

ASTAXANTHIN

**Natural Antioxidant for Neuro-protection,
Vision Enhancement & Skin Rejuvenation**

■ **Astaxanthin-5**

(Oil, Food Grade)

■ **Astaxanthin-20**

(Oil, Food Grade)

■ **Astaxanthin-P1**

(Powder, Food Grade)

■ **Astaxanthin-LS1**

(Water-soluble Liquid, Food Grade)

■ **Astaxanthin-5C**

(Oil, Cosmetic Grade)

■ **Astaxanthin-20C**

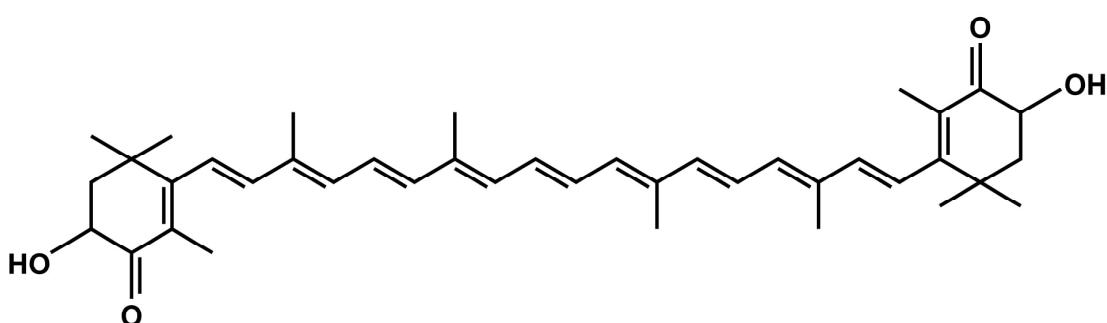
(Oil, Cosmetic Grade)

■ **Astaxanthin-PC1**

(Powder, Cosmetic Grade)

■ **Astaxanthin-LSC1**

(Water-soluble Liquid, Cosmetic Grade)



ORYZA OIL & FAT CHEMICAL CO., LTD

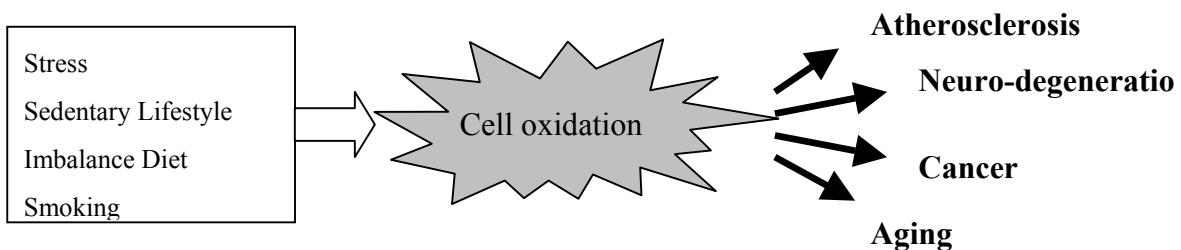
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ASTAXANTHIN

Natural Antioxidant for brain,
Vision Enhancement and Skin Rejuvenation

1. Introduction

Aging and degenerative diseases (e.g. cerebral stroke, atherosclerosis, cancer etc.) are inevitable healthcare problems of the modern society. Free radicals have been documented to be the damaging element for health. Stress, irregular lifestyle, unbalance diet & smoking are contributing factors to the generation of free radicals, active oxygen which accelerates the process of ageing. Antioxidants are becoming popular for the health improvement.



Astaxanthin is a naturally occurring carotenoid pigment with excellent antioxidant effect extracted and purified from *Haematococcus* algae. Astaxanthin has been documented to protect against free radicals and promote numerous health functions.

2. Astaxanthin

Astaxanthin is a red-pigment carotenoid occurring naturally in a wide variety of living organisms and classified as a xanthophyll. It has a chemical structure similar to that of the familiar carotenoid β-carotene (Fig. 1). It is commonly found in crustaceans (e.g. shrimps, crawfish, crabs and lobster) and produced by microalgae. The pink flesh of a healthy wild salmon is due to the presence of astaxanthin. It has been suggested that astaxanthin protects muscle cells from damaging effects of active oxygen produced upon swimming upstream. Meanwhile, astaxanthin contained in salmon roe is considered to protect the roe from reactive oxygen species generated by UV rays.

The antioxidant of astaxanthin is stronger than β-carotene and vitamin E by **40x and 1,000x** respectively. Besides, astaxanthin differs from other antioxidants in its ability to penetrate the blood brain and retina barriers. Therefore, it is believed to protect the brain and nervous system from neurodegenerative diseases (e.g. cerebral thrombosis

and stroke) and aging.

Meanwhile, astaxanthin has been documented to prevent age-related macular degeneration (AMD) and enhance immune functions. Furthermore, recent studies revealed the wrinkling and moisturizing effect of astaxanthin which suggest its potential cosmeceutical applications in protection against skin aging.

Oryza Oil & Fat Chemical Co., Ltd. has commissioned the production of astaxanthin from *Haematococcus* algae in high concentration and various dosage forms for applications in nutraceutical, cosmeceuticals, food and feed industries.

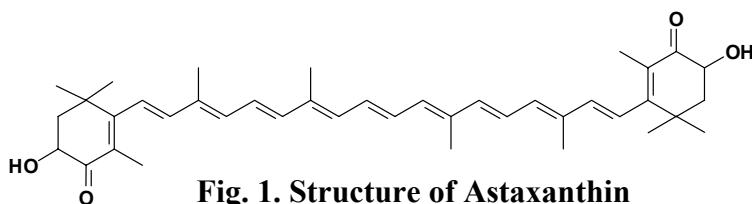


Fig. 1. Structure of Astaxanthin

Table 1: Equivalent amount of Astxanthin in food relative to recommended daily intake of 6mg per day

| | |
|----------------------------------|-----------------|
| Sockeye (1 piece 80g) | 2.4 pieces |
| Kirk Salmon (1 piece 80g) | 5 pieces |
| Salmon roe (1 table spoon 25g) | 30 table spoons |
| Prawns (1 huge prawn weight 70g) | 30 huge prawns |
| Crabs (1 cup 500g) | 1.8 cups |
| Krill | 30 g |

(equivalent to 30mg of Astaxanthin-20)

3. Antioxidant Effect

Astaxanthin has unique chemical properties based on its molecular structure ^{1) 2)}. As illustrated in Fig. 2, the presence of hydroxyl (OH) and ketone (C=O) moieties on each ionone ring along with an extension of conjugated double bond system explained the potency of astaxanthin with higher antioxidant activity compared to β -carotene and vitamin E. The 2 most prominent antioxidant activities of astaxanthin are quenching of singlet oxygen and inhibition of lipid peroxidation.

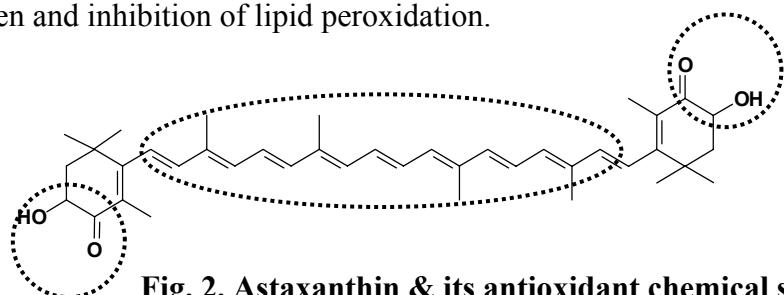


Fig. 2. Astaxanthin & its antioxidant chemical site

- 1) Goto,S.,Kogure,K.,Abe,K. *et al.*: Efficient radical trapping at the surface and inside the phospholipids membrane is responsible for highly potent antioxidative activity of the carotenoid astaxanthin, *Biochem. Biophys.Acta*, **1521**,251-258(2001)
- 2) W.Miki: Biological functions and activities of animal carotenoids, *Pure Appl. Chem.*, **63**,141-146(1991).

(1) Singlet Oxygen (${}^1\text{O}_2$) Quenching Ability

Singlet Oxygen (${}^1\text{O}_2$) is produced by photosensitization upon exposure to light. It is highly reactive as it damages proteins structure in the body and oxidizes protein residues such as methionine, triptophan, histidine, and cysteine. In addition, singlet oxygen oxidizes unsaturated fatty acids of cell membrane producing lipid peroxides.³⁾

Among series of studies, one research group compared the singlet oxygen quenching abilities of several carotenoids: astaxanthin, canthaxanthin, zeaxanthin, β -carotene, fucoxanthin and halocynthiaxanthin. Singlet oxygen (${}^1\text{O}_2$) was generated by naphthalene-derived endoperoxide; known amount of carotenoids was added and the quenching rate constant (Kq) was calculated from the decrease in singlet oxygen-generated infrared chemiluminescence ³⁾⁴⁾⁵⁾ The Kq value of astaxanthin was reported to be the highest; 3.3×10^{-9} , while the commonly known β -carotene has a Kq value of 0.089×10^{-9} (Table 2). Hence, astaxanthin has an increased singlet oxygen quenching ability over the corresponding alkyl carotenoid, β -carotene.

Table 2. Singlet Oxygen Quenching Rate Constants
[Test Reagent : CDCl₃/CD₃OD(2:1)]

| Carotenoids | Singlet Oxygen Quenching Rate (10 ⁻⁹ Kq) | |
|--------------------|--------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
| Astaxanthin | 3.3 |  Approximately 40x |
| Cantaxanthin | 2.1 | |
| Zeaxanthin | 0.22 | |
| β-carotene | 0.089 | |
| Fucoixanthin | 0.009 | |
| Halocynthiaxanthin | 0.004 | |

- 3) Shimizu N., Miki A.: Measurement of carotenoids' singlet oxygen scavenging ability using a chemiluminescence detector, Summary of Lectures, Speing Meeting of Japanese Society of Fisheries Science 1992, 322 (1992)
- 4) Shimizu N., Goto M., Miki A., et al.: Carotenoids' singlet oxygen scavenging ability, Summary of Lectures, 6th Carotenoid Study Meeting, 26 (1992)
- 5) Miki A.: Carotenoids from Marine World – Metabolism and Bioactivity, Science of Fisheries Series 94, 1997, Koseisha Koseikaku, Tokyo

(2) Inhibition of Lipid Peroxidation

Lipid Peroxidation is believed to contribute to the development of diseases such as atherosclerosis, inflammation, aging and cancers. Peroxidation of unsaturated fatty acids destroy structure of cell membrane leading to functional disorders of receptors and proteins within cell membrane⁶⁾⁷⁾⁸⁾.

Xanthophylls such as astaxanthin and zeaxanthin have been documented to inhibit lipid peroxidation by inhibiting the production of lipid hydroperoxide (LOOH) upon oxidative damage of fatty acids and other lipids⁶⁾⁷⁾⁸⁾. Astaxanthin has been found to inhibit lipid peroxidation in rat hepatic mitochondria induced by ADP/Fe²⁺. Its inhibitory activity (ED₅₀) has been reported to be **1,000 times more potent than conventional α-tocopherol (vitamin E)** (Fig.3)⁹⁾¹⁰⁾.

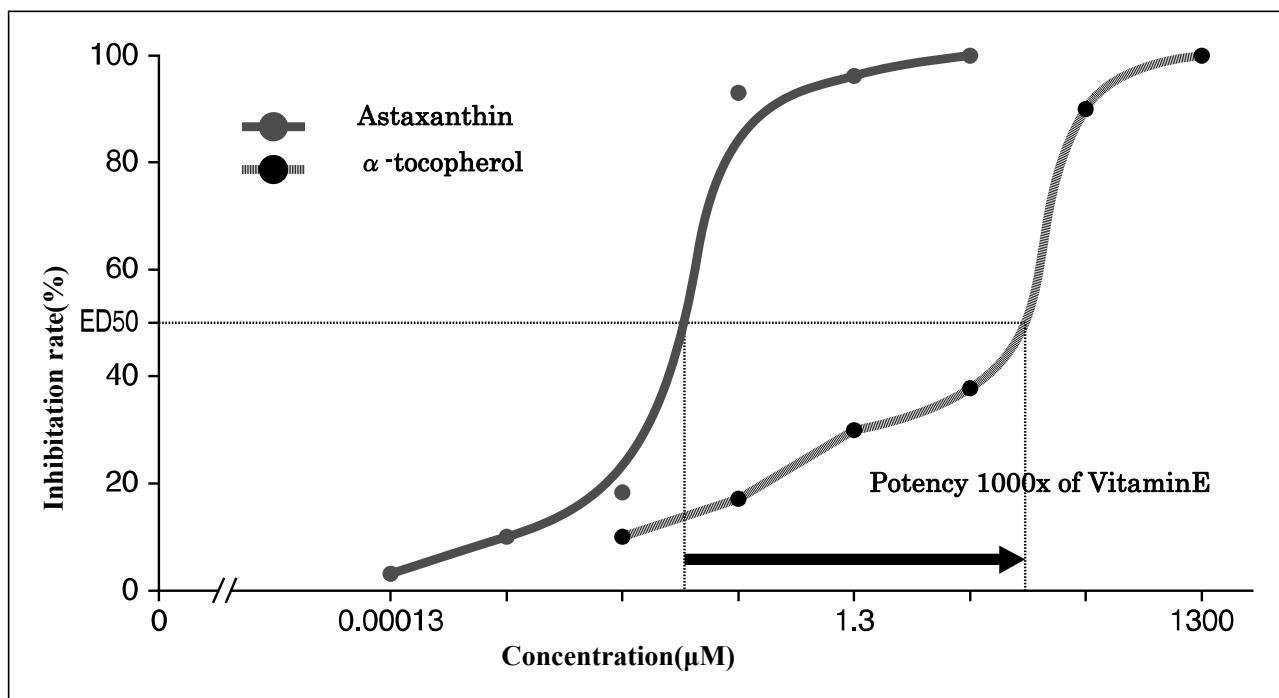


Fig. 3. The Effect of Astaxanthin & α -tocopherol on Lipid Peroxidation on Rat Hepatic Mitochondria induced by Fe^{2+} .

(Cyto-protection & Biology, 7, 383-391 (1989); Modified and updated)

- 6) B. P. Lim, A. Nagano, J. Terao et al.: Antioxidant activity of xanthophylls on peroxy radical-mediated phospholipid peroxidation, *Biochem. Biophys. Acta*, **1126**, 178-184 (1992).
- 7) J. Terao: Antioxidant activity of β -carotene related carotenoids in solution, *Lipids*, **24**, 659-661 (1989)
- 8) Miki A.: Carotenoids from Marine World – Metabolism and Bioactivity, Science of Fisheries Series 94, 1997, Koseisha Koseikaku, Tokyo
- 9) W. Miki: Biological functions and activities of animal carotenoids, *Pure Appl. Chem.*, **63**, 141-146 (1991)
- 10) Kurashige Y., Okazoe Y., et al: Prevent of Free Radical Induced Biomembrane Disorders by Astaxanthin, *Cyto-protection & Biology*, **7**, 383-391 (1989).

4. The Effect of Astaxanthin on Cell Membrane

Lipid peroxidation occurs when free radicals such as reactive oxygen species oxidizes cell membrane which leads to cell damage. There are 3 main protective antioxidants in the human body, namely, vitamin E, vitamin C & β -carotene. These antioxidants supplement each other in protecting the cell membrane from multiple oxidative chained reactions¹¹⁾.

Vitamin E is present in the hydrophobic region of the cell membrane (Fig. 4). Upon lipid peroxidation by free radicals, Vitamin E will donate its free electrons to neutralize free radicals. Oxidized Vitamin E will be recycled by Vitamin C which is located in the hydrophilic region of the cell membrane. Hence, Vitamin E continues its antioxidant activity while oxidized Vitamin C is metabolism and excreted from the body.

β -carotene as a lipophilic compound, is present in the hydrophobic region of cell membrane and trap free radicals generated within the cell membrane. Astaxanthin has been reported to span the cell membrane bilayer (fat /water) because of its unique structure with polar terminal rings¹²⁾. The polar OH-group in two terminal rings of astaxanthin is likely to be oriented at/near the membrane surface while the polyene chain in the interior of the membrane¹³⁾¹⁴⁾. Accordingly, astaxanthin could be effective in scavenging reactive oxygen species at membrane surface while its polyene chain inhibit oxidative chain reaction in the membrane¹⁴⁾. Astaxanthin is an excellent antioxidant that protects the entire cellular components and cells from free radicals damage and degradation.

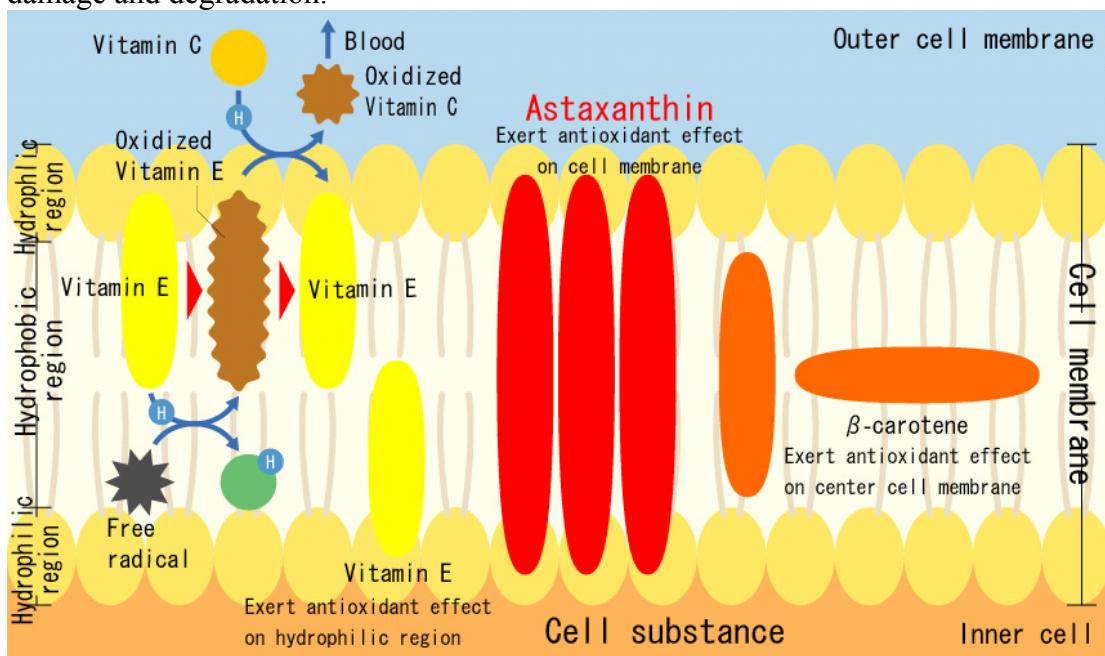


Fig. 4. Antioxidant on cell membrane

- 11) Yoshikawa S.: Introduction to Free Radicals, 1996, Sentan Igakusha, Tokyo
- 12) H.Y. Yamamoto et al.: Thermal transition and special properties of carotenoid-containing liposomes, *Biochem. Biophys. Acta*, **507**, 119-127 (1978)
- 13) Goto, S., Kogure, K., Abe, K., et al.: Efficient radical trapping at the surface and inside the phospholipids membrane is responsible for highly potent antioxidative activity of the carotenoid astaxanthin, *Biochem. Biophys. Acta.*, **1521**, 251-258 (2001)
- 14) W. Miki: Biological functions and activities of animal carotenoids, *Pure Appl. Chem.*, **63**, 141-146(1991).

5. Prevention Against Cerebral Ischemia

Previous studies documented that astaxanthin prevents brain damages due to ischemia¹⁵⁾. Astaxanthin has been documented as an antioxidant that crosses the blood-brain barrier making its availability to alleviate oxidative stresses of the brain, ocular & central nervous system. It is believed to prevent against cerebral ischemia due to oxidative stresses.

An in-house research was conducted in Oryza Oil & Fat Chemical Co., Ltd. to investigate the effect of astaxanthin on middle cerebral artery occlusion and reperfusion model of cerebral ischemia in rats.

Cerebral ischemia/Ischemia stroke occurs upon occlusion of cerebral blood flow to the core region of major brain artery resulting in cell death and infarction in the brain. Oxidative stress is a major factor in cerebral ischemia damage as the brain consumes large amount of oxygen. Transient focal ischemia/reperfusion model in rat was used to investigate the protective effect of astaxanthin as it is an antioxidant that penetrates the blood-brain barrier. Total area of infarction was measured and compared between groups treated with oral astaxanthin (equivalent to free astaxanthin 100mg/kg) and controlled.

As illustrated in Fig. 5, astaxanthin is preventive against cerebral ischemia as the total area of infarction is reduced by 40% ($p<0.01$) (Fig. 6).

Thus, astaxanthin is protective against oxidative stress of the brain at dosage of free astaxanthin 100mg/kg.

[Method of Experiment]

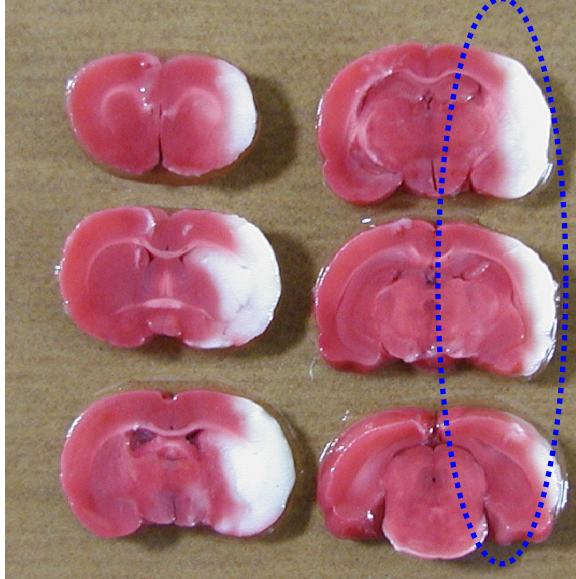
Male Wistar rats were given oral astaxanthin, 500mg/kg of Astaxanthin-20 (equivalent to free astaxanthin 100mg/kg) at 24-hour and 1-hour prior to cerebral artery occlusion. Cerebral ischemia was induced by occluding the right middle arteries using a stopper. Cerebral occlusion persisted for 1-hour followed by reperfusion of cerebral blood flow.

Rats were then given once again oral astaxanthin of similar dosage 2-hour prior to removal of brain. Brain were removed (24-hour post reperfusion of cerebral blood flow) to produce specimens of 2mm intervals. Meanwhile, living brain cells of cerebral cortex were coloured with 2% TTC solution (2, 3, 5-triphenyltetrazolium chloride/phosphate buffered saline) for 15 min at 37°C (Fig. 5). Total area of infarction (white areas as illustrated in Fig. 5) was measured using image analysis software, NIH image to determine cerebral stroke. Results were compared with rats in control group which olive oil was given as placebo. Rats with neurological symptoms with extension of left foreleg after 15 min of middle cerebral artery occlusion were used in the middle cerebral artery occlusion/reperfusion model.

Table. 3: Experimental Procedures – Middle Artery Occlusion / Reperfusion Model

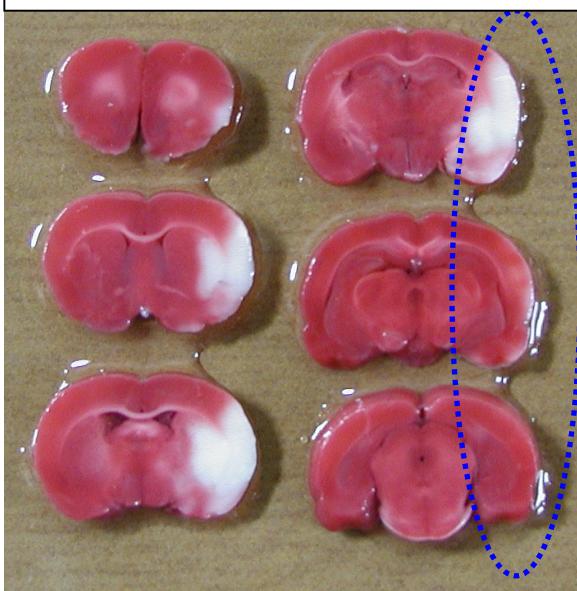
| <u>Time (hour)</u> | <u>Experiment Procedures</u> |
|--------------------|-------------------------------------------------------------------------------------------------------------|
| 0 hr | → Oral administration of 500mg/kg of Astaxanthin-20 (24-hour prior to cerebral artery occlusion) |
| 23 hr | → Oral administration of 500mg/kg of Astaxanthin-20 (1-hour prior to cerebral artery occlusion) |
| 24 hr | → Induction of Cerebral Ischemia: Occlusion of right middle cerebral arteries ↓ |
| 25 hr | → Reperfusion of cerebral blood flow |
| 47 hr | → Oral administration of 500mg/kg of Astaxanthin-20 (2-hour prior to removal of cerebral cortex) |
| 49 hr | Removal of brain → (24-hour post reperfusion of cerebral blood flow) Determination of infarction area |

Cerebral Ischemia damage of cell death (white area) in middle artery occlusion / reperfusion model



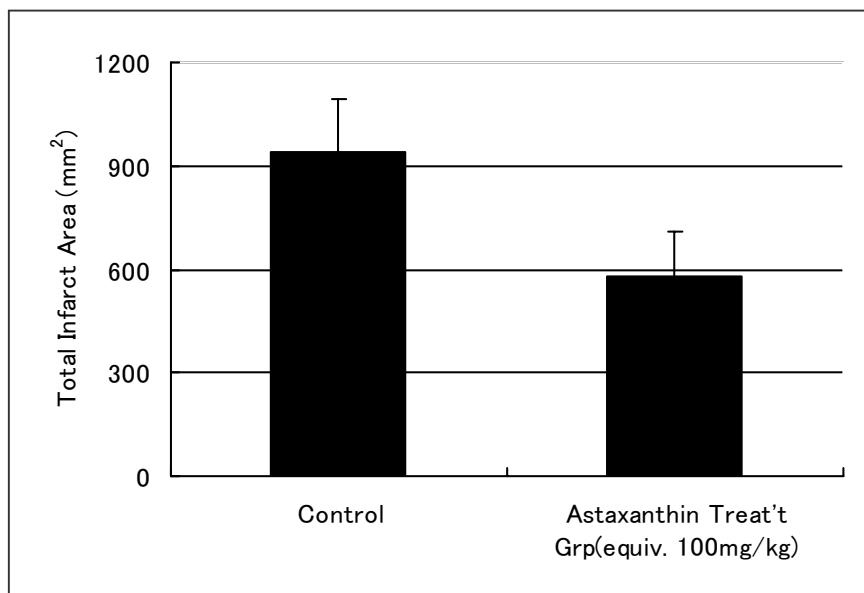
Control Group

Reduction in Cerebral Ischemia damage in the presence of astaxanthin (white area) in middle artery occlusion reperfusion model.



Astaxanthin Treatment Group

Fig. 5. Brain Specimens of Rats



** : comparison with control group ($p < 0.01$)

Fig. 6. Total Area of Infarction in Rat Model (Ave ±S.E., n=6)

- 15) Kudo,Y., Nakajima,R., Matsumoto,N. *et al.*, : Effects of astaxanthin on brain damages due to ischemia, *Carotenoid Science*, 5, 25(2002)

6. Prevention against Asthenopia & promote healthy visual accommodation

A double-blind human trial was prompted to evaluate the effect of astaxanthin on visual accommodation and asthenopia. 40 healthy subjects with complaints of asthenopia were divided into 2 groups, one group under treatment of astaxanthin 6mg daily while the other group was on placebo. The trial lasted for a duration of 4-week¹⁶⁾¹⁷⁾.

Sub-objective accommodation power, positive accommodation power and negative accommodation power were measured before and after the trial to evaluate the degree of asthenopia. As illustrated in Table 4, Figs. 7 & 8, the following findings confirmed the beneficial effects of astaxanthin:

1. Sub-objective accommodation power (rate of change) in astaxanthin treatment group was significantly higher than that of the control group ($p<0.01$)
2. Astaxanthin treatment group has higher rate of positive and negative accommodation times (rate of change) compared to those of control group.
3. The subjective degree of asthenopia in astaxanthin treatment group, namely, “blinking eye-feeling” and “tendency of irritation” significantly reduced as measured by VAS (visual analogue scale).

Oral administration of Astaxanthin improved accommodation power and subjective symptoms of asthenopia safely.

Table 4. The evaluation on ophthalmic function

(Sub-objective accommodation power, positive accommodation time & negative accommodation time)

| | Test Subjects | | | | | |
|---------------------------------------|---------------|------------|------------|-----------------------|--------------|--------------|
| | Control | | | Astaxanthin Treatment | | |
| Indexes | Before | 2-wk later | 4-wk later | Before | 2-wk later | 4-wk later |
| Sub-objective accommodation power (%) | 100 | 103.2±19.2 | 107.8±25.2 | 100 | 156.0±47.7** | 164.4±52.8** |
| Positive accommodation power (D/sec) | 3.60±2.07 | 3.61±2.10 | 3.78±2.04 | 2.95±1.18 | 3.88±1.35** | 4.27±1.52** |
| Negative accommodation power (D/sec) | 5.14±3.24 | 4.99±3.49 | 5.75±3.13 | 3.78±1.88 | 5.34±2.58** | 5.68±2.02** |

Value: average±SE

**: significance compared with before oral administration ($p<0.01$)

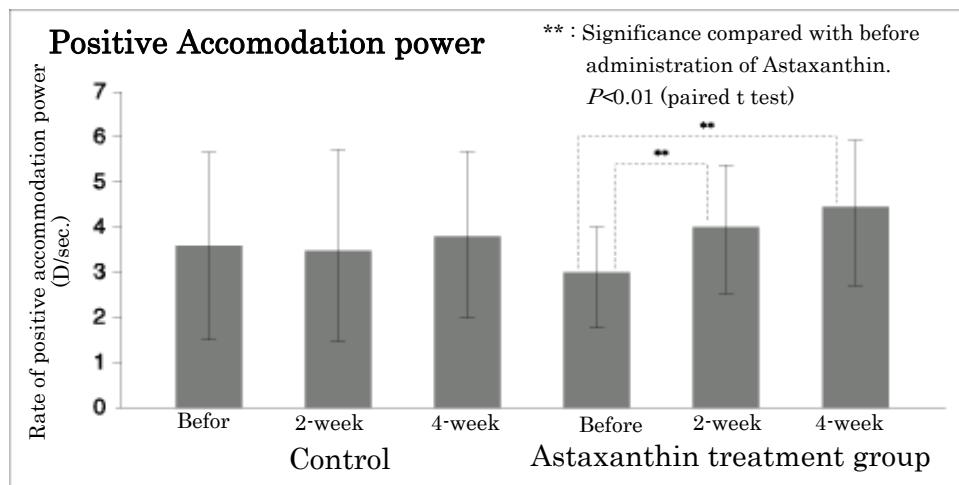


Fig. 7. Changes in Positive Accommodation Power (mean±S.D.)

(Clinical Medicine, 21 (6), 637-650(2005) modified)

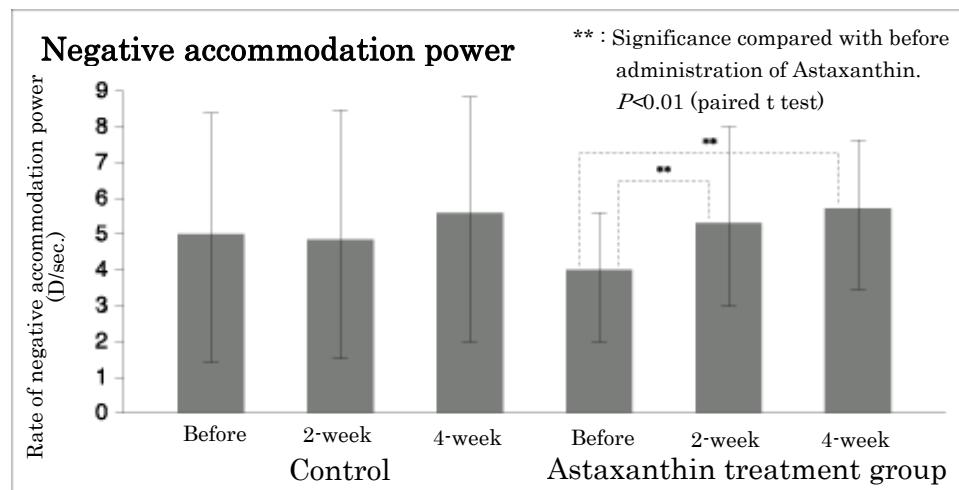


Fig. 8 Changes in Negative Accommodation Power (mean±S.D.)

(Clinical Medicine, 21 (6), 637-650(2005)modified)

- 16) Shiratori K., Ogami K., Nitta T., et al.: The effects of Astaxanthin on Accomodation and Asthenopia – Efficacy Identification Study in Healthy Volunteers, *Clinical Medicine*, 21 (6), 637-650 (2005)
- 17) Nitta T., Ogami K., Shiratori K., et al.: The effects of Astaxanthin on Accomodation and Asthenopia – Dose Finding Study in Healthy Volunteers, *Clinical Medicine*, 21 (5), 543-556 (2005)

7. Inhibition of Atherosclerosis

Atherosclerosis occurs when the walls of arteries are damaged and narrowed by deposits of plaque formed from cholesterol and other fatty substances. Consequently, blood circulation is obstructed. High blood cholesterol levels, especially LDL cholesterol are associated with the development of atherosclerosis upon oxidation by free radicals. Macrophage cells then pick up the oxidized LDL to form “foam cells” which ultimately accumulate in fatty streaks to develop as atheromatous plaque. There are 2 types of atheromatous plaque: stable atheromas & unstable atheromas. Stable atheromas are atheromas covered with thick fibrous tissue with thick intima while unstable atheromas are atheromas covered with thin fibrous tissue with thin intima (Fig. 9). Unstable atheromas can result in haemorrhage, vascular occlusion, formation of thrombus (blood clot) and necrosis. Inflammation occurs in response to macrophage infiltration in unstable atheromas.

In a 24-week study conducted by Li, W., Hlisten, A., et al, found that astaxanthin 100mg/kg significantly decreased macrophage infiltration, improved plaque stability & significantly diminished apoptosis in Watanabe heritable hyperlipidaemic (WHHL) rabbits model. However, astaxanthin has no effect on the lesion size and lipid accumulation¹⁸⁾.

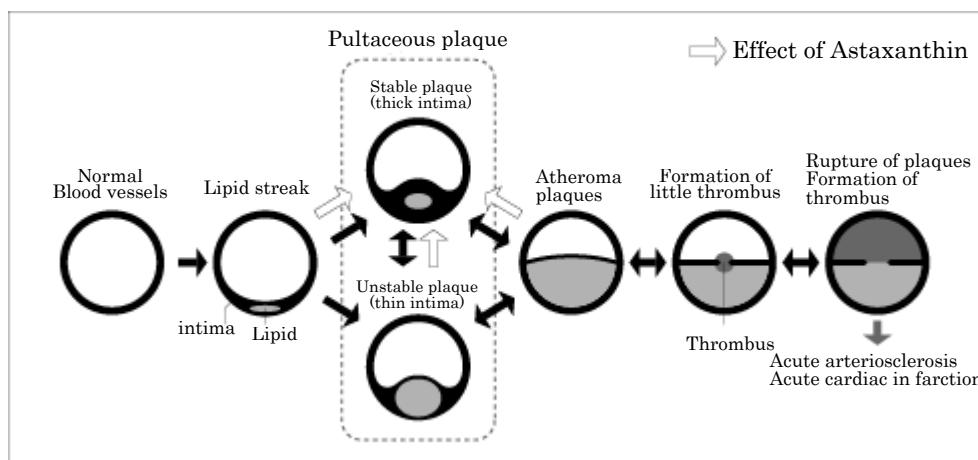


Fig. 9 Plaque Formation

18) Li, W., Hlisten, A., et al.: α -tocopherol and astaxanthin decrease macrophage infiltration, apoptosis and vulnerability in atheroma of hyperlipidaemic rabbits, *J. Mol. Cell Cardiol.*, **75**, 969-978 (2004).

8. Anti-inflammatory Effect

The anti-inflammatory effect of astaxanthin has been documented¹⁹⁾²⁰⁾. Inflammation is the very initial response of the immune system to infection or irritation. It is characterized by redness, heat, swelling, pain and dysfunction of organs involved. Phagocytosis occurs upon invasion of pathogens where pathogens are ingested by macrophages followed by the release of cytokines IL-1 and TNF- α at inflammation site. Meanwhile, enzyme iNOS is stimulated to produce NO (carbon monoxide). Illustration on cascade of inflammation is shown in Fig. 11. IL-1 and TNF- α further activate NF- κ B to increase the production of IL-1. Increased IL-1 in turn activated COX-2 and produces PGE₂ (prostaglandin E₂). NO, TNF- α and PGE₂ are inflammatory factors that mediate the immune system.

However, elevated blood NO level may result in tissue disorders such as cancer and aging. Meanwhile, excessive production of PGE₂ can result in feverish symptoms and pain such as rheumatoid arthritis (RA).

Study found that astaxanthin prevent the **anti-inflammatory response of macrophages**. In a mice experimental model, the effect of astaxanthin on inflammation induced by lipopolysaccharides (LPS) was evaluated and compared with prednisolone, a steroid anti-inflammatory drug. Findings indicated that astaxanthin regulated the production of NO, TNF- α and PGE₂ while its activity was 1/10 of prednisolone (Fig. 11).

In vitro, astaxanthin demonstrated inhibitory effect against NF- κ B and IL-1 β in mice's RAW cells which are similar to macrophages.

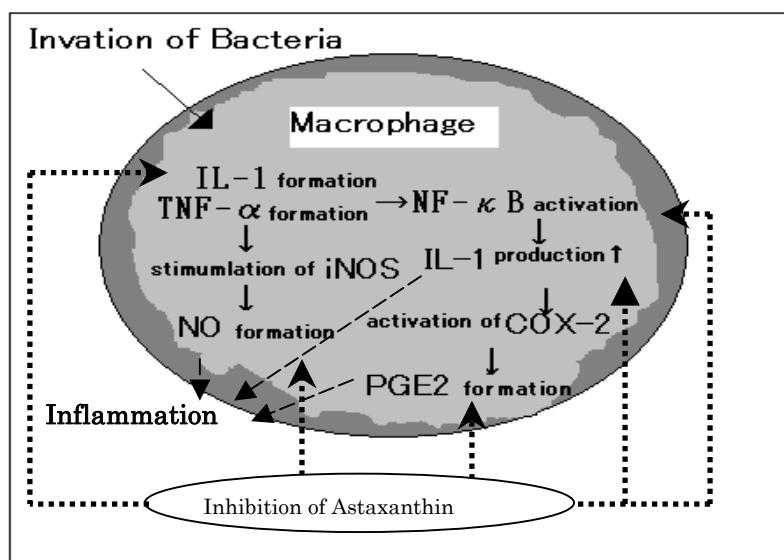


Fig. 10.The mechanism of macrophages during inflammation and the effect of Astaxanthin

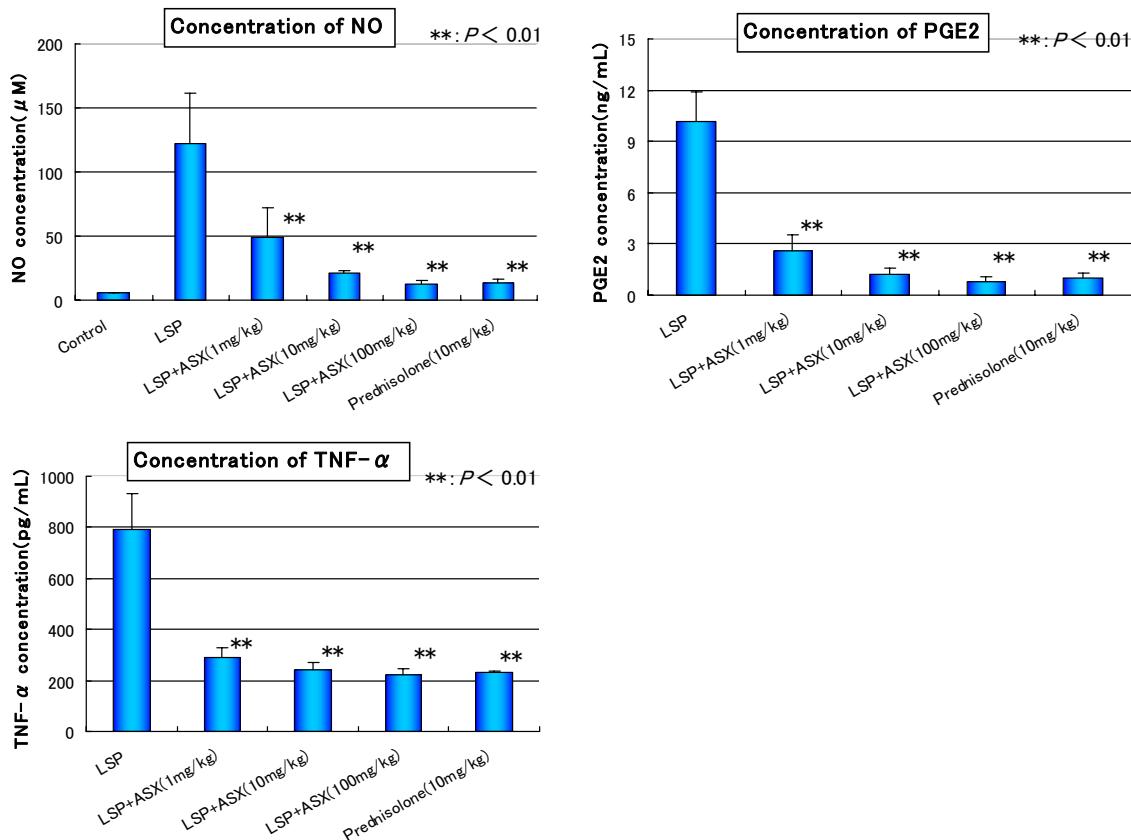


Fig. 11. The Inhibitory Effect of Astaxanthin on NO, TNF- α & PGE₂ productions

- 19) Ohgami, K., Shiratori, K., et al.: Effects of astaxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo, *Invest Ophthalmol Vis. Sci.*, **44**(6), 2694-2701(2003)
- 20) Lee, S.J., Bai, S.K., et al.: Astaxanthin inhibits nitric oxide production and inflammatory gene expression by suppressing I κ B kinase independent, *Mol. Cells.*, **16**, 97-105 (2003)

9. Cosmetic Effect

Previous study conducted revealed that oral administration of astaxanthin improves skin's moisture content, reduces fine lines and wrinkles while restores skin elasticity²¹⁾. A single-blind test was conducted to evaluate the effect of oral astaxanthin 4mg/day on 28 health female subjects for 6-week. Results confirmed that astaxanthin improved skin moisture, promoted skin elasticity while reduced wrinkling skin. Likewise, skin elasticity improved upon dermatologist examination (Fig. 12).

As mentioned earlier, astaxanthin quenches singlet oxygen generated by UV rays. So, it is suggested 6-week oral administration of astaxanthin exert protective effect on skin collagen against oxidative cross-linking and degradation of new collagen.

Alternatively, another study revealed the photo-aging protective effect of astaxanthin

on bald mice where formation of wrinkles reduced with improvement on skin elasticity (Fig. 13)²².

Astaxanthin, being an excellent antioxidant is protective against photo-aging, skin wrinkling while improves skin elasticity.

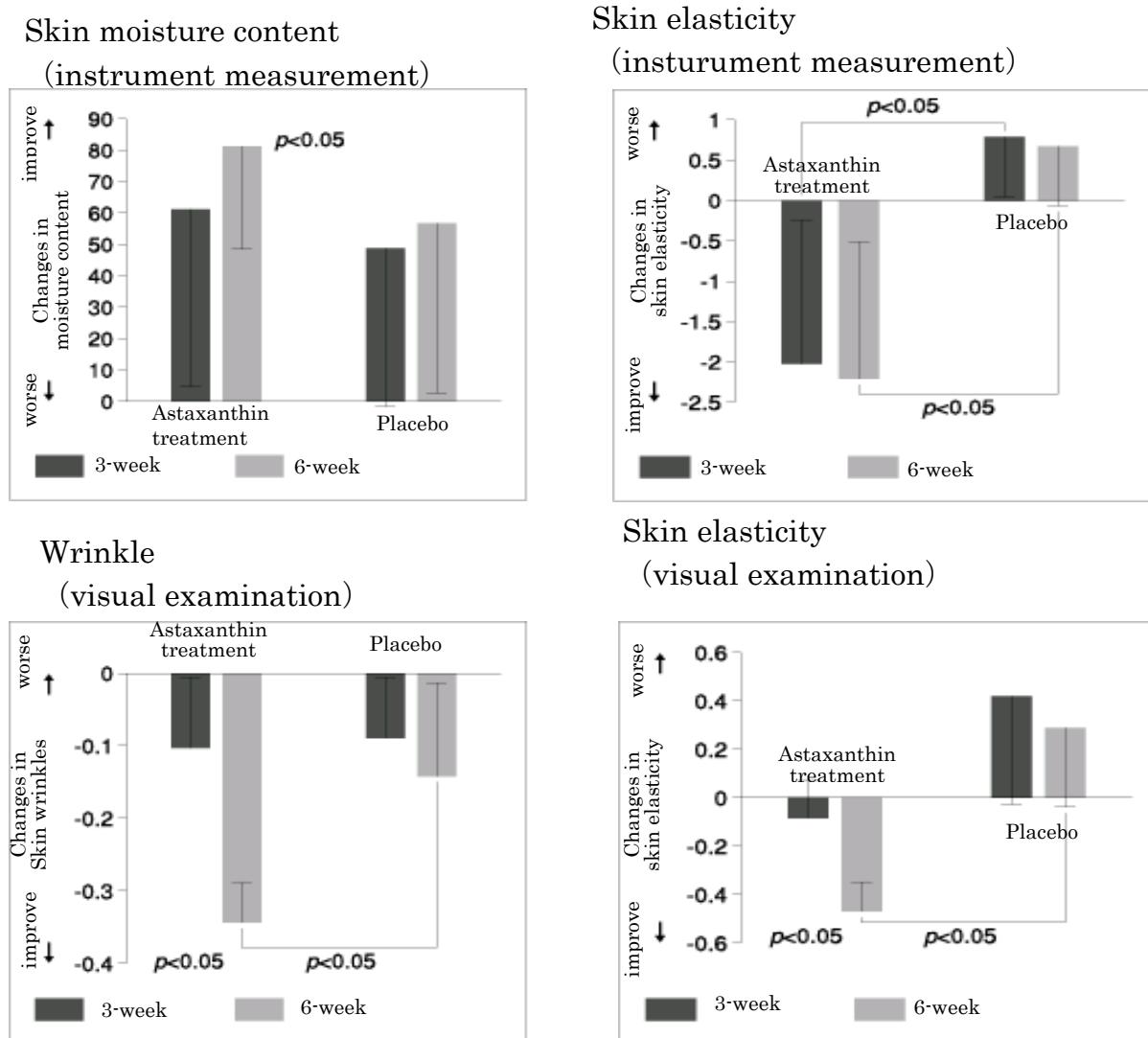


Fig.12. Skin changes upon Oral administration of Astaxanthin 4mg/day for 6-week

(FOOD Style 21, 9, 72-75(2005) modified)

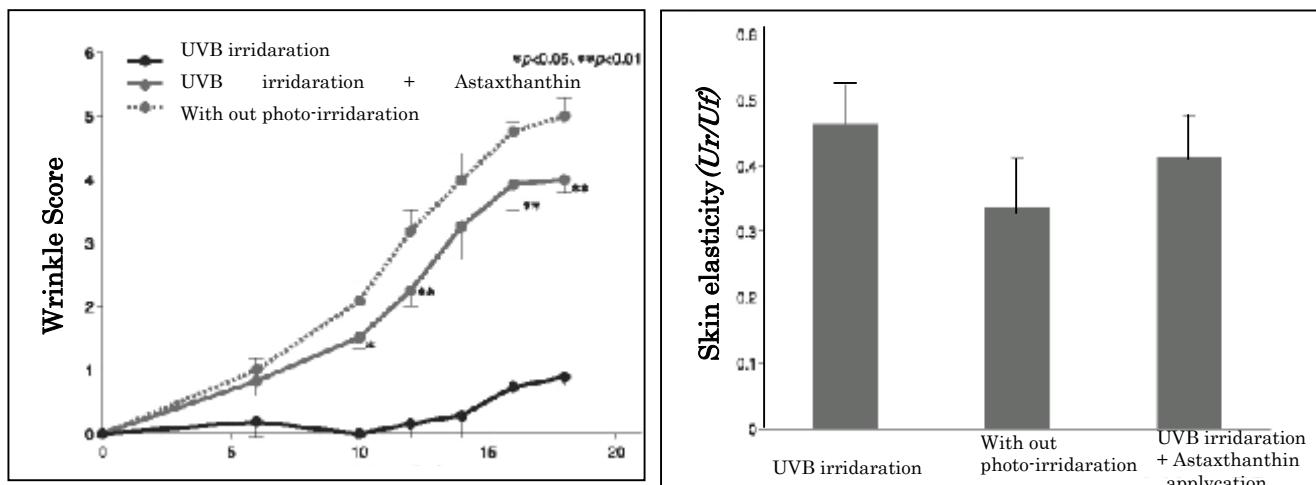


Fig. 13. The Photo-aging Protective Effect of Asataxanthin

(Reduction in wrinkles formation and improvement on skin elasticity)

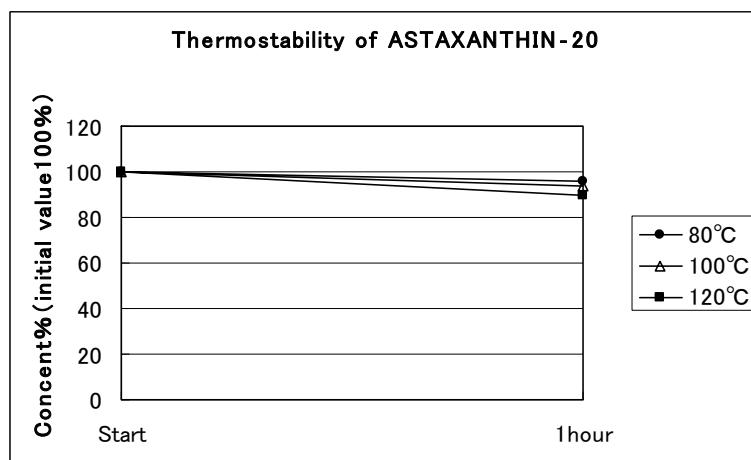
(*Japanese Cosmetic Science Society Journal*, 29(1), 9-19(2005) modified)

- 21) Yamashita E.: The cosmetic effect of food supplement containing Astaxanthin, Food Style 21, 9, 72-75 (2005)
- 22) Mizutani Y., Sakata O., Hoshino T., et al.: The photo-aging protective effect of carotenoids and its application in cosmetics, Japanese Cosmetic Science Society Journal, 29 (1), 9-19 (2005)

10. Stability of Astaxanthin

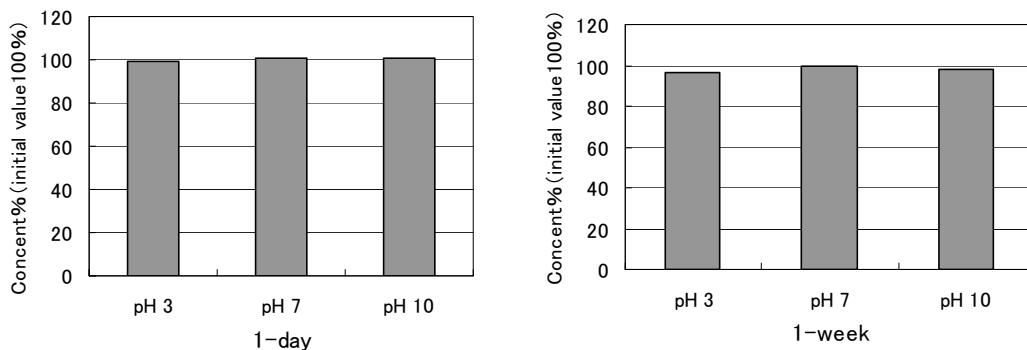
(1) Thermostability of Astaxanthin-20

The thermostability of Astaxanthin-20 was conducted at 80°C, 100°C & 120°C for one hour. Content of astaxanthin was highly stable at normal food processing temperature, i.e. 80°C, 100°C while there was 10% decomposition at temperature of 120°C.



(2) pH Stability of Astaxanthin-LS1

The evaluation on pH stability of Astaxanthin-LS1 was conducted. Astaxanthin 1.2% concentration in solution was prepared and stored at different pH for 1-day and 1-week respectively under darkness. Results showed that astaxanthin is highly stable at all pH ranges.



(3) Stability of Astaxanthin-P1

(comparison with other commercially available astaxanthin, Astaxanthin A)

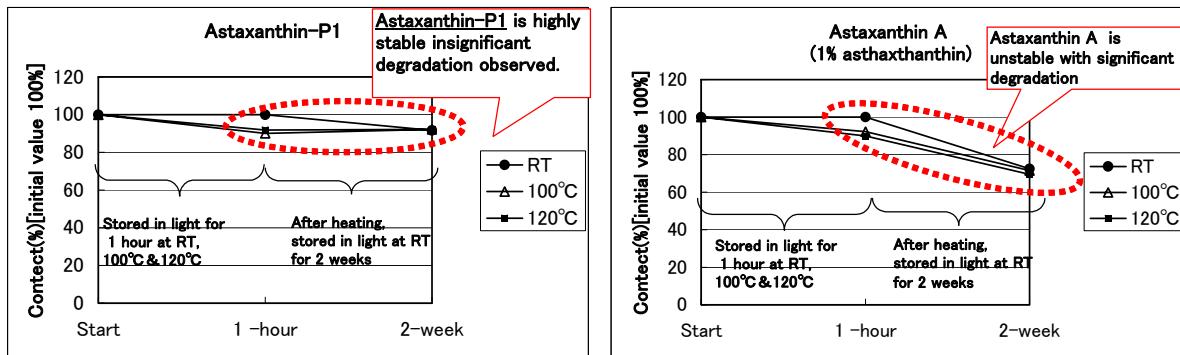
① Thermostability, Photo-stability and Oxidation comparisons

The thermostability, photo-stability and Oxidation of Astaxanthin-P1 by Oryza Oil & Fat Chemical Co., Ltd. was compared with other commercially available astaxanthin, Astaxanthin A. Both Astaxanthin-P1 and Astaxanthin A are in powder form containing astaxanthin 1%.

Astaxanthin-P1 and Astaxanthin A were stored at room temperature, 100°C and 120°C openly for 1-hour in light. The content of astaxanthin was verified and found that both Astaxanthin-P1 and Astaxanthin A decompose by approximately 10% at 100°C and 120°C. The test samples were continued to be kept under room temperature for 2-week openly in light for further evaluation.

Results revealed that there was no further decomposition of astaxanthin in Astaxanthin-P1 while there was additional 30% astaxanthin decomposition in Astaxanthin A at room temperature, 100°C and 120°C after 2-week.

Hence, Astaxanthin-P1 is a powdered astaxanthin with excellent stability against heat, light and oxygen.

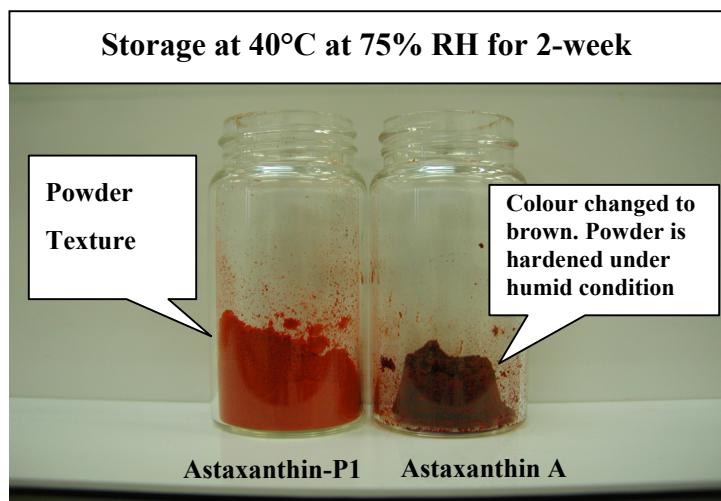


② Stability against Humidity (Hygroscopic)

Further stability test was conducted to differentiate Astaxanthin-P1 from other commercially available product. Astaxanthin-P1 and Astaxanthin A were stored at 40°C, 75% RH (Relative Humidity) for 2-week to compare the stability on humidity. No physical changes observed on Astaxanthin-P1 while its content decomposed by approximately 15%.

On the other hand, there was colour changes observed in Astaxanthin A where the powder turned brown and hardened. Content of astaxanthin could not be determined due to property deterioration of Astaxanthin A.

Accordingly, Astaxanthin-P1 is more superior as compared to other commercially available products with its excellent stability and scientific datas.



11. Nutritional Information

| Items | ASTAXANTHIN-2 0 | ASTAXANTHIN- 5 | ASTAXANTHIN-P 1 | ASTAXANTHIN-LS 1 |
|--------------|-----------------------|-------------------|--------------------|---------------------|
| Water | 0.05 g/100 g | 0.01 g/100 g | 0.00 g/100 g | 14.7 g/100 g |
| Protein | 0.5 g/100 g | 0.13 g/100 g | 0.03 g/100 g | 0.03 g/100 g |
| Fat | 99.3 g/100 g | 99.8 g/100 g | 22.0 g/100 g | 30.3 g/100 g |
| Ash | Less than 0.1 g/100 g | 0.0 g/100 g | 0.0 g/100 g | 0.0 g/100 g |
| Carbohydrate | 0.2 g/100 g | 0.03 g/100 g | 78.0 g/100 g | 55.0 g/100 g |
| Sodium | 3 m g/100 g | 0.75 m g/100 g | 0.17 m g/100 g | 0.16 m g/100 g |
| Energy | 897 kcal/100 g | 899 kcal/100 g | 510 kcal/100 g | 493 kcal/100 g |

The values are calculated from nutritional analysis of Haematococcus Extract (ASTAXANTHIN-20).

Test Trustee : Japan Food Research Center Foundation

Date of analysis : April 17, 2006

Test number : 506040061-001

Test Trustee : SRL, Inc

Date of analysis : March 17, 2006

Test number : 13504-000-80000

12. Safety Profile of Astaxanthin

(1) Acute Toxicity (LD₅₀)

Fasting ddY mice (average weight of male mice 30g, female mice 25, aged 5-week old) were given oral Astaxanthin -20 at 2000mg/kg and kept for 14-day at 23±2°C, RH50±10% with free access to feed and water. No abnormalities and fatal event observed at the end of test. No abnormalities of internal organs detected with naked eyes upon autopsy performance. Thus, LD₅₀ of astaxanthin is deduced to be >2000mg/kg in both male and female mice.

(2) Ethoxyquin

Ethoxyquin is not detected in Astaxanthin. (Detection Limit 0.05mg/kg)

Test Trustee : Japan Institute of Oil & Fats, Other Foods Inspection Foundation.

Date of analysis : March 10, 2006

Reference Number : 06100354-001—1

13. Recommended Daily Dose of Astaxanthin

The universal recommended daily dose of Astaxanthin (equiv. free astaxanthin) is 4 to 6 mg/day. Therefore, the equivalent recommended daily dose of range of astaxanthin as follows:

| | | |
|-----------------|------------------------|-------------------|
| Astaxanthin-5 | (oil) | 80 to 120 mg/day |
| Astaxanthin-20 | (oil) | 20 to 30 mg/day |
| Astaxanthin-P1 | (powder) | 400 to 600 mg/day |
| Astaxanthin-LS1 | (water soluble liquid) | 400 to 600 mg/day |

14. Applications of Astaxanthin

| | Applications | Indication | Examples |
|----------|----------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| Food | Health Food Beauty Food | 1) Antioxidant 2) Promote Brain Functions 3) Prevent Asthenopia 4) Skin Rejuvenation | Beverages, tonic, hard & soft capsule, tablets, candies, chewing gum, gummy, cookies, chocolates etc. |
| Cosmetic | Cosmetics | | Toner, lotion, mask etc. |

15. Packaging

Astaxanthin-5 (oil, food application)

Astaxanthin-5C (oil, food application)

1kg, 5kg Interior packaging : Blik

Exterior packaging : Cardboard box

Others: Nitrogen filling

Astaxanthin-20 (oil, food application)

Astaxanthin-20C (oil, cosmetics application)

1kg Interior packaging : Blik

Exterior packaging : Cardboard box

Others: Nitrogen filling

0.1kg Interior packaging : Polyethylene container

Exterior packaging : Aluminium bag

Others: Nitrogen filling

Astaxanthin-P1 (powder, food application)

Astaxanthin-PC1 (powder, cosmetics application)

1kg, 5kg Interior packaging : Polyethylene bag (inner bag),
aluminium bag (outer bag)

Exterior packaging : Cardboard box

Others : Nitrogen filling in inner bag

Deoxidizer, and silica pack in outer bag

Astaxanthin-LS1 (Water soluble liquid, food application)

Astaxanthin-LSC1 (Water soluble liquid, cosmetic application)

1kg, 5kg Interior packaging : Blik (lining ; apply epoxy)

Exterior packaging : Cardboard box

Others: Nitrogen filling

16. Storage

Store in cool (below 5°C), dry dark place. Avoid places with high humidity.

Astaxanthin-LS1 & Astaxanthin-LSC1 are recommended to be refrigerated.

17. Expression

Astaxanthin – 20, - 5, - P1, - LS1

Expression : Haematococcus extract

Haematococcus extract containing astaxanthin

Haematococcus extract (containing astaxanthin)

Astaxanthin – 20C

INCI Name: Haematococcus Pluvialis Extract, Astaxanthin

Astaxanthin – 5C

INCI Name: Caprylic/Capric Triglyceride, Haematococcus Pluvialis Extract, Astaxanthin, Tocopherol

Astaxanthin – PC1

INCI Name: Cyclodextrin ,Caprylic/Capric Triglyceride, Haematococcus Pluvialis Extract, Tocopherol, Astaxanthin

* Please refer to your nation's standard.

PRODUCT STANDARD

PRODUCT NAME
ASTAXANTHIN - 5
(FOOD)

This product is liquid oil extracted from *Haematococcus Pluvialis*. It guarantees a minimum of 5.0 % astaxanthin.

Appearance Red to dark-red liquid with light unique smell

Astaxanthin (Free) Min. 5.0 % (Spectrophotometry)

Purity Test

(1)Heavy Metal Max. 10 ppm (The Japanese Standards of Food Additives)

(2)Arsenic Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation)

Standard Plate Count Max. 1×10^3 cfu/g (Analysis for Hygienic Chemists)

Moulds & Yeasts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Coliform Negative (Analysis for Hygienic Chemists)

| <u>Composition</u> | <u>Ingredient</u> | <u>Content</u> |
|---------------------------|--------------------------|-----------------------|
| | Haematococcus Extract | 25 % |
| | Natural Tocopherol | 1 % |
| | Triglyceride | 74 % |
| | Total | 100 % |

PRODUCT STANDARD

PRODUCT NAME
ASTAXANTHIN - 20
(FOOD)

This product is liquid oil extracted from *Haematococcus Pluvialis*. It guarantees a minimum of 20.0 % astaxanthin.

Appearance Red to dark-red liquid with light unique smell.

Astaxanthin (Free) Min. 20.0 % (Spectrophotometry)

Purity Test

(1) Heavy Metals Max. 10 ppm (The Japanese Standards of Food Additives)

(2) Arsenic Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation)

Standard Plate Count Max. 1×10^3 cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

| <u>Composition</u> | <u>Ingredient</u> | <u>Content</u> |
|---------------------------|--------------------------|-----------------------|
| | Haematococcus Extract | 100 % |

PRODUCT STANDARD

PRODUCT NAME
ASTAXANTHIN – P1
(FOOD)

This powdered product is extracted from *Haematococcus Pluvialis*. It guarantees a minimum of 1.0 % astaxanthin.

Appearance Red to dark-red coloured powder with light unique smell.

Astaxanthin (Free) Min. 1.0 % (Spectrophotometry)

Purity Test

(1)Heavy Metal Max. 10 ppm (The Japanese Standards of Food Additives)

(2)Arsenic Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation)

Standard Plate Count Max. 1×10^3 cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Coliform Negative (Analysis for Hygienic Chemists)

| <u>Composition</u> | <u>Ingredients</u> | <u>Contents</u> |
|---------------------------|----------------------------|------------------------|
| | Haematococcus Extract | 5.6 % |
| | Natural Tocopherols | 2.0 % |
| | Triglyceride | 14.4 % |
| | <u>Cyclodextrin</u> | 78.0 % |
| | Total | 100 % |

PRODUCT STANDARD

PRODUCT NAME
ASTAXANTHIN-LS1
(FOOD)

This product is an emulsion of constituents extracted from *Haematococcus Pluvialis*. It guarantees a minimum of 1.0 % astaxanthin

Appearance Red to dark-red liquid with unique smell.

Astaxanthin (Free) Min. 1.0 % (Spectrophotometry)

Purity Test

(1) Heavy Metals Max. 10 ppm (The Japanese Standards of Food Additives)

(2) Arsenic Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation)

Standard Plate Counts Max. 1×10^3 cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

| <u>Composition</u> | <u>Ingredients</u> | <u>Contents</u> |
|---------------------------|------------------------------|-----------------|
| | Haematococcus Extract | 5.3 % |
| | Natural Tocopherols | 1.0 % |
| | Edible Vegetable Oil | { |
| | Glycerin Ester of Fatty Acid | |
| | Enzymatic Lysolecithin | |
| | Glycerin | |
| | Purified Water | 93.7 % |
| | Total | 100% |

PRODUCT STANDARD

| |
|-------------------------|
| PRODUCT NAME |
| ASTAXANTHIN – 5C |
| (COSMETICS) |

This product is liquid oil extracted from *Haematococcus Pluvialis*. It guarantees a minimum of 5.0 % astaxanthin.

Appearance Red to dark-red liquid with light unique smell.

Astaxanthin (Free) Min. 5.0 % (Spectrophotometry)

Purity Test

(1) Heavy Metals Max. 10 ppm (The Second method)

(2) Arsenic Max. 1 ppm (The Third method)

Standard Plate Counts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

| <u>Composition</u> | <u>Ingredients</u> | <u>Contents</u> |
|---------------------------|---------------------------------|-----------------|
| | Caprylic/Capric Triglyceride | 74 % |
| | Haematococcus Pluvialis Extract | 20% |
| | Astaxanthin | 5 % |
| | <u>Tocopherol</u> | <u>1 %</u> |
| | Total | 100 % |

Ref : The Japanese Standards of Quasi-Drug Ingredients.

PRODUCT STANDARD

PRODUCT NAME

ASTAXANTHIN – 20C

(COSMETICS)

This product is liquid oil extracted from *Haematococcus Pluvialis*. It guarantees a minimum of 20.0 % astaxanthin.

Appearance

Red to dark-red liquid with light unique smell.

Astaxanthin (Free)

Min. 20.0 % (Spectrophotometry)

Purity Test

(1) Heavy Metals Max. 10 ppm (The Second method)

(2) Arsenic Max. 1 ppm (The Third method)

Standard Plate CountsMax. 1×10^2 cfu/g (Analysis for Hygienic Chemists)**Moulds and Yeasts**Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)**Coliforms**

Negative (Analysis for Hygienic Chemists)

Composition

| Ingredient | Content |
|---------------------------------|---------|
| Haematococcus Pluvialis Extract | 80 % |
| Astaxanthin | 20 % |
| Total | 100 % |

Ref : The Japanese Standards of Quasi-Drug Ingredients.

PRODUCT STANDARD

PRODUCT NAME
ASTAXANTHIN – PC1
(COSMETICS)

This powdered product is extracted from *Haematococcus Pluvialis*. It guarantees a minimum of 1.0 % astaxanthin.

Appearance Red to dark-red powder with light unique smell.

Astaxanthin (Free) Min. 1.0 % (Spectrophotometry)

Purity Test

(1) Heavy Metals Max. 10 ppm (The Second method)

(2) Arsenic Max. 1 ppm (The Third method)

4.Standard Plate Counts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

5.Moulds and Yeasts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

6.Coliforms Negative (Analysis for Hygienic Chemists)

| <u>7.Composition</u> | <u>Ingredients</u> | <u>Contents</u> |
|-----------------------------|---------------------------------|-----------------|
| | Cyclodextrin | 78.0 % |
| | Caprylic/Capric Triglyceride | 14.4 % |
| | Haematococcus Pluvialis Extract | 4.6% |
| | Tocopherol | 2.0 % |
| | <u>Astaxanthin</u> | 1.0 % |
| | Total | 100 % |

Ref : The Japanese Standards of Quasi-Drug Ingredients.

PRODUCT STANDARD

| |
|---------------------------|
| PRODUCT NAME |
| ASTAXANTHIN – LSC1 |
| (COSMETICS) |

This product is an emulsion of constituents extracted from *Haematococcus Pluvialis*. It guarantees a minimum of 1.0 % astaxanthin.

Appearance Red to dark-red liquid with unique smell.

Astaxanthin (Free) Min. 1.0 % (Spectrophotometry)

Purity Test

(1) Heavy Metals Max. 10 ppm (The Second method)

(2) Arsenic Max. 1 ppm (The Third method)

Standard Plate Counts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

| <u>Composition</u> | Ingredients | Contents |
|---------------------------|---------------------------------|-----------------|
| | Haematococcus Pluvialis Extract | 4.3 % |
| | Astaxanthin | 1.0 % |
| | Tocopherol | 1.0 % |
| | Vegetable Oil | |
| | Glycerin Ester of Fatty Acid | |
| | Lysolecithin | |
| | Glycerin | |
| | Water | |
| | Total | 100% |

Ref : The Japanese Standards of Quasi-Drug Ingredients.

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***Correction**

- Change Recommended Daily Dose.(P.20)

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