACIFIC COAST CO.	Healthy nutrition supplement containing astaxanthin
WO02058483	BYUNCORED	Use of astaxanthin in nutraceuticals
NZ299641	BURTOR AND HARGREAVES	Use of astaxanthin in pharmaceuticals
US6277417	YOUNG AND RUBICAM	Method of inhibiting astaxanthin synthesis
US2001778304	ANDERSON AND HETHERINGTON	Method of inhibiting the synthesis of astaxanthin
EP 0276771	SUNTORY AND HANEDA	Method of producing astaxanthin



Fig 4: Algatechnologies "Red-stage" solar photobioreactors - general view

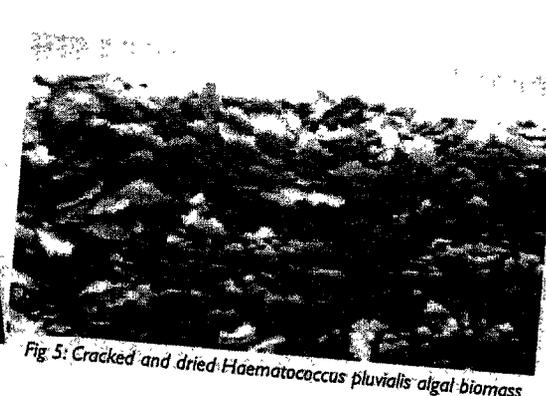


Fig 5: Cracked and dried-Haematococcus pluvialis algal biomass

Algatechnologies produces its *Astaxanthin* from the microalga *Haematococcus pluvialis* according to its patented biocontrolled growing process¹. The plant is located in the southern part of Israel, in the Arava Desert, near the resort city of Eilat, thus exploiting the area's high solar radiation year-round.

The major parameters used to assess high-quality commercial *Haematococcus* biomass and oleoresins are high *Astaxanthin* content in the product, low levels of biological and chemical contamination, and excellent stability of the *Astaxanthin* in the product. Producing *Astaxanthin* in a closed system throughout the entire process ("Green" and "Red") in an area with high solar-radiation intensity year-round, as in the case of Algatechnologies, yields high-quality *Astaxanthin* products (see Fig. 5). This algal biomass contains ~4% of its dry weight as *Astaxanthin*. The production of the algal

biomass in flake form (as with Algatechnologies' dry biomass), offers additional clear advantages when an extraction process is required for the production of high-quality oleoresin with ~10% *Astaxanthin* concentration.

Medical and nutraceutical applications of astaxanthin

Medical researchers have shown that *Astaxanthin* may have significant pharmaceutical applications. In-vitro experiments, in-vivo pre-clinical studies and early-stage clinical trials have clearly indicated the possibility that *Astaxanthin* itself, or in conjunction with other components, behaves like a prophylactic and curing agent against various diseases and health conditions (See Tables 2 and 3):

Conclusions and Product Future

Numerous scientific papers indicate that natural *Astaxanthin* has great potential as a

superb antioxidant with beneficial effects on various human diseases and physiological phenomena. The advanced commercial production of natural *Astaxanthin* from the microalga *Haematococcus pluvialis* supplies the market with high-quality products rich in *Astaxanthin*, suitable for human applications. Further clinical data will strengthen the scientific basis for the role of natural *Astaxanthin* as a unique and efficient anti-oxidant and for its use in human health. We expect that in a few years' time, the natural *Astaxanthin* market will significantly increase its volume and line of applications. New specific *Astaxanthin*-based products will be designed for the treatment of specific diseases. Some of these new products will contain other components as well (the "cocktail concept"), such as other carotenoids, antioxidants, vitamins, poly-unsaturated fatty acids, minerals, and more. The natural *Astaxanthin* market will become sophisticated and multi-product, and will include products for the food, food coloring, cosmetics and pharmaceutical industries as well.

Table 3: Selected articles on Astaxanthin applications for human and mammalian health

Disease	Reference
CENTRAL NERVOUS SYSTEM AND NEURODEGENERATIVE DISEASES	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100
EYE HEALTH	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100
JOINT HEALTH/MUSCLE ENDURANCE	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100
INFLAMMATION AND IMMUNE SYSTEM	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100
CARDIOVASCULAR HEART LIPID PEROXIDATION AND BLOOD	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100
CANCER	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100
FERTILITY	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100
SUNBURN AND SKIN HEALTH, ANTIAGING AND ANTIWINKLING	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100
BENIGN PROSTATIC HYPERPLASIA (BPH)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100

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Photos courtesy Rubi Rubenstein

References

- Boussiba S.; Voneshik A.; Cohen Z.; Richmond A. (1997) A procedure for large scale production of *Astaxanthin* from *Haematococcus*. WO 97/28274.
- Papas, A.M. (1999) Antioxidant Status, Diet, Nutrition, and Health. CRC Press.
- Rakaza, P. and Kinsley, N.L. (1992) Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. Arch. Biochem. Biophys. 297: 291-295.
- Naguib, Y.M.A. (2000) Antioxidant activities of astaxanthin and related carotenoids. J. Agric. Food Chem. 48: 1150-1154.
- Chew B.P. and Park J.S. (October 2003) Dissertation in Supply Side West, Las Vegas.
- Tso, M.O.M. and Lam, T.-T. (1996) Method of Retarding and Ameliorating Central Nervous System and Eye Damage. Assigned to U. S. Nutraceuticals. U.S. Patent #5527533.
- Faccinetti F.; Dawson V.L.; Dawson T.M. (1998) Free radicals as mediators of neuronal injury. Cell Mol Neurobiol. 18(6):667-82.
- Lorenz, R.T. and Cysewski, G.R. (2000) Commercial potential for *Haematococcus* micro-algae as a natural source of astaxanthin. Trends in Biotechnol. 18: 160-167.
- Guerin, M.; Huntley M. E.; and Olajola M. (2003) *Haematococcus* astaxanthin: applications for human health and nutrition. Trends in Biotechnol. 21: 210-216.
- Shibata A.; Kiba Y.; Akai N.; Fukuzawa K.; Terada H. (2001) Molecular characteristics of astaxanthin and beta-carotene in the phospholipid monolayer and their distributions in the phospholipid bilayer. Chem Phys Lipids 113(1-2): 11-22.
- Goto S.; Kogure K.; Abe K.; Kimata Y.; Kitahara K.; Yamashita E.; Terada H. (2001) Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent anti-oxidative activity of the carotenoid astaxanthin. Biochim Biophys Acta 1512(2): 251-8.
- Oshima S.; Ojima F.; Sakamoto H.; Ishiguro Y.; Terao J. (1993) Inhibitory effect of beta-carotene and astaxanthin on photosensitized oxidation of phospholipid bilayers. J Nutr Sci Vitaminol (Tokyo) 39(6): 607-15.
- Kurashige M.; Okimasu E.; Inoue M.; Utsuki K. (1990) Inhibition of oxidative injury of biological membranes by astaxanthin. Physiol Chem Phys Med NMR 22(1): 27-38.
- Nakagawa K.; Kang S.D.; Park D.K.; Handelman G.J.; Miyazawa T. (1997) Inhibition of beta-carotene and astaxanthin of NADPH-dependent microsomal phospholipid peroxidation. J. Nutr. Sci. Vitaminol (Tokyo) 43(3): 345-55.
- Bahner, B. (1993) Beta -carotene use grows: natural sector in flux. Chemical Market Reporter 244:26-27.
- Cyentech Corporation - Technical paper available at <http://www.cyentech.com/ods/axbul60.pdf>
- Mera Pharmaceuticals (1999) *Haematococcus pluvialis* and astaxanthin safety for human consumption. Technical Report TR:3005.001 available at <http://www.astaxfactor.com/techreports/tr3005.001.htm>
- La Hays - US Nutraceuticals technical paper available at <http://www.lahays.com/safety.html>
- Spiller GA.; Dewell A. (2003) Safety of an Astaxanthin-Rich *Haematococcus pluvialis* Algal Extract: A Randomized Clinical Trial. J Med Food 6(1): 51-6.
- Turjuman, S.A. et al. (1997) Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. J AOAC Int. 80: 822-822.
- Miercke Ojalongo, J.; Lippell A.; Pettersson A.; Hoglund P. (2003) Oral bioavailability of the anti-
- oxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations. Eur J Pharm Sci 19(4): 293-304.
- Gierk RM.; Yao L.; She L.; Furr HC. (2000) A comparison of lycopene and astaxanthin absorption from corn oil and olive oil emulsions. Lipids 35(7): 803-8.
- Snodderly D.M. (1995) Evidence for protection against age related macular degeneration by carotenoids and antioxidant vitamins. Amer J. Clinical Nutrition. 63(6):1448S-1461S.
- Ogami K.; Shiratori K.; Kotake S.; Nishida T.; Mizuki N.; Yazawa K.; Ohno S. (2003) Effects of astaxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo. Invest Ophthalmol Vis Sci. 44(6): 2694-701.
- Waagbø R.; Hamre K.; Bjerkes E.; Berge R.; Watnø E.; Lie O.; Torstensen B. (2003) Cataract formation in Atlantic salmon, *Salmo salar* L., smolt relative to dietary pro- and antioxidants and lipid level. J Fish Dis. 26(4): 213-229.
- O'Connor L.; O'Brien N. (1999) Modulation of UVA light-induced oxidative stress by beta-carotene, lutein and astaxanthin in cultured fibroblasts. J Dermatol Sci. 16(3): 226-30.
- Malmsten C. (1998) Dietary supplementation with

- astaxanthin rich algal meal improves muscle endurance—a double blind study on male students. Unpublished study from the Karolinska Institute, Gustavberg, Sweden.
- 28 Lignell A. (2001) Medicament for improvement of duration of muscle function or treatment of muscle disorders or diseased. US patent # 6,245,818 Astaxantolene AB Sweden.
- 29 Bennedson, M. et al. (1999) Treatment of *H. pylori* infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes. *Immunol Lett.* 70: 185-189.
- 30 Wang X.; Willen R.; Wadstrom T. (2000) Astaxanthin-rich algal meal and vitamin C inhibit *Helicobacter pylori* infection in BALB/c mice. *Antimicrob Agents Chemother* 44(9): 2452-7.
- 31 Lignell A. (1998) Treatment of Dyspepsia. Patent no. WO 00/23064. Available at www.ubave.com/muscle_endurance_whiletapars.html
- 32 Anderson T.; Petterson S. (2001) Method of inhibiting the expression of inflammatory cytokines. Patent no. WO 01/72266.
- 33 Lorenz T. et al. (2000) Method of retarding Ameliorating Carpal tunnel syndrome. US Patent #. 6,258,855.
- 34 Hughes D.A. (1999) Effects of dietary antioxidants on the immune function of the middle aged. *Proc. Nutr. Soc.* 58(1): 79-84.
- 35 Lignell A. (2001) Use of xanthophylls, Astaxanthin e.g. for the treatment of auto-immune diseases, chronic viral and intracellular bacterial infections. Patent # WO 01/24787.
- 36 Lignell A. et al. (2000) Method for the prophylactic treatment of mastitis. WO99/30701.
- 37 Jyonouchi, H. et al. (1995) Effect of carotenoids on in vitro immunoglobulin production by human peripheral blood mononuclear cells: astaxanthin, a carotenoid without vitamin A activity, enhances in vitro immunoglobulin production in response to a T-dependent stimulant and antigen. *Nutr. Cancer* 23: 171-183.
- 38 Lee SJ.; Bai SK.; Lee KS.; Namkoong S.; Na H.J.; Ha KS.; Han J.A.; Yim S.V.; Chang K.; Kwon Y.G.; Lee S.K.; Kim Y.M. (2003) Astaxanthin inhibits nitric oxide production and inflammatory gene expression by suppressing I κ B kinase-dependent NF- κ B activation. *Mol Cells Korea* 16(1): 97-105.
- 39 Chew BP.; Wong MW.; Park J.S.; Wong T.S. (1999) Dietary beta-carotene and astaxanthin but not canthaxanthin stimulate splenocyte function in mice. *Anticancer Res.* 19(6B): 5223-7.
- 40 Jyonouchi H.; Zhang L.; Tomita Y. (1993) Studies of immunomodulating actions of carotenoids. II. Astaxanthin enhances in vitro antibody production to T-dependent antigens without facilitating polyclonal B-cell activation. *Nutr Cancer* 19(3): 269-80.
- 41 Jyonouchi H.; Hill R.J.; Tomita Y.; Good RA. (1991) Studies of immunomodulating actions of carotenoids. I. Effects of beta-carotene and astaxanthin on murine lymphocyte functions and cell surface marker expression in vitro culture system. *Nutr Cancer* 16(2): 93-105.
- 42 Akyon Y. (2002) Effect of antioxidants on the immune response of *Helicobacter pylori*. *Clin Microbiol Infect.* 8(7): 438-41.
- 43 Okai Y.; Higashi-Okai K. (1996) Possible immunomodulating activities of carotenoids in in vitro cell culture experiments. *Int J Immunopharmacol* 18(12): 753-8.
- 44 Jyonouchi H.; Sun S.; Mäkelä M.; Gross MD. (1996) Effects of various carotenoids on cloned, effector-stage T-helper cell activity. *Nutr Cancer* 26(3): 313-24.
- 45 Jyonouchi H.; Sun S.; Tomita Y.; Gross MD. (1995) Astaxanthin, a carotenoid without vitamin A activity, augments antibody responses in cultures including T-helper cell clones and suboptimal doses of antigen. *J Nutr* 125(10): 2483-92.
- 46 Jyonouchi H.; Zhang L.; Gross M.; Tomita Y. (1994) Immunomodulating actions of carotenoids: enhancement of in vivo and in vitro antibody production to T-dependent antigens. *Nutr. Cancer* 21(1): 47-58.
- 47 Murillo E. (1992) Cholesterolemic effects of canthaxanthin and astaxanthin in rats. *Arc Latinoam Nutr.* 42: 409-13.
- 48 Iwamoto I.; Hoosoda K.; Hirano R.; Kurata H.; Matsumoto A.; Miki W.; Kamiyama M.; Nakasu H.; Yamamoto S.; Kondo K. (2001) Inhibition of low-density lipoprotein oxidation by astaxanthin. *J Atheroscler Thromb* 7: 216-22.
- 49 Zhang L.X.; Cooney RV.; Bertram JS. (1991) Carotenoids enhance gap junction communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemo-preventative action. *Carcinogenesis* 12(11): 2109-14.
- 50 Aoi W.; Nario Y.; Sakuma K.; Kuchida M.; Tokuda H.; Maoka T.; Toyokuni S.; Oka S.; Yasuhara M.; Yoshikawa T. (2003) Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice. *Antioxid Redox Signal* 5(1): 139-44.
- 51 Kang J.O.; Kim S.J.; Kim H. (2001) Effect of astaxanthin on the hepatotoxicity, lipid peroxidation and antioxidant enzymes in the liver of CCl₄-treated rats. *Methods Find Exp Clin Pharmacol.* 23(2): 79-84.
- 52 Murillo E. (1992) Hypercholesterolemic effect of canthaxanthin and astaxanthin in rats. *Arch Latinoam Nutr* 42(4): 409-13.
- 53 Rengel D.; Diez-Navajas A.; Serra-Rico A.; Veiga P.; Muga A.; Milcua J.C. (2000) Exogenously incorporated ketocarotenoids in large unilamellar vesicles. Protective activity against peroxidation. *Biochim Biophys Acta* 1463(1): 179-87.
- 54 Jyonouchi H.; Sun S.; Iijima K.; Gross MD. (2000) Antitumor activity of astaxanthin and its mode of action. *Nutr cancer* 36(1): 59-65.
- 55 Kurihara H.; Koda H.; Asami S.; Kiso Y.; Tanaka T. (2002) Contribution of the anti-oxidative property of astaxanthin to its protective effect on the promotion of cancer metastasis in mice treated with resistant stress. *Life Sci.* 70(21): 2539-20.
- 56 Tanaka T.; Makita H.; Ohnishi M.; Mori H.; Satoh K.; Hara A. (1995) Chemoprevention of rat oral carcinogenesis by naturally occurring xanthophylls, astaxanthin and canthaxanthin. *Cancer Res.* 55 (19): 4059-4064.
- 57 Tanaka T.; Morishita Y.; Suzuki M.; Kojima T.; Okumura A.; Mori H. (1994) Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoid astaxanthin. *Carcinogenesis* 15(1): 15-19.
- 58 Tanaka T.; Kawamori T.; Ohnishi M.; Makita H.; Mori H.; Satoh K.; Ha A. (1995) Suppression of azoxymethane-induced rat colon carcinogenesis by dietary administration of naturally occurring xanthophylls, astaxanthin and canthaxanthin during the postinitiation phase. *Carcinogenesis* 16(12): 2957-63.
- 59 Rousseau E.J.; Dawson A.J.; Dunn B. (1992) Protection by beta-carotene and related compounds against oxygen-mediated cytotoxicity and genotoxicity: implications for carcinogenesis and anticarcinogenesis. *Free Radic Biol Med* 13(4): 407-33.
- 60 Kozuki Y.; Miura Y.; Yagasaki K. (2000) Inhibitory effects of carotenoids on the invasion of rat ascites hepatoma cells in culture. *Cancer Lett* 151(1): 111-5.
- 61 Caradet S.; Le Bon AM.; Barges R.; Suschietti M.; Astorg P. (1998) Dietary carotenoids inhibit aflatoxin B₁-induced liver neoplastic foci and DNA damage in the rat: role of the modulation of aflatoxin B₁ metabolism. *Carcinogenesis* (3): 403-11.
- 62 Black HS. (1998) Radical interception by carotenoids and effects on UV carcinogenesis. *Nutr Cancer* 31(3): 212-7.
- 63 Nishino H.; Murakoshi M.; Ii T.; Takemura M.; Kuchida M.; Kanazawa M.; Mou XY.; Wada S.; Masuda M.; Otsuka Y.; Yagasaki S.; Satomi Y.; Jinno K. (2002) Carotenoids in cancer chemoprevention. *Cancer Metastasis Rev.* 21(3-4): 257-64.
- 64 Li Z.; Wang Y.; Mo B. (2002) The effects of carotenoids on the proliferation of human breast cancer cell and gene expression of bcl-2. *Zhonghua Yu Fang Yi Xue Za Zhi.* 36(4): 254-7.
- 65 Chew BP.; Park J.S.; Wong MW.; Wong T.S. (1999) A comparison of the anticancer activities of dietary beta-carotene, canthaxanthin and astaxanthin in mice in vivo. *Anticancer Res* 19(3A): 1849-53.
- 66 Hansen KB.; Tauson AH.; Inbror J. (2001) Effect of supplementation with the antioxidant astaxanthin on reproduction, pre-weaning growth performance of kits and daily milk intake in milk. *J Reprod Fertil Suppl.* 57: 331-4.
- 67 Lignell A. et al. (1998) Method of increasing the production and improving the quality of semen. WO99/29313.
- 68 Lyons NM.; O'Brien NM. (2002) Modulatory effects of an algal extract containing astaxanthin on UVA-irradiated cells in culture. *J Dermatol Sci* (1): 73-84.
- 69 Savours N.; Briand G.; Armony-Touz MC.; Combre A.; Maudet M.; Nicol M. (1995) Vitamin A status and metabolism of cutaneous polyamines in the hairless mouse after UV irradiation: action of beta-carotene and astaxanthin. *Int J Vitam Nutr Res* 65(2): 79-86.
- 70 Sun et al. (2001) Effects of astaxanthin from *Haematococcus pluvialis* on human skin. *Fragrance Journal* 12: 98-103.
- 71 Yamahita et al. (1995) Suppression of post-UVB hyperpigmentation by topical astaxanthin from krill. *Fragrance Journal* 14: 180-185.
- 72 R. Todd Lorenz. Method for retarding and preventing sunburn by UV light. R. Patent no. US 6,433,025 B1 Cyanotech corporation.
- 73 Anderson, M. (2001) Method of inhibiting 5- α Reductase with Astaxanthin to Prevent and Treat Benign Prostate Hyperplasia (BPH) and Prostate Cancer in Human Males. US Patent #6277417.
- 74 Østerlie M.; Bjerkeng B.; Låaen-Jensen S. (1999) On bioavailability and deposition of beta-Z-isomers of astaxanthin in pigments. *In Food Technology, Proceedings of 1st International Congress PFT (Miguez Mosquera, M. I.; Galan, M. J.; Mendez, D. H., eds) pp: 157-161. International Congress on Pigments in Food Technology, Sevilla, Spain*
- 75 Østerlie M.; Bjerkeng B.; Låaen-Jensen S. (1999) Blood appearance and distribution of astaxanthin E/Z isomers among plasma lipoproteins in humans administered in a single meal with astaxanthin. Abstract 2A-13. Presented at the 12th International Symposium on Carotenoids in Cairns, Queensland, Australia, July 18-23, 1999.
- 76 Østerlie M.; Bjerkeng B.; Låaen-Jensen S. (2000) Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration of astaxanthin. *J. Nutr. Biochem.* 11: 462-490.