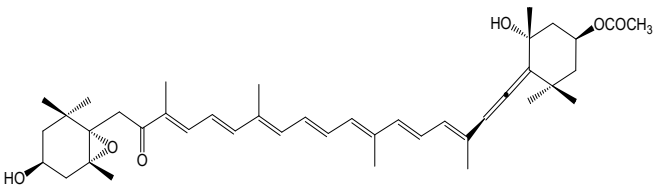
Oryza

ORYZA OIL & FAT CHEMICAL CO., LTD.

FUCOXANTHIN

Dietary Ingredient for Prevention of Metabolic Syndrome, Antioxidation and Cosmetics

- **FUCOXANTHIN-5KW**
  (Oil, Food Grade)
- **FUCOXANTHIN-1KW**
  (Oil, Food Grade)
- **FUCOXANTHIN-P1KW**
  (Powder, Food Grade)
- **FUCOXANTHIN-WSP0.1KW**
  (Water-soluble Powder, Food Grade)
- **FUCOXANTHIN-5CKW**
  (Oil, Cosmetic Grade)
- **FUCOXANTHIN-1CKW**
  (Oil, Cosmetic Grade)
- **FUCOXANTHIN-PC1KW**
  (Powder, Cosmetic Grade)
- **FUCOXANTHIN-WSPC0.1KW**
  (Water-Soluble Powder, Cosmetic Grade)



ORYZA OIL & FAT CHEMICAL CO., LTD.

ver. 3.0 YF

FUCOXANTHIN

Dietary Ingredient for Prevention of Metabolic Syndrome and Beauty Enhancement

1. Introduction

[Kelp]

Kelp is a type of seaweed with the classification Laminariaceae, Laminariales, Phaeophyceae, Heterokontophyta. Seaweeds are broken into different categories according to their colors, such as blue seaweeds, diatom, green seaweeds, brown seaweeds, and red seaweeds. Kelp is categorized as brown seaweed.

There are 45 types and 14 genus of kelp in Japan. Around the world, it is believed that 26 genus grow in the Northern Hemisphere and 9 genus grow in the Southern Hemisphere. Kelp grows in cold currents and can be found along the Pacific coast of Japan in Miyagi Prefecture and northern prefectures as well as the ocean around Hokkaido, its main region of production. Ranked by order, Laminaria japonica, Laminaria diabolica, Laminaria ochotensis, and Laminaria angustata are all known as high-grade kelp.

Approximately 95% of the kelp produced in Japan comes from Hokkaido. The remaining 5% comes from the prefectures of Aomori, Iwate, and Miyagi in the Tohoku region.

Fig. 1 Laminaria japonica and harvested kelp

[History of Kelp as Food – the Kelp Road]

In many parts of the world, seaweed is used as a source of food. The people of China, South Korea, Pacific islands such as Hawaii, and other coastal regions eat kelp relatively often.

However, the Japanese are the only people that consume kelp on almost a daily basis.
Long ago, when rice, labor, and produce were collected as taxes, kelp was used to pay taxes in rural areas. Kelp was also used as an offering to the gods and Buddha.
During the Muromachi Era, when ship building technology advanced and trade grew, the use of kelp spread throughout Japan.

Fig. 2 “Kelp Harvest”, painting of June from the “Folding Screen Paintings of the Twelve Months’ Custum of the Ainu People” by Biozann Hirayama

Fig. 3 Painting of the kelp harvest of the Ainu and Japanese people in the Edo era

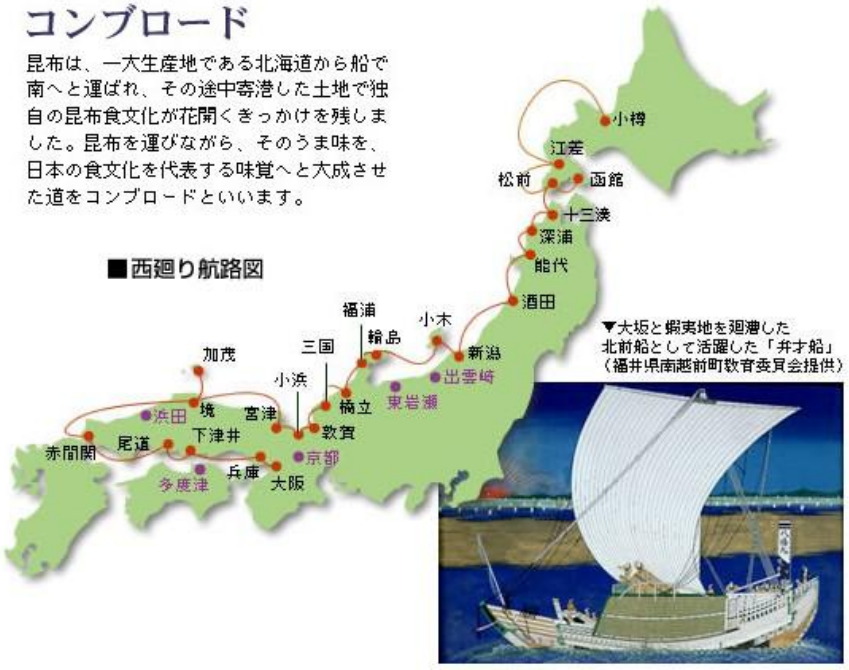


Fig. 4 An illustration of the Kelp Road

Merchant ships known as “Kitamaebune” containing kelp departed from Esa, Matsumae, and Hakodate in Hokkaido, sailed south and made port in Tsuruga or Wakasa. The kelp was then transported to Kyoto by land.
The ships then continued further south, entered the Seto Inland Sea through Shimonoseki, and finally made port in Osaka. It was here that the kelp was unloaded and processed. This is why Osaka has many salted kelp processors even today. Over time, salted kelp made in Osaka became widely known and it was shipped to Edo (currently Tokyo).
A merchant ship once arrived in Sakai and was unable to find its business partner. At a loss, the crew luckily came across a ship carrying sugar from Okinawa and made a deal to exchange cargos. This is how kelp was introduced to Okinawa. Kelp soon became popular there because kelp went so well with the pork dishes that Okinawans often prepared and ate.
The sea route that kelp was transported on was known as the “Kelp Road.” As kelp was transported from north to south by ship, unique ways of eating kelp developed in different regions along the way. Over time, kelp became an integral part of the Japanese diet.

2. What is Fucoxanthin?

Fucoxanthin is only contained in brown seaweeds such as kelp, *hijiki*, and *wakame* seaweed in small quantities. It is a type of non-provitamin A carotenoid and it belongs to xanthophyll. Fucoxanthin has the allene structure and epoxide and hydroxyl groups as shown in Fig. 5. As studies on functionality of carotenoids as health food are actively carried out lately, functionality of fucoxanthin has been clarified as well. As a result of the studies, fucoxanthin has been reported to have activities to prevent obesity and diabetes[1-6], combat cancer[7-15], prevent oxidation of the body[16-17], prevent vascularization[18], and combat inflammations[19].

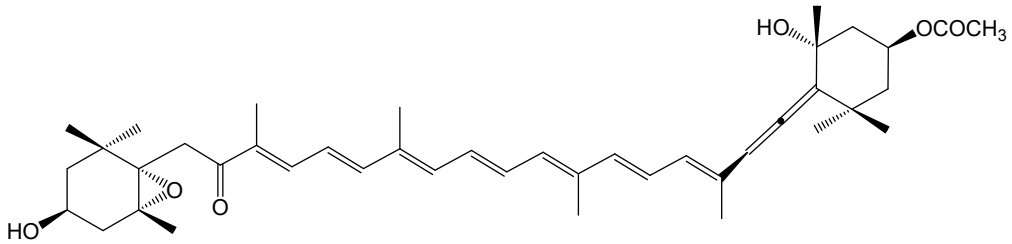


Fig. 5 Structure of Fucoxanthin

ORYZA OIL & FAT CHEMICAL CO., LTD. developed high-concentration fucoxanthin from kelp taken from Hokkaido (Japan) with the cooperation of Professor Kanazawa from the Graduate School of Agricultural Science in Kobe University, Ogurayayamamoto Corporation, and Nihon Shokuhin Kaihatsu Kenkyusho Co., Ltd. through an industry-government-academia joint study activity as part of the Ministry of Agriculture, Forestry and Fisheries’ project to assist multi-field research activities for the creation of bio-oriented industries (held by the Bio-oriented Technology Research Advancement Institution, National Agriculture and Food Research Organization in FY 2006 to 2010). ORYZA’s unique advanced technology to extract and purify natural materials was also applied in the project.

We evaluated new functions of fucoxanthin on our own and discovered that fucoxanthin has activities to inhibit various enzymes involved in skin turnover (collagenase, hyaluronidase, and elastase) and promote collagen production. We also confirmed its activities to inhibit tyrosinase, inhibit melanin production in melanoma cells, reduce pigmentation on guinea pigs of which skin was exposed to UV ray, and inhibit acne germ-derived lipase. This indicates that fucoxanthin may have skin-lightening and enhancing activities. We believe that we can provide fucoxanthin as a new functional food and cosmetic ingredient with anti-metabolic syndrome activity (anti-obesity, anti-diabetes).
This catalog mainly describes fucoxanthin's anti-metabolic syndrome activities (anti-obesity, anti-diabetes) and beauty-enhancing activities (skin-enhancing, skin-lightening, anti-acne).

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5) Maeda H, Hosokawa M, Sashima T, Takahashi N, Kawada T, Miyashita K. Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *Int J Mol Med.* **18**(1): 147-52 (2006).
6) Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K. Fucoxanthin from edible seaweed, Undaria pinnatifida, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem Biophys Res Commun.* **332**(2): 392-7 (2005).
7) Das SK, Hashimoto T, Kanazawa K. Growth inhibition of human hepatic carcinoma HepG2 cells by fucoxanthin is associated with down-regulation of cyclin D. *Biochim Biophys Acta.* **1780**(4): 743-9 (2008).
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3. Pharmacological Functions of Fucoxanthin

(1) Anti-metabolic syndrome activity

There are believed to be nearly 860 million metabolic syndrome patients in six major countries in the world. The number of obese people is increasing in Japan due to more Westernized and irregular dietary habits and lack of exercise resulting from a more convenient lifestyle. According to a trial calculation of the Ministry of Health, Labour and Welfare, approximately 190 million people (aged between 40 and 70) either have metabolic syndrome or will so in the future, which is one out of two men and one out of five women in the age group. Although obesity, high blood pressure, hyperlipidemia, and diabetes were formerly considered to be independent lifestyle diseases, accumulated visceral fat must also be considered as a common cause of them. Accumulated visceral fat increases free fatty acid in the blood and triggers hyperlipidemia and insulin resistance. Various physiologically active substances, namely adipocytokines, are secreted from visceral fat tissues. Excessively accumulated visceral fat has been confirmed to destroy the secretion balance or these substances and cause metabolic syndrome.

White adipose tissues and brown adipose tissues are found in human fat tissues and perform different functions. White adipose tissues store excessive calories as fat. Increased white adipose tissues signify obesity. On the contrary, brown adipose tissues maintain body temperature at a certain level and consume excessive calories by degrading fat and generating heat. These activities are performed by uncoupling protein 1 (UCP1) existing selectively in mitochondrial inner membrane of brown adipose tissues. Various biogenic factors are involved in the expression of UCP1. Food components capsaicin, capsiate, and caffeine increase the UCP1 expression by increasing the secretion of noradrenaline and EPA and DHA do the same by becoming PPARγ ligand. However, in human bodies, the amount of brown adipose tissues decreases with age so the increase of brown adipose tissues does not necessarily contribute to the prevention of obesity. Therefore, the expression of UCP1 in white adipose tissues is desired so that UCP1 can accelerate the oxidation of white fat and conversion of energy to heat and in turn decrease white adipose tissues.

1) Activity on adipose cells (differentiated 3T3-L1) (in vitro)

3T3-L1 cell strain is often used for screening functional components having anti-obesity activity. When it is cultivated under specific conditions, it is differentiated into adipose cells and oil drops are accumulated within the cells. A study group lead by Professor Miyashita from the Graduate School of Fisheries Sciences of Hokkaido University added 10 μm and 25 μm of pure fucoxanthin to the cell strain and evaluated fat stores by the oil red method and discovered that fucoxanthin significantly inhibits fat stores. (Fig. 6)

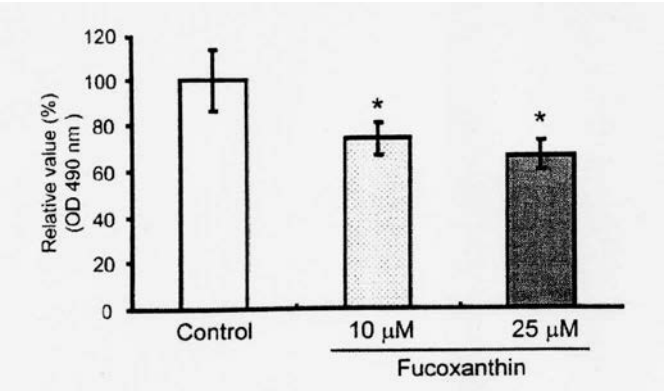


Fig. 6 Effect of Fucoxanthin on lipid accumulation of 3T3-L1 cells during adipocyte differentiation
*: *P<0.01*

Reference:
Maeda H, Hosokawa M, Sashima T, Takahashi N, Kawada T, Miyashita K. Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *Int J Mol Med.* **18**(1): 147-52 (2006).

2) Anti-obesity activity (in vivo)

Professor Miyashita presented a further report about fucoxanthin's anti-obesity activity. The study group fed fucoxanthin fraction (fucoxanthin content: 67.4%) mixed in feed (0.4%) to KKAy obese mice for 4 weeks and measured their visceral fat (WAT, white adipose tissue) weight. As a result, WAT weight of the fucoxanthin fraction-fed group significantly reduced as compared to that of the control group. No activity to reduce fat weight was found in the glycolipid fraction-fed group (Fig. 7). They studied UCP1 expression in WAT in Western blot analysis and confirmed a significant increase of the expression. As shown in Fig. 8A, no UCP1 band was found in WAT of mice in the control and glycolipid fraction-fed groups in Western blot analysis. In the fucoxanthin fraction-fed group, however, prominent UCP1 band expression was confirmed. Expression of β-Actin, that expresses constantly, needed to be at a similar level in all groups. Therefore, UCP1 expression was corrected with β-Actin level for calculation.

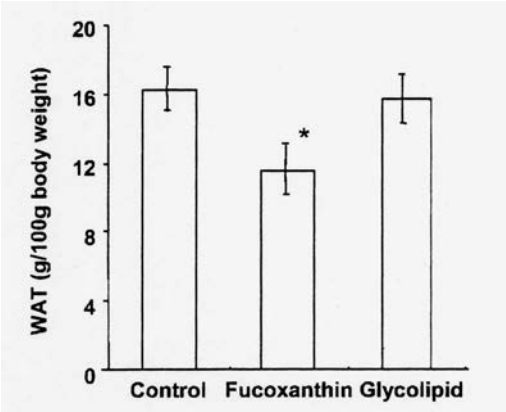


Fig. 7 Inhibition of WAT weight of obese KKAy mice fed fucoxanthin rich fraction, glycolipids fraction and control diet
*: *P<0.01*

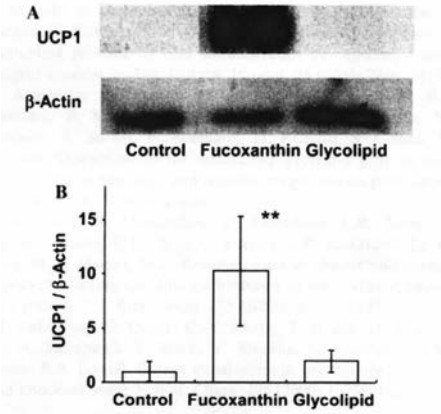


Fig. 8 UCP1 expression in WAT of obese KKAy mice fed fucoxanthin rich fraction, glycolipids fraction and control diet
**: *P<0.05*

There is a growing awareness about fucoxanthin throughout the world as the only dietary constituent which causes UCP1 expression in white adipose tissues. Fucoxanthin is now expected to be applied in foods with an anti-metabolic syndrome effect.

Reference:
Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K. Fucoxanthin from edible seaweed, Undaria pinnatifida, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem Biophys Res Commun.* **332**(2): 392-7 (2005).

3) Anti-diabetes activity (in vivo)

Characteristics of spontaneously diabetic, obese model KKAy mice are hyperglycemia, obesity, and elevated levels of serum insulin and leptin. Professor Miyashita's group discovered

fucoxanthin's anti-diabetes activity using the mice. The study group fed fucoxanthin (purity: 97%) mixed in feed (0.1% and 0.2%) to KKAy mice for 4 weeks and measured their blood glucose levels, serum insulin levels, and serum leptin concentrations. As a result, blood glucose levels, serum insulin levels, and serum leptin concentrations of the fucoxanthin-fed group significantly lowered as compared to the ones of the control group (Fig. 9). Since leptin is secreted from adipose tissues, fucoxanthin's activity to lower the serum leptin concentration is believed to be performed by reducing white adipose tissues. Thus, fucoxanthin is expected to reduce high blood glucose levels caused by the accumulation of visceral fat (obesity).

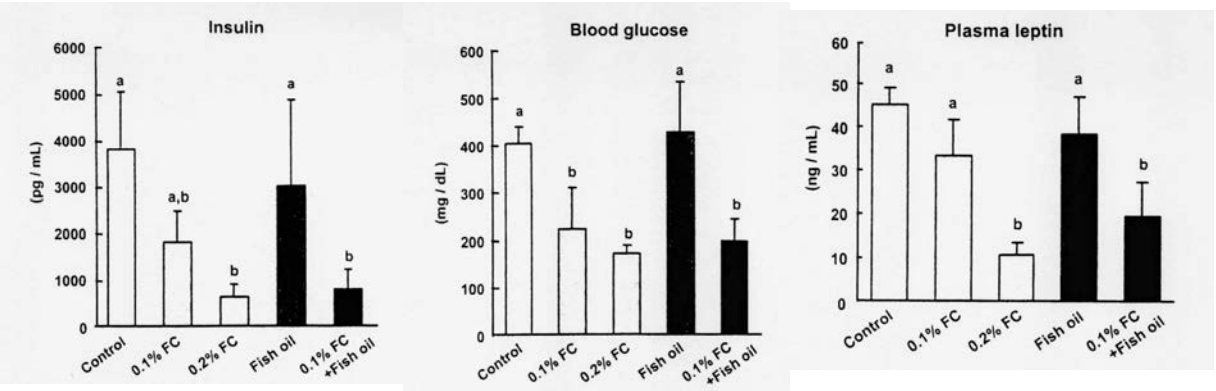


Fig. 9 Improvement effect on plasma insulin, glucose and leptin levels of obese KKAy mice fed fucoxanthin (FC) and/or fish oil. The different letters were significantly different from each other at *P<0.01*

Reference:
Maeda H, Hosokawa M, Sashima T, Miyashita K. Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-Ay mice. *J Agric Food Chem.* **55**(19): 7701-6 (2007).

(2) Skin care and skin-whitening activities

1) Collagenase inhibitory activity

Collagen makes up 90% of the dermis of the skin. It is distributed throughout the dermis, making the skin adequately elastic and strong. When collagenase is activated and collagen is degraded, aging phenomena such as wrinkles and sagging occur. Laminaria japanica extract (LJE for short, fucoxanthin content: 8.6%) was confirmed to inhibit the activation of collagenase concentration-dependently in the concentration range from 30 μg/mL to 1,000 μg/mL, inhibiting the degradation of collagen (Fig. 10). (The results were obtained through a joint study with Ogurayayamamoto Corporation.)

[Method of experiment]
LJE was dissolved in 10% DMSO. Collagenase enzyme solution 0.05 mL and PZ-peptide substrate solution 0.04 mL were added to 0.05 mL of the sample. They were reacted for 30 minutes at 37°C and 1 mL of 25 mM citric acid solution was added to stop the reaction. The sample was then extracted with 5 mL of ethyl acetate and the amount of PZ-peptide cleaved by collagenase was measured by the absorbance of the ethyl acetate layer.

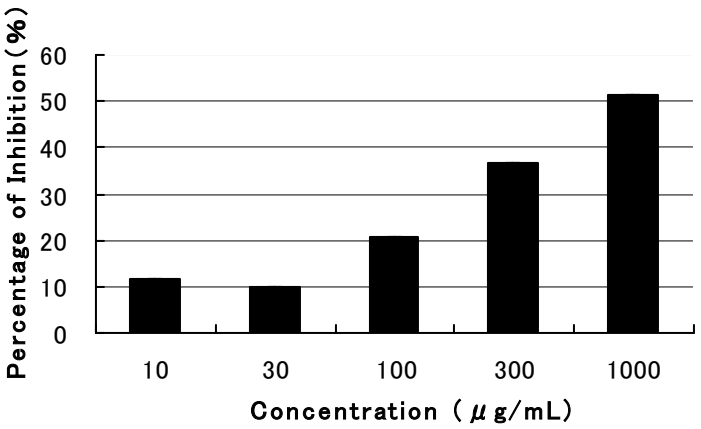


Fig. 10 Inhibitory activity of LJE (fucoxanthin content: 8.6%) on collagenase

2) Activity to promote collagen generation in normal human fibroblasts

Collagen is essential to keep the skin in a beautiful condition. However, collagen production declines after people reach the age of 20. Keeping the skin beautiful may be possible if the production of new collagen is promoted so that collagen can be continuously provided to the skin. In a test using normal fibroblasts from the dermis of a normal female, LJE (fucoxanthin content: 8.6%) was confirmed to promote collagen production in normal human fibroblasts (cells of a person at the age collagen production capacity declines) (Fig. 11). (The results were obtained through a joint study with Ogurayayamamoto Corporation.)

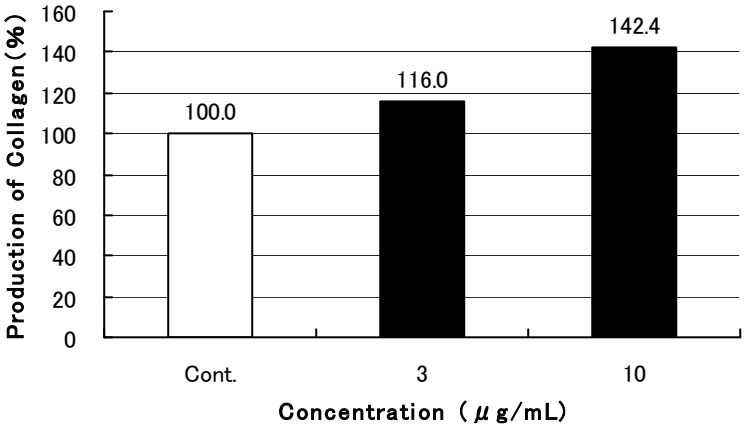


Fig. 11 Enhancement of collagen production in normal human fibroblasts by LJE (fucoxanthin content: 8.6%)

[Method of experiment]
Fibroblast from a 36 year-old Caucasian female (facial dermis) was prepared at 5x10 4 pieces/mL in a medium and 0.1 ml each was inseminated in 48-well plates. 0.2 mL of medium was added to each plate, the samples were cultivated for one day, and the medium was removed. Ultra DOMA-PF Liquid (protein-free medium) was dispensed by 0.38 mL, 20 μL of LJE solution was added, and the samples were cultivated for 3 days. Then, collagen quantity in the supernatant was measured using the Procollagen type I C-peptide (PIP) EIA Kit.

3) Hyaluronidase inhibitory activity

Hyaluronic acid is widely distributed in the body including the skin, joint fluids, vitreous bodies, and ligaments. It performs various functions in the skin such as bonding and protecting cells, forming skin tissues, retaining moisture in tissues, and maintaining elasticity of the skin. When the amount of hyaluronic acid declines, the skin becomes dehydrated and looses firmness which results in wrinkles and sagging. Hyaluronic acid generated in the body is degraded by hyaluronidase. LJE (fucoxanthin content: 8.6%) was confirmed to inhibit the activation of hyaluronidase concentration-dependently in the concentration range from 30 μg/mL to 1,000 μg/mL, inhibiting the degradation of hyaluronic acid (Fig. 12). (The results were obtained through a joint study with Ogurayayamamoto Corporation.)

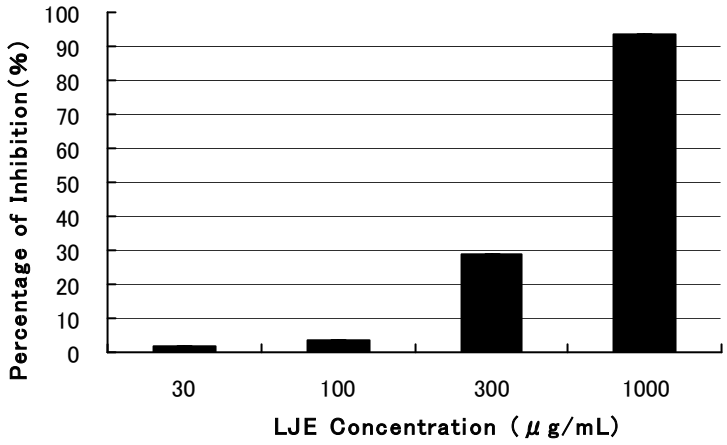


Fig. 12 Inhibitory activity of LJE (fucoxanthin content: 8.6%) on hyaluronidase

[Method of experiment]
LJE was dissolved in 10% DMSO and was reacted with hyaluronic acid and hyaluronidase reaction system. It was then reacted with p-dimethylaminobenzaldehyde and the absorbance was measured.

4) Elastase inhibitory activity

Elastin is a major protein making up the skin just like collagen and often found in highly-extensible tissues such as skin. Since it is involved in skin's elasticity, when elastin is degraded, the skin becomes less elastic which results in wrinkles and sagging. Elastin generated in the body is degraded by elastase. LJE (fucoxanthin content: 8.6%) was confirmed to inhibit the activation of elastase concentration-dependently in the concentration range from 10 μg/mL to 300 μg/mL, indicating the possibility of inhibiting the degradation of elastin (Fig. 13).

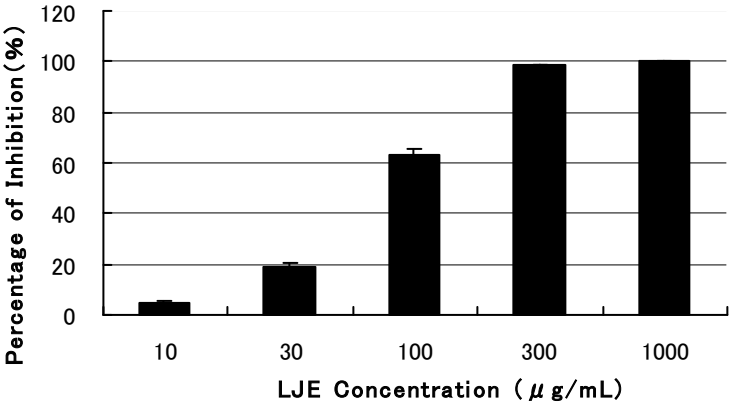


Fig. 13 Inhibitory activity of LJE (fucoxanthin content: 8.6%) on elastase

[Method of experiment]

LJE was dissolved in 10% DMSO and the elastase enzyme activation inhibitory rate was measured using the Enz Chek Elastase Assay Kit (Molecular Probes).

5) Tyrosinase inhibitory activity

Dullness, darkish tone, and dark spots on the skin are caused by melanin. Dopaquinone is produced out of tyrosine in the body due to actions of tyrosinase. Then, melanin is produced by the advancement of oxidation reaction and other processes.

In our test, LJE (fucoxanthin content: 8.6%) performed a concentration-dependent activity to inhibit tyrosinase in the concentration range from 10 μg/mL to 1000 μg/mL. Pure fucoxanthin purified from kelp extract was also confirmed to inhibit actions of tyrosinase concentration-dependently in the concentration range from 1 μg/mL to 30 μg/mL (Fig. 14).

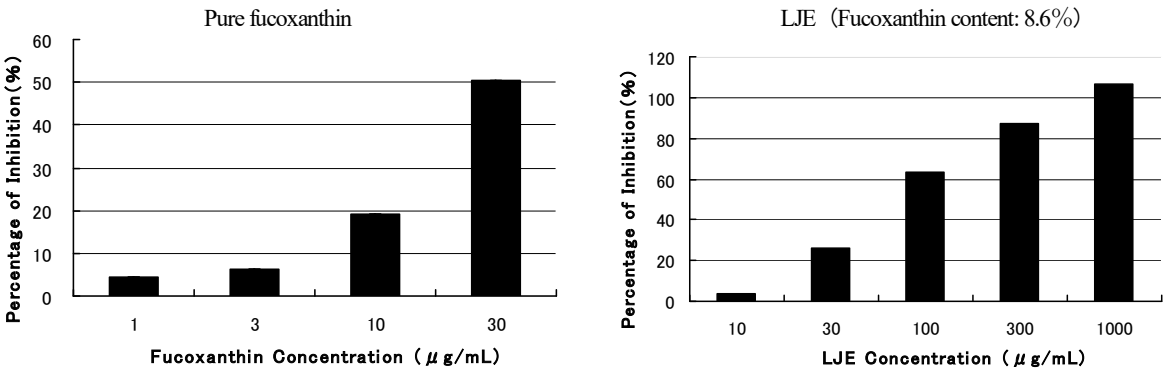


Fig. 14 Inhibitory activity of pure fucoxanthin and LJE (fucoxanthin content: 8.6%) on tyrosinase

[Method of experiment]
0.3% L-DOPA (70 μL/well) was added to sample solutions with different concentrations (70 μL/well) and the samples were preliminarily heated (5 min. at 37°C). Then, tyrosinase (mushroom-derived, 1.6 units/mL) solution (70μL/well) was added and reacted for 5 minutes at 37°C. After the reaction, absorbance (492 nm) was measured using a micro plate reader.

6) Melanin production inhibitory activity on B16 melanoma cells

We examined LJE and fucoxanthin for the effect to inhibit melanin production using B16 mice melanoma cells in order to measure their skin-lightening activity.

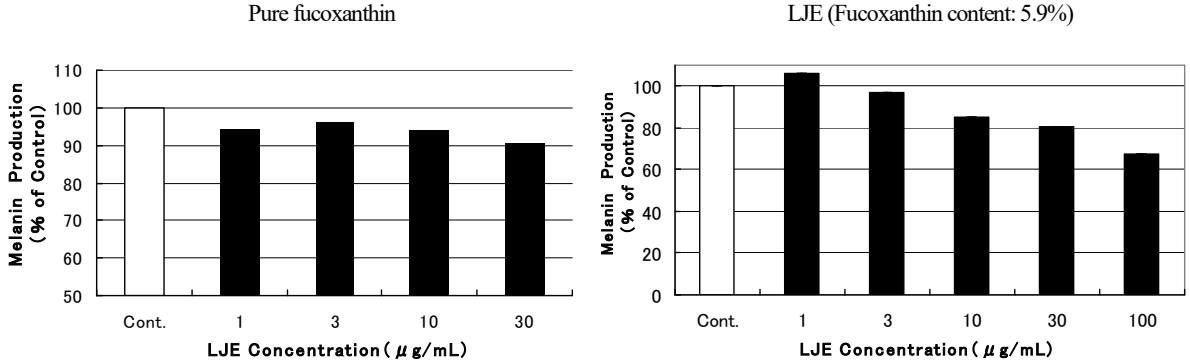


Fig. 15 Inhibitory activity of pure fucoxanthin and LJE (fucoxanthin content: 5.9%) on melanin production in B16 melanoma cells

Kelp extract (fucoxanthin concentration: 5.9%) demonstrated concentration-dependent melanin production inhibitory activity in the concentration range from 3 μg/mL to 100 μg/mL (Fig. 15). Pure fucoxanthin also demonstrated melanin production inhibitory activity in the concentration range from 1 μg/mL to 30 μg/mL. These results clarified that fucoxanthin inhibits melanin production at the cellular level as well. (The results were obtained through a joint study with Ogurayayamamoto Corporation.)
[Method of experiment]
B16 melanoma cells were suspended (1.8×105 cells/mL) in 2 mM theophylline-containing MEM medium (10% FCS, penicillin/streptomycin-containing) and 500 μL each was inseminated in a 24-hole plate. Sample solution (55 μL) was added and cultivated for 3 days. Then, the medium was removed, PBS (300 μL) was added, and cells were ultrasonic-fragmented. The fragmented solution was collected in a 96-hole plate and absorbance was measured (measurement wavelength: 415 nm, reference wavelength: 700 nm). The melanin production inhibition rate was calculated by the formula (absorbance of the sample/absorbance of the control x 100).

7) Pigmentation inhibitory activity (*in vivo*)

We had brown guinea pigs to freely intake Fucoxanthin-P1 (fucoxanthin: 1%) and examined the influence on darkening of the skin (pigmentation) caused by UV ray exposure. As a result, L* value (lightness value. The lower the value is, the closer to black) of the Fucoxanthin-P1-fed

group lowered while remaining higher (lighter) than the value of the control group from the last day of UV ray exposure to day 12. On day 15, L* value of the Fucoxanthin-P1-fed group significantly increased (Fig. 16). These results clearly indicate that Fucoxanthin-P1 inhibits pigmentation and accelerates the process to eliminate pigment deposits. Anti-pigmentary activity of fucoxanthin was published following journal.

Shimoda H., Tanaka J., Shan S., Maoka T. Anti-pigmentary activity of fucoxanthin and its influence on skin mRNA expression of melanogenic molecules. *J. Pharmacy Pharmacol*. **62**, 1137-1145 (2010).

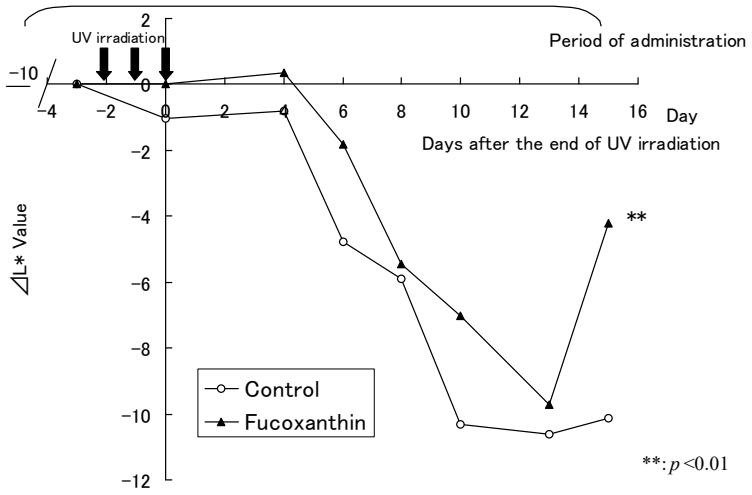


Fig. 16　Inhibitory activity of fucoxanthin-P1 on pigmentation (n=4)

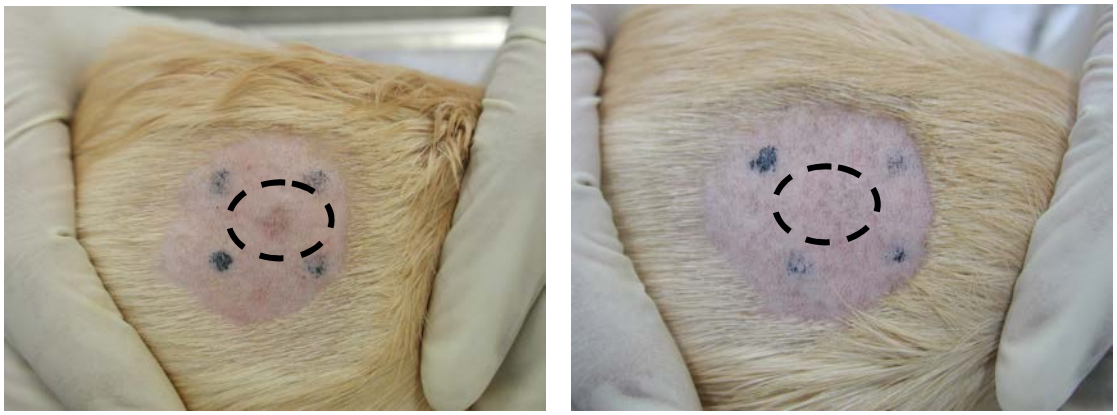


Fig. 17　Pictures of the UV irradiation parts on the sixteenth day (left: control, right: fucoxanthin-P1)

[Method of experiment]

We had guinea pigs (Weiser-Maples brown guinea pigs, male, 4-week old) to freely intake feed containing 1% Fucoxanthin-P1 starting on 7 days before UV ray exposure (day -10). Then, we exposed them to UV ray (UV-B, 2,000 mJ/cm2) for 3 days (days -2 to 0) using a UV ray irradiator (Solar Simulator, USHIO INC.) Feed containing 1% Fucoxanthin-P1 was fed to the guinea pigs continuously during the exposure period and for 16 days after exposure (days 0 to

16). We measured the lightness (L* value) of their skin using a spectrocolorimeter before UV ray exposure and 4, 6, 8, 10, 13, and 15 days after UV ray exposure was started.

8) Melanogenesis inhibitory effect and its mechanism in mice

As described above, fucoxanthin inhibited tyrosinase activity, melanogenesis in melanoma and UVB-induced skin pigmentation in guinea pig. We further discussed its melanogenesis inhibitory effect and its mechanism in hairless mice by topical application and oral administration. Topical application of fucoxanthin (1%) significantly suppressed mRNA expression of cyclooxygenase (COX)-2, endothelin receptor A, p75 neurotrophin receptor (NTR), prostaglandin E receptor 1 (EP1), melanocortin 1 receptor (MC1R) and tyrosinase related protein 1 (Table 1). The suppression of p75NTR, EP1 and MC1R expressions were observed at 0.01% application. Fig. 18 shows microscopic images of a cross-section of skin topically treated with fucoxanthin and stained by Fontana-Masson.

Table 1 Effect of topical treatment with fucoxanthin on skin mRNA expression in UVB-irradiated hairless mice

			Fucoxanthin (%)		
	Ct of control	Control	0.01	0.1	1
Released cytokine from epidermal cell					
ET-1	27.2	1.00±0.02	1.12±0.18	1.23±0.09*	0.97±0.11
NT-3	30.7	1.00±0.02	1.84±0.13	2.98±0.07**	1.16±0.05
COX-2	27.1	1.00±0.01	1.01±0.22	0.85±0.18	0.73±0.05*
Receptor in melanocyte					
EDNRA	25.3	1.00±0.01	1.03±0.08	0.98±0.11	0.83±0.05*
NT3R	28.7	1.00±0.02	0.98±0.09	1.29±0.24**	0.81±0.14
p75NTR	27.8	1.00±0.04	0.70±0.08**	0.70±0.18*	0.57±0.16**
EP1	27.7	1.00±0.02	0.73±0.10**	0.86±0.16	0.66±0.07**
MC1R	27.2	1.00±0.05	0.79±0.02**	0.75±0.03**	0.66±0.03**
Melanogenesis in melanocyte					
Tyr	26.3	1.00±0.03	0.91±0.07	0.85±0.16	0.77±0.15
Tyrp1	25.0	1.00±0.03	0.85±0.11	0.74±0.08**	0.64±0.11**

Each value represents mean±SE of 5 mice. Asterisks denote significant difference from control at *: $p<0.05$, **: $p<0.01$, respectively. Each mRNA expression was corrected by that of β-actin.

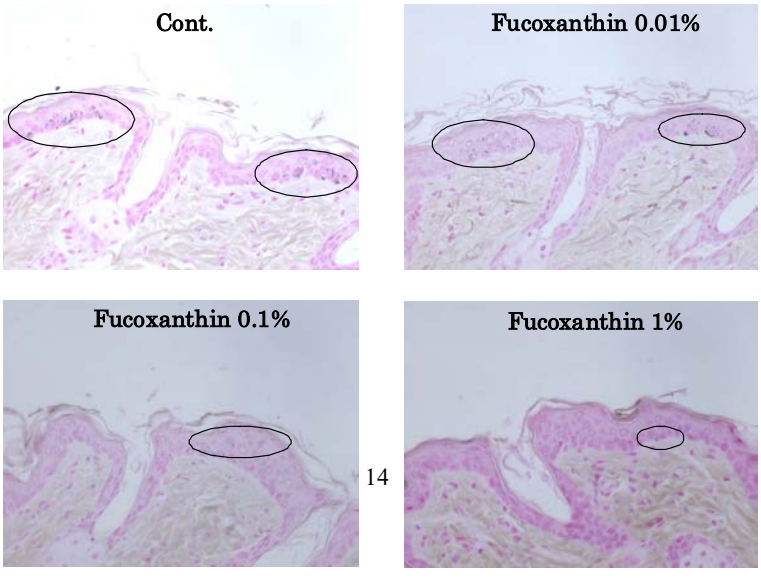


Fig. 18 Microscopic images of a cross-section of skin topically treated with fucoxanthin. Black dots shows melanin stained by Fontana-Masson. Fucoxanthin inhibited melanin production in the UVB-irratiated skin dose-dependently.

Similar results were obtained with oral application of fucoxanthin as shown in Table 2. Fucoxanthin (10 mg/kg) significantly suppressed expression of COX-2, p75NTR, EP1 and MC1R.

Table 2 Effect of oral treatment with fucoxanthin on skin mRNA expression in UVB-irradiated hairless mice

			Fucoxanthin (mg/kg)		
	Ct of control	Control	0.1	1	10
Released cytokine from epidermal cell					
ET-1	27.3	1.00±0.01	1.23±0.10*	1.40±0.13**	1.27±0.16*
NT-3	29.3	1.00±0.02	0.90±0.05	0.82±0.06	0.86±0.01
COX-2	27.9	1.00±0.01	0.86±0.16	0.89±0.11	0.74±0.09*
Receptor in melanocyte					
EDNRA	25.5	1.00±0.01	0.96±0.04	1.09±0.05	0.96±0.06
NT3R	28.8	1.00±0.02	1.01±0.16	1.24±0.11*	0.96±0.10
p75NTR	28.9	1.00±0.04	0.84±0.18	0.87±0.06	0.69±0.07**
EP1	28.2	1.00±0.01	1.05±0.08	0.89±0.07	0.80±0.09*
MC1R	25.5	1.00±0.01	0.94±0.17	0.87±0.08	0.76±0.06*
Melanogenesis in melanocyte					
Tyr	27.1	1.00±0.03	0.99±0.16	1.04±0.14	0.96±0.09
Tyrp1	28.0	1.00±0.03	0.95±0.20	1.01±0.19	0.69±0.16

Each value represents mean±SE of 5 mice. Asterisks denote significant difference from control at *: $p<0.05$, **: $p<0.01$, respectively. Each mRNA expression was corrected by that of β-actin.

[Method of experiment]

To examine the effect of topical and oral treatment of fucoxanthin on skin mRNA changes, hairless mice were used. For topical treatment, fucoxanthin was mixed with white petrolatum (0.01, 0.1 and 1%). The ointment (50 μL) was applied once a day to the back skin of the mice shortly after UVB-irradiation. For oral treatment, fucoxanthin was suspended in water (0.1, 1 and 10 mg/kg) with 5% acacia and given once a day, 2 hr before UVB-irradiation. UVB (160 mJ/cm^2) was irradiated to back skin for 7 days followed by increased UVB (320 mJ/cm^2) irradiation for 7 more days. On the day after the last UVB-irradiation, the irradiated skin area was removed and the specimens were soaked in 10% neutralized formaldehyde or RNA later™ attached to an RNeasy Protect Mini Kit, respectively. Each specimen soaked in formaldehyde was stained by Fontana-Masson staining for melanin detection. Microscopic observation was

performed at a magnification of ×400. The specimens in RNA later™ were stored at 4°C for RT-PCR analysis.

(3) Anti-acne activity

P. acnes is an acne bacterium always present in hair follicles on the skin. Growth of *P. acnes* is believed to be closely involved in the development and worsening of acne. Acne bacteria grow using sebum as a nutrient source. Grown acne bacteria produce lipase which degrades triglyceride in sebum and releases fatty acids which causes a series of inflammatory reactions, including the migration of white blood cells to the dermis, invasion, and release of inflammatory factors. Inflammatory factors irritate the skin and accelerate inflammation and also cornification of the epidermis. This is how acne occurs and worsens.

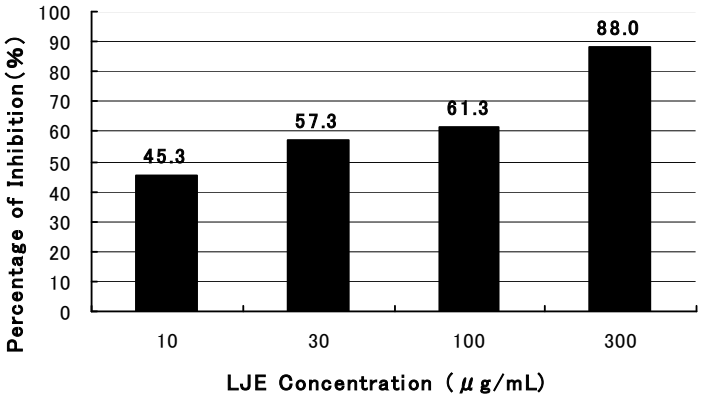


Fig. 19 Inhibitory activity of LJE on lipase from *P. acnes*

In our test, kelp extract (fucoxanthin concentration: 3.9%) inhibited the activation of lipase derived from acne bacteria concentration-dependently in the concentration range from 10 μg/mL to 300 μg/mL. This test result indicates that kelp extract may have an anti-acne activity (Fig. 19).

[Method of experiment]

P. acnes was cultivated in a GAM liquid medium and fungus bodies were collected by centrifugation (3,000 rpm, 10 min). PBS was added to the collected fungus bodies, the mixture was ultrasonic-fragmented and centrifuged, and the supernatant was collected. After the supernatant was dialyzed with PBS at 4°C for 3 days, P. acnes-derived crude lipase was obtained by freeze dehydration. Kelp extract's lipase inhibitory activity was measured using the Lipase Kit S (Dainippon Pharmaceutical). A color-producing solution (390 μL), kelp extract solutions in different concentrations (25 μL), 2 μL of P. acnes-derived crude lipase (50 mg/mL), and elastase inhibitory agent (10 μL) were put in a test tube and were preliminarily heated in a thermostatic chamber at 30°C for 5 minutes. Then, substrate solution (50 μL) was added and reacted with the mixture at 30°C for 30 minutes in a condition shaded from light. Lastly, the absorbance at 415 nm was measured.

(4) Clinical test on humans

We examined Fucoxanthin-P1's effect on various serum parameters related to metabolic syndrome and beauty enhancement when it is ingested continuously. We had 12 volunteer subjects (male and female) from our company freely take Fucoxanthin-P1 (50 mg/capsule/day, equivalent to 0.5 mg of fucoxanthin) for 14 days and compared their metabolic syndrome indexes (male subjects only) and serum parameters before and after the ingestion period. We also measured moisture content (moisture), pH, and sebum amount of the skin of female subjects (6 people) before and after the intake of the capsules.

Method of Experiment: Moisture: CORNEOMETER SM825 (CK electronic GmbH), pH: SKIN-pH-METERPH900 (CK electronic GmbH), and sebum amount: SEBUMETER SM810 (CK electronic GmbH) of the subjects' skin were measured. Skin's elasticity was measured using the Modulus face care sensor.
Tested parts: Face, inner side of an upper arm
Test conditions: Temperature: 24°C, Humidity: 60%

1) Effects on metabolic syndrome indexes
We compared metabolic syndrome indexes before and after the intake of fucoxanthin in male subjects. As a result, we confirmed that their abdomen subcutaneous fat thickness significantly reduced ($P<0.05$) and their weight and waist sizes slightly reduced (Table 3, Fig. 20). According to blood test results, their blood neutral fat level lowered within the normal range (before intake: 178.8±144.8, after intake: 103.6±32.9) and HDL cholesterol increased (before intake: 63.6±12.2, after intake: 68.6±6.5) (Table 4, Fig. 21). The test results indicate that fucoxanthin has an anti-metabolic syndrome activity.

Table 3 Improvement effect on metabolic syndrome indexes

	Before ingestion	After ingestion
Body weight (kg)	71.8±8.3	71.4±7.8
Body fat rate (%)	23.6±4.0	23.7±4.4
BMI (kg/m^2)	25.1±2.3	25.1±2.3
impedance (Ω)	460.0±77.7	459.8±61.4
Weight of fat (kg)	17.0±3.8	17.1±4.6
Obesity index (%)	14.0±10.7	13.9±10.5
Waist (cm)	87.2±6.7	85.9±7.1
Hip (cm)	95.8±6.8	97.6±3.7
Waist/hip ratio	0.9 ±0.1	0.88±0.1
Abdomen subcutaneous fat thickness (mm)	23.8±3.5	$20.5\pm2.9^{P<0.05}$

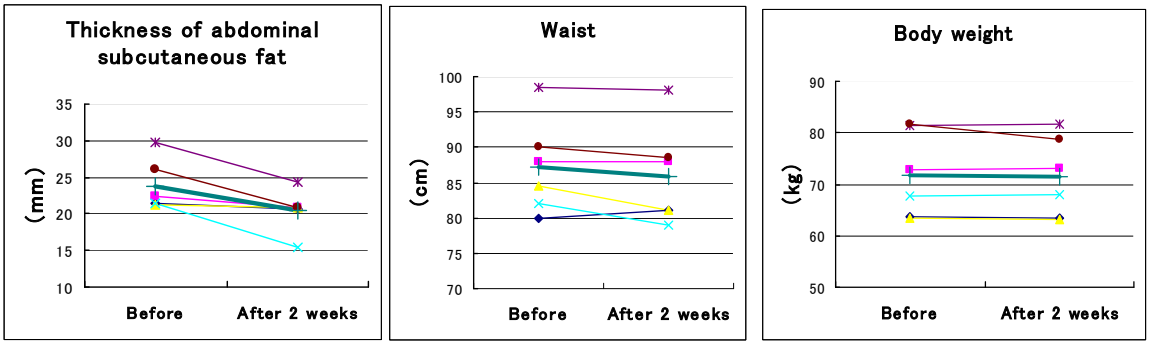


Fig. 20　Improvement effect of fucoxanthin on metabolic syndrome indexes

Table 4　Changes of blood parameters related to metabolic syndrome

	Before Ingestion	After ingestion
HDL-cholesterol	63.6±12.2	68.6±6.5
TG	178.8±144.8	103.6±32.9
Total cholesterol	203.2±17.5	202.0±27.4
LDL-cholesterol	114.6±20.9	120.2±28.4
Free fatty acid	0.4±0.3	0.5±0.3
BUN	17.08±5.3	16.1±2.4
Creatinine	1.0±0.2	1.0±0.1
uric acid	8.3±2.2	7.9±2.1
Ketone bodies	26.4±12.7	36.0±36.4
Glucose	101.4±19.3	96.4±8.6

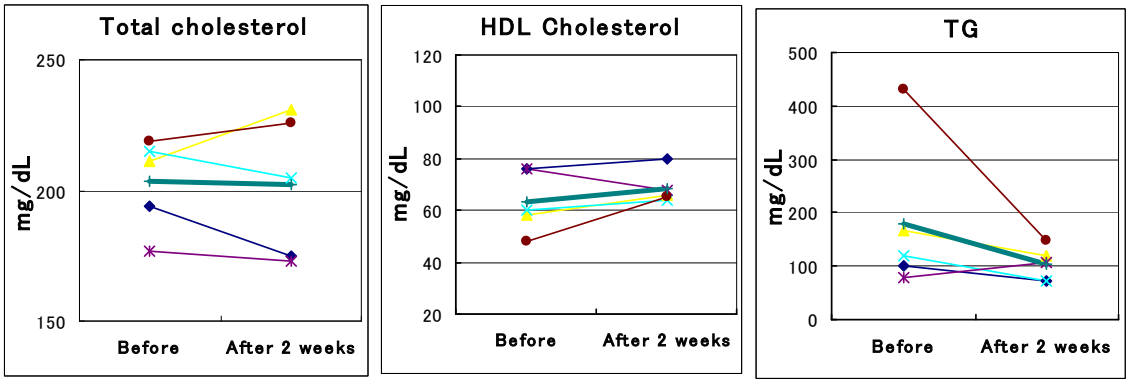


Fig. 21　Improvement effect of fucoxanthin on blood metabolic syndrome parameters

2) Beauty enhancement effects

We compared skin conditions before and after the intake of fucoxanthin in female subjects. Although fucoxanthin did not demonstrate any moisturizing effect, it improved skin's pH level and sebum amount. It also increased skin elasticity of 4 subjects out of 6 (Fig. 22). The test results indicate that fucoxanthin has a beauty-enhancing effect.

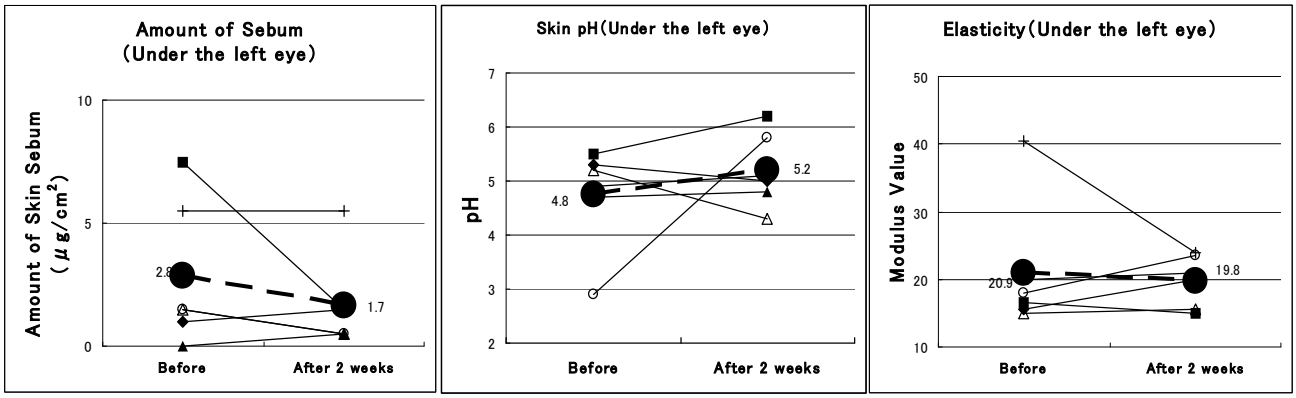


Fig. 22 Improvement effect of fucoxanthin on skin conditions

(5) Antioxidative activity in the body

Active oxygen is generated in the human body due to irritation such as mental stress. Active oxygen causes oxidative damages such as damage on cells and is closely related to various lifestyle-related diseases and acceleration of aging.
We evaluated the antioxidative activity of LJE (fucoxanthin content: 8.2%) using the 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability as the index. As a result, kelp extract demonstrated a DPPH radical scavenging ability concentration-dependently in the concentrations shown in Fig. 23. (The results were obtained through a joint study with Ogurayayamamoto Corporation.)

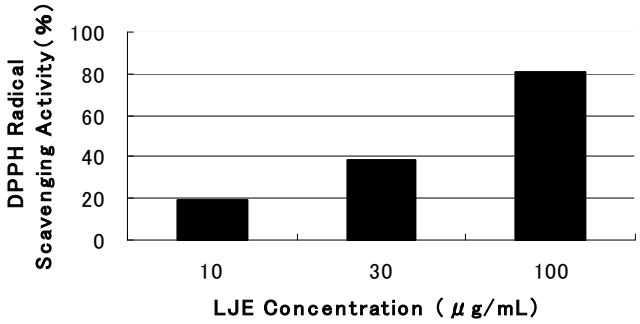


Fig. 23 DPPH radical scavenging activity of LJE(fucoxanthin content: 8.2%)

As carotenoids' activities, antioxidative activity is the most known. Fucoxanthin has strong antioxidative activity just like other carotenoids. Although other carotenoids such as beta carotene, beta cryptoxanthin, zeaxanthin, astaxanthin, lycopene, and rutin perform their antioxidative activity under aerophilic conditions, fucoxanthin performs higher antioxidative activity under a low-oxygen partial pressure. This is a major characteristic of fucoxanthin.

Reference:
Nomura T, Kikuchi M, Kubodera A, Kawakami Y. Proton-donative antioxidant activity of fucoxanthin with 1,1-diphenyl-2-picrylhydrazyl (DPPH). *Biochem Mol Biol Int.* **42**(2): 361-70 (1997).

(6) Anticancer activity

As a type of carotenoid, fucoxanthin has been attracting people's attention for its effect to prevent cancer. Fucoxanthin has been reported to prevent colon cancer, duodenal cancer, leukemia, prostate cancer, and liver cancer (Literatures 7 to 15).

4. Pharmacokinetics of Fucoxanthin

(1) Metabolic disposition of fucoxanthin

CaCo-2 human colon cancer cells are known to differentiate into small intestinal epithelial

cell-like form after approximately 3 weeks of monolayer culture and are often used in model experiments to study digestive absorption of food components and drugs.
Sugawara et al.[1] examined fucoxanthin absorption in Caco-2 cells. As a result, fucoxanthin and fucoxanthinol with hydrolyzed acetyl group (Fig. 24) were found and identified within Caco-2 cells. It was assumed that acetyl group was hydrolyzed by the actions of esterolytic enzyme existing in the digestive tract and free fucoxanthinol was absorbed.

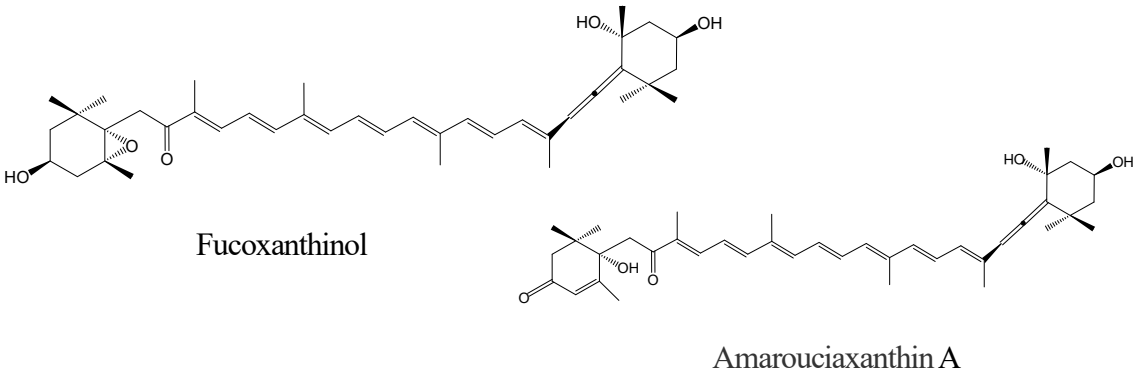
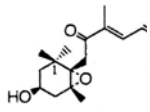
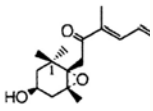


Fig. 24 Chemical structures of fucoxanthinol and amarouciaxanthin A

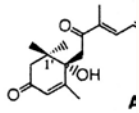
Metabolic disposition of fucoxanthin was studied using mice[2]. 40 nmol of fucoxanthin was orally administrated to each mouse and their blood plasma was analyzed one hour later. Although no fucoxanthin was found, its metabolite fucoxanthinol and amarouciaxanthin A (Fig. 24) were found and identified.
According to the test results, metabolic disposition of fucoxanthin was estimated as shown in Fig. 25. After the intake, fucoxanthin is hydrolyzed into fucoxanthinol by lipase from pancreatic fluid, fatty acid ester-degrading enzyme such as cholesterol esterase, or esterase from small intestinal epithelial cells. Fucoxanthin is then absorbed by gastrointestinal epithelial cells, carried to the liver through lymph fluid and blood, and metabolized into amarouciaxanthin A.



Hy
gastroin



Dehydrogenation/
in
liver



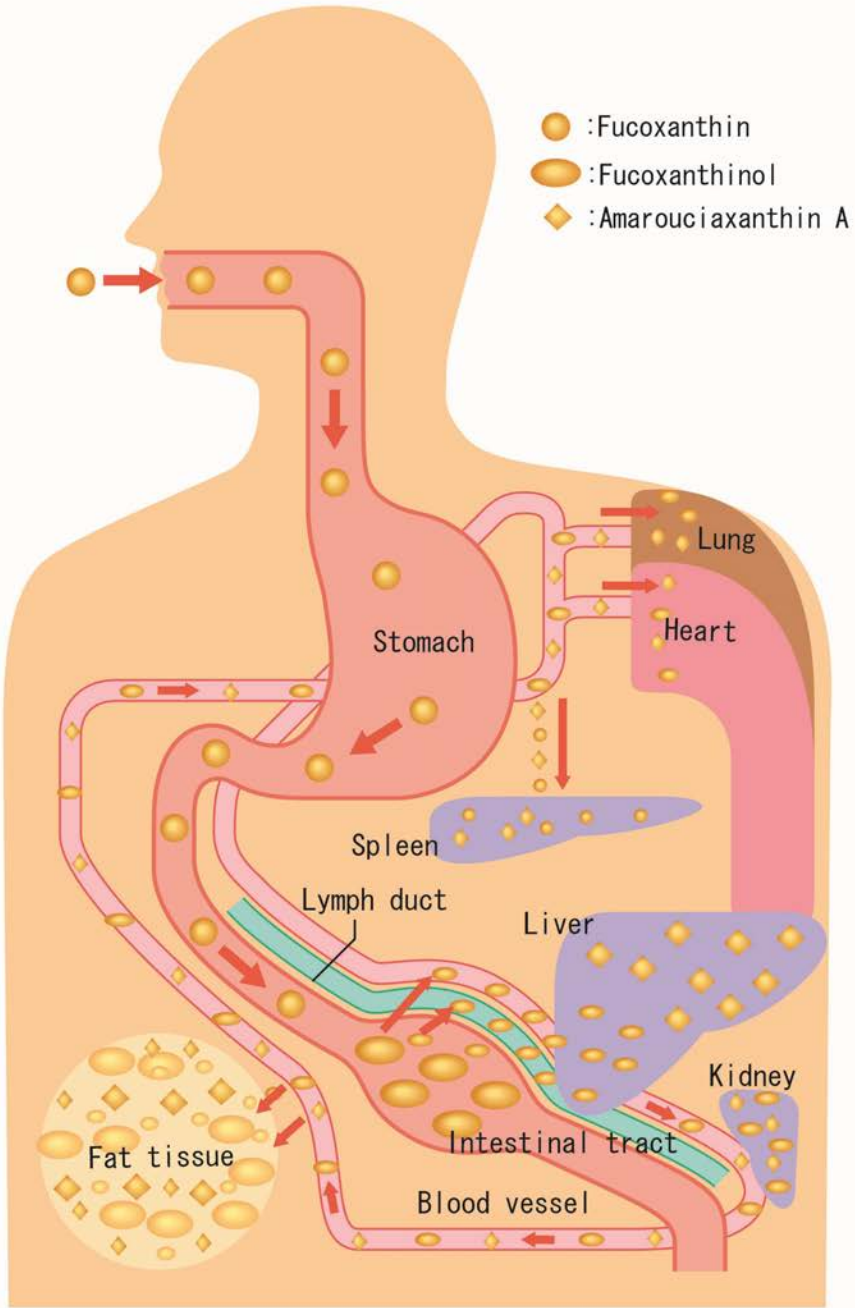


Fig. 25 Postulated biotransformation pathway of fucoxanthin

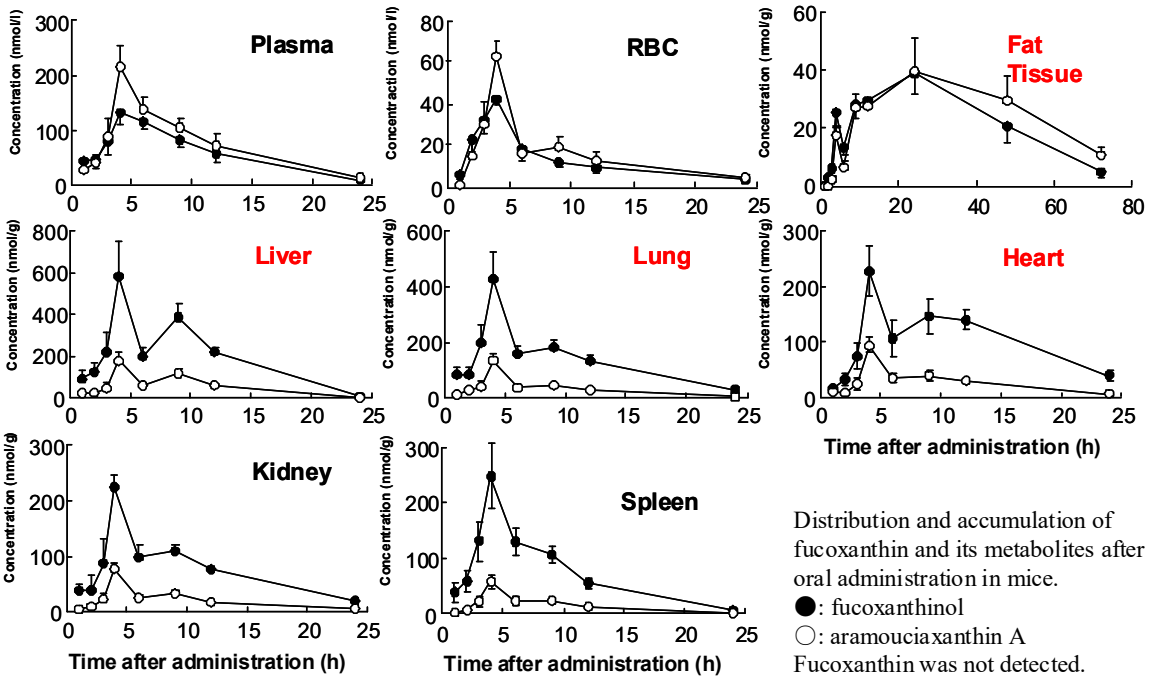
References:

1） Sugawara T, Baskaran V, Tsuzuki W, Nagao A. Brown algae fucoxanthin is hydrolyzed to fucoxanthinol during absorption by Caco-2 human intestinal cells and mice. *J Nutr.* **132**(5): 946-51 (2002).

2） Asai A, Sugawara T, Ono H, Nagao A. Biotransformation of fucoxanthinol into amarouciaxanthin A in mice and HepG2 cells: formation and cytotoxicity of fucoxanthin metabolites. *Drug Metab Dispos.* **32**(2): 205-11 (2004).

(2) Distribution and accumulation of fucoxanthin in mice

The distribution and accumulation of fucoxanthin in mice were investigated by Prof. Kanazawa of the Graduate School of Agricultural Science, Kobe University. The mice were given a single dose of fucoxanthin (160 nmol/mouse) and then fucoxanthin and its metabolites were determined in plasma, red blood cell, fat tissue, liver, lung, heart, kidney and spleen in a time course as shown in Fig. 26. Fucoxanthin was not detected in all the tissues and organs investigated. Instead, two metabolites of fucoxanthin, fucoxanthinol and amarouciaxanthin A were detected after 1 hr of administration. They both reached peak concentration at about 4 hr and then died away gradually and diminished at about 25 hr except for the fat tissue. The metabolites reached peak concentration in the fat tissue at about 25 hr and diminished very slowly at about 72 hr.



Distribution and accumulation of fucoxanthin and its metabolites after oral administration in mice.
●: fucoxanthinol
○: aramouciaxanthin A
Fucoxanthin was not detected.

Modified from Hashimoto et al., Br. J. Nutr., 102 (2), 242-248 (2009)

Fig. 26 Distribution and accumulation of fucoxanthin and its metabolites after oral administration in mice

Although fucoxanthin was not detectable in a single dose oral administration, however, it could be detected in red blood cell (erythrocytes), fat tissue (adipose tissue), liver, lung, heart, kidney and spleen except for the plasma when fucoxanthin was consecutively administrated to mice for one week at a dosage of 160 nmol/mouse/day (Fig. 27).

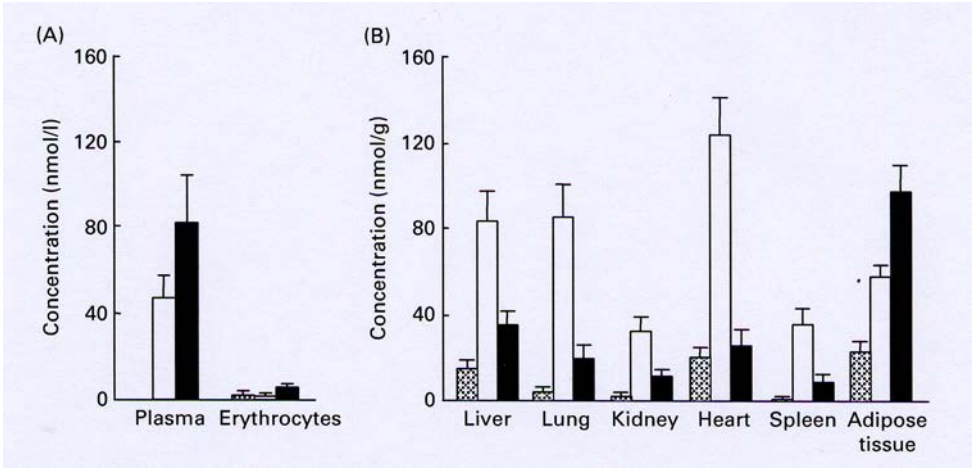


Fig. 27 Detection of fucoxanthin and its metabolites in mice after one week consecutive oral administration. Left: fucoxanthin, Middle: fucoxanthinol, Right: amarouciaxanthin A.

References:
Hashimoto T., Ozaki Y., Taminato M., Das S. K., Mizuno M., Yoshimura K., Maoka T. and Kanazawa K. The distribution and accumulation of fucoxanthin and its metabolites after oral administration in mice. *Br. J. Nutr.,* **102** (2), 242-248 (2009)

5. Stability of fucoxanthin

(1) Thermostability

The thermostability of fucoxanthin was evaluated on Fucoxanthin-1 and Fucoxanthin-P1 at 80°C and 100°C for one hour. The result shows that Fucoxanthin-1 is relatively stable at 80°C for one hour. However, to some extent it degraded at 100°C for one hour. Therefore, it is recommended that Fucoxanthin-1should be processed under 100°C.
As for Fucoxanthin-P1, it is stable at 80°C and 100°C for one hour. Therefore, Fucoxanthin-P1 can be manipulated under the conventional processing temperature for food.

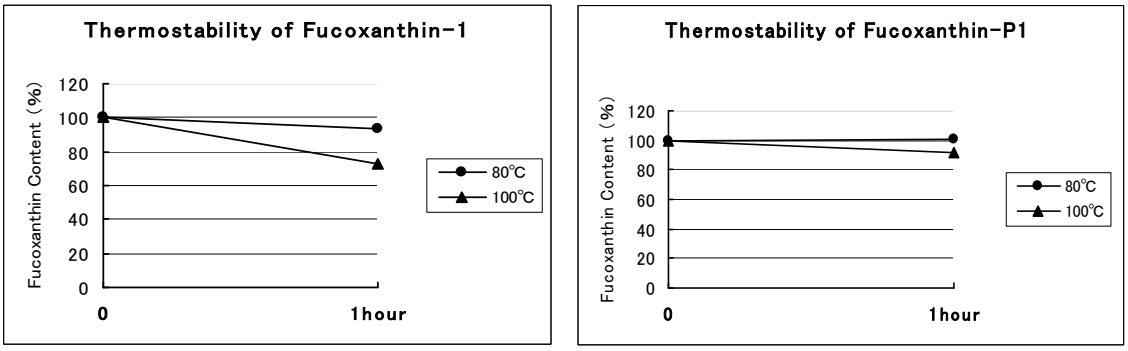


Fig. 28 Thermostability of fucoxanthin

(2) pH stability

Fucoxanthin-WSP0.1 was used for pH stability test. Solution of Fucoxanthin-WSP0.1 (1.0%) was prepared and adjusted to the required pH level. The solutions were stored under light-proof condition at room temperature for 1 day and 1 week, respectively. Result showed that content of fucoxanthin remained stable at a wide pH range from acidic condition to basic condition.

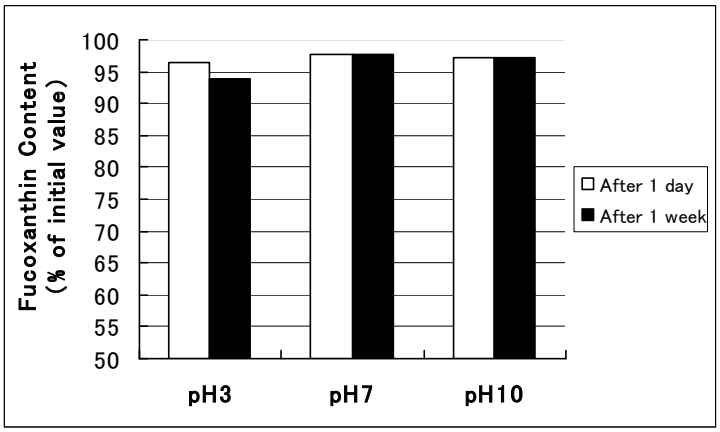


Fig. 29 pH stability of Fucoxanthin-WSP0.1

6. Recommended Dosage

It is suggested that 0.5~1.0 mg of fucoxanthin should be taken a day, i.e.,

Fucoxanthin-5KW (oil)	10~20 mg/day
Fucoxanthin-1 KW (oil)	50~100 mg/day
Fucoxanthin-P1 KW (powder)	50~100 mg/day
Fucoxanthin-WSP0.1 KW (powder)	500~1000 mg/day (Please do not exceed 1% in beverages)

7. Nutritional Composition

Items	Fucoxanthin -5 (100 g)	Fucoxanthin -1 (100 g)	Note	Analytical Method
Water	4.0g	0.8 g		Heat-drying at reduced atmospheric pressure
Protein	0.5 g	0.1 g	1	Kjeldahl Method
Fat	75.0 g	83.6 g		Acid degradation
Ash	0.0 g	0.0 g		Direct Incineration
Carbohydrate	20.5g	15.5 g	2	
Energy	759kcal	815 kcal	3	Atwater Method (Revised)
Sodium	90 mg	18 mg		Atomic absorption spectrophotometory
Table Salt	0.2 g	0.0 g		Sodium Equiv. value
Dietary fiber	0.0 g	0.0 g		Prosky Method

Items	Fucoxanthin-P1 (100 g)	Fucoxanthin-WSP0.1 (100 g)	Note	Analytical Method
Water	1.5 g	0.2 g		Heat-drying at reduced atmospheric pressure
Protein	0.2 g	0.0 g	1	Kjeldahl Method
Fat	10.2 g	1.0 g		Acid degradation
Ash	0.2 g	0.0 g		Direct Incineration
Carbohydrate	87.9 g	98.8g	2	
Energy	444 kcal	404 kcal	3	Atwater Method (Revised)
Sodium	43 mg	4.3 mg		Atomic absorption spectrophotometory
Table Salt	0.1 g	0.0 g		Sodium Equiv. value
Dietary fiber	0.0 g	0.0 g		Prosky Method

Notes: 1) Nitrogen, protein conversion factor: 6.25

2) Carbohydrate expression standard (Ministry of Health and Welfare's announcement No. 176)
Calculation: 100 – (water + protein + fat + ash)

3) Energy expression standard (Ministry of Health and Welfare's announcement No. 176)
Conversion factor: Protein 4, fat 9, sugar 4, dietary fiber 2

4) The nutritional compositions of Fucoxanthin-5and Fucoxanthin-WSP0.1 were calculated according to the analytical values of Fucoxanthin-1and Fucoxanthin-P1.

Test trustee: SRL, Inc

Date of analysis: October, 2, 2008
Test No.:200809250026

8. Safety Profile of Fucoxanthin

(1) Residual agricultural chemicals

Fucoxanthin (kelp extract with no diluents added) was examined for 507 residual agricultural chemical compounds following the provisions of the Food Hygiene Law and pesticide legislation. As a result, contents of all compounds were confirmed to be below the standard values (measurable limits).
Test trustee: Food Safety Evaluation and Analysis Center, Masis Co., Ltd.
Date of test report issued: October 1, 2008
Report No. 24007

(2) Acute toxicity (LD50)

LJE (fucoxanthin content: 3.0%) was orally administrated to 5-week old male and female Sprague-Dawley rats (weight: male 154 g to 168 g, female 120 g to 138 g) in a dose of 2000 mg/kg. The rats were housed at 23 ± 2°C and at 50 ± 10% humidity with free access to feed and drinking water for 14 days. No abnormal change was found in their weight as compared to the control group. No abnormalities were found in their organs upon autopsy after the test either. LD50 of kelp extract (fucoxanthin content: 3.0%) is deduced to be 2,000 mg/kg for rats.

(3) Micronucleus test

LJE (fucoxanthin content: 3.0%) was orally administrated to 8-week old male and female ICR mice (weight: 32 g to 36 g) in a dose of 500 mg/kg, 1,000 mg/kg, and 2,000 mg/kg. Bone-marrow cells of the mice were taken 24 hours later to create bone-marrow smear. The slide smear was created by Giemasa staining, polychromatic erythrocytes were observed using a microscope, and expression rate of micronucleate polychromatic erythrocytes was calculated. The ratio of polychromatic erythrocytes for all red blood cells was also calculated as an index of inhibiting the growth of bone-marrow cells. As a result of the test, there were no incidences of death for any of the dosages and there was no change in the mice's general physical conditions or weight. There was no statistically significant increase in the micronucleus-inducing frequency as compared to the negative control group. No dosage dependence was confirmed either. On the contrary, the micronucleus-inducing frequency of the positive control group prominently increased as compared to the negative control group. There was no significant difference in the ratio of polychromatic erythrocytes for all red blood cells for any of the dosages, indicating no activity to inhibit the growth of bone-marrow cells.

(4) 90-day repeated dose toxicity

A 90-day repeated dose toxicity test was carried out by orally administrating LJE (fucoxanthin content: 3.0%), mixed in diet at 1.0%, 2.0%, and 4.0% concentrations, to F344/DuCrj rats. There was no change in general conditions for both male and female rats and no animal died

through the entire test period. There was no significant change in their weight as compared to the control group.

9. Commercial Application

	Applications	Claims	Examples
Foods	Prevention of metabolic syndrome, anti-obesity, diet, anti-diabetes, anti-cancer, skin care・skin whitening	1) Anti-metabolic syndrome 2) Anti-obesity, diet 3) Anti-diabetes 4) Skin care・skin whitening 5) Anti-cancer	Beverages, hard & soft capsules, tablets, candies, chewing gums, chocolates, wafers, jellies, etc
Cosmetics	Functional cosmetics		Body lotions, body gel, mask, etc.

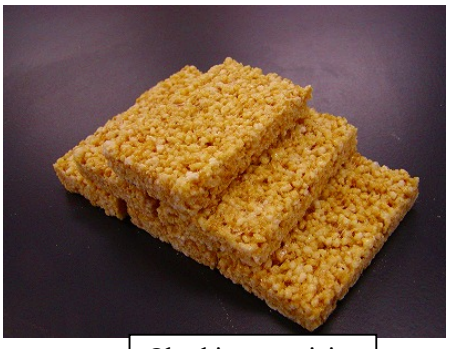
Spread containing fucoxanthin

Pie containing fucoxanthin

Stick potato containing fucoxanthin

Powdered green tea shortbread containing fucoxanthin

Okoshi containing fucoxanthin



Okoshi containing fucoxanthin

Application examples of fucoxanthin (provided by Ogurayayamamoto Corporation)

10. Package

Fucoxanthin-5KW、-1 KW (Oil, Food grade)
Fucoxanthin -5C KW、-1C KW (Oil, Cosmetic grade)

1kg、5kg Interior: Tin can
Exterior: Cardboard
Others: Fill with nitrogen gas, store below 5℃

Fucoxanthin- P1 KW (Powder, Food grade)
Fucoxanthin-WSP0.1 KW (Powder, Food grade)
Fucoxanthin- PC1 KW (Powder, Cosmetic grade)
Fucoxanthin-WSPC0.1 KW (Powder, Cosmetic grade)

1kg、5kg Interior packaging: Aluminium bag
Exterior packaging: Cardboard
Others: Package in vaccum, store below 5℃

11. Storage

The product is vacuum-packed or nitrogen-filled. Store the product sealed in a cool, dark place (5°C or below). Avoid high temperature and humidity. Once the package is open, remove air or fill nitrogen and use the product up as soon as possible.

12. Expression

Food grade:

Fucoxanthin-5 KW**, 1** KW

Expression: Caprylic/Capric Triglyceride, Laminaria Japanic Extract Containing Fucoxanthin or Kombu Extract Containing Fucoxanthin and Natural Tocopherol

Fucoxanthin-P1 KW**, -WSP0.1** KW

Expression: Caprylic/Capric Triglyceride, Laminaria Japonica Extract Containing Fucoxanthin or Kombu Extract Containing Fucoxanthin, Cyclodextrin and Natural Tocopherol

＊Please refer to your nation's laws and regulations.

Cosmetic grade:

INCI name:

Fucoxanthin-PC1 KW**:** Cyclodextrin, Caprylic/Capric Triglyceride, Tocopherol, Laminaria Japonica Extract, Fucoxanthin

Fucoxanthin-5C KW**, -1C** KW**:** Caprylic/Capric Triglyceride, Laminaria Japonica Extract, Fucoxanthin, Tocopherol

Fucoxanthin-WSPC0.1 KW**:** Maltosyl Cyclodextrin, Cyclodextrin, Maltose, Caprylic/Capric Triglyceride, Tocopherol, Laminaria Japonica Extract, Fucoxanthin

PRODUCT STANDARD

PRODUCT NAME : **FUCOXANTHIN-5KW** (FOOD)

This product is oily liquid extracted with ethanol from kombu (Laminaria japonica) or wakame (Undaria pinnatifida) and then purified. It contains minimum of 5.0 % fucoxanthin.

Appearance	Reddish brown liquid with light unique smell.	
Fucoxanthin	Min. 5.0 %	(HPLC)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
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)

Composition

Ingredient	Content
Triglyceride	79.0 %
Seaweed Extract	20.0 %
Mixed Tocopherol	1.0 %
Total	100.0 %

PRODUCT STANDARD

PRODUCT NAME : **FUCOXANTHIN-1KW** (FOOD)

This product is oily liquid extracted with ethanol from kombu (Laminaria japonica) or wakame (Undaria pinnatifida) and then purified. It contains minimum of 1.0 % fucoxanthin.

Appearance	Reddish brown liquid with light unique smell.	
Fucoxanthin	Min. 1.0 %	(HPLC)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
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)

Composition

Ingredient	Content
Triglyceride	96.5 %
Seaweed Extract	4.0 %
Mix Tocopherol	1.0 %
Total	100.0 %

PRODUCT STANDARD

PRODUCT NAME : **FUCOXANTHIN-P1KW** (FOOD)

This product is a powder extracted with ethanol from kombu (Laminaria japonica) or wakame (Undaria pinnatifida) and then purified. It contains minimum of 1.0 % fucoxanthin.

Appearance	Orangey powder with light unique smell.	
Fucoxanthin	Min. 1.0 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
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)

Composition

Ingredient	Content
Cyclodextrin	76.00 %
Triglyceride	18.96 %
Seaweed Extract	4.86 %
Mixed Tocopherol	0.24 %
Total	100.0 %

PRODUCT STANDARD

PRODUCT NAME : **FUCOXANTHIN-WSP0.1KW** (FOOD)

This product is a powder extracted with ethanol from kombu (Laminaria japonica) or wakame (Undaria pinnatifida) and then purified. It contains minimum of 0.1 % fucoxanthin.

Appearance	Orangey powder with light unique smell.	
Fucoxanthin	Min. 0.1 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
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)

Composition

Ingredient	Content
Cyclodextrin	96.75 %
Triglyceride	2.38 %
Seaweed Extract	0.60 %
Mixed Tocopherol	0.27 %
Total	100.00 %

PRODUCT STANDARD

PRODUCT NAME : **FUCOXANTHIN-5CKW** (COSMETIC)

This product is oily liquid extracted with ethanol from kombu (Laminaria japonica) or wakame (Undaria pinnatifida) and then purified. It contains minimum of 5.0 % fucoxanthin.

Appearance	Reddish brown liquid with light unique smell.	
Fucoxanthin	Min. 5.0 %	(HPLC)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(The Second method of The Japanese Standards of Quasi-Drug Ingredients)
(2) Arsenic (as As_2O_3)	Max. 2 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
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)

Composition

Ingredient	Content
Caprylic/Capric Triglyceride	79.0 %
Algae Extract	20.0%
Tocopherol	1.0 %
Total	100.0 %

PRODUCT STANDARD

PRODUCT NAME : **FUCOXANTHIN-1CKW** (COSMETIC)

This product is oily liquid extracted with ethanol from kombu (Laminaria japonica) or wakame (Undaria pinnatifida) and then purified. It contains minimum of 1.0 % fucoxanthin.

Appearance	Reddish brown liquid with light unique smell.	
Fucoxanthin	Min. 1.0 %	(HPLC)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(The Second method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As_2O_3)	Max. 2 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
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)

Composition

Ingredient	Content
Caprylic/Capric Triglyceride	95.0 %
Algae Extract	4.0%
Tocopherol	1.0 %
Total	100.0 %

PRODUCT STANDARD

PRODUCT NAME : **FUCOXANTHIN-PC1KW** (COSMETIC)

This product is a powder extracted with ethanol from kombu (Laminaria japonica) or wakame (Undaria pinnatifida) and then purified. It contains minimum of 1.0 % fucoxanthin.

Appearance	Orangey powder with light unique smell.	
Fucoxanthin	Min. 1.0 %	(HPLC)
Loss on Drying	Max. 10.0 %	(1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(The Second method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As_2O_3)	Max. 2 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
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)

Composition

Ingredient	Content
Cyclodextrin	76.00 %
Caprylic/Capric Triglyceride	18.96 %
Algae Extract	4.80 %
Tocopherol	0.24 %
Total	100.0 %

PRODUCT STANDARD

PRODUCT NAME : **FUCOXANTHIN-WSPC0.1KW**

(COSMETIC)

This product is a powder extracted with ethanol from kombu (Laminaria japonica) or wakame (Undaria pinnatifida) and then purified.
It contains minimum of 0.1 % fucoxanthin. This product is water-soluble

Appearance	Orangey powder with light unique smell.	
Fucoxanthin	Min. 0.1 %	(HPLC)
Loss on Drying	Max. 10.0 %	(1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(The Second method of The Japanese Standards of Quasi-Drug Ingredients)
(2) Arsenic (as As_2O_3)	Max. 2 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
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)

Composition

Ingredient	Content
Maltosyl Cyclodextrin Cyclodextrin Dimaltosyl Cyclodextrin	96.75 %
Caprylic/Capric Triglyceride	2.38 %
Algae Extract	0.60 %
Tocopherol	0.25 %
Total	100.0 %

ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

From product planning to OEM - For any additional information or assistance, please contact：

Headquarters:
ORYZA OIL & FAT CHEMICAL CO., LTD.

No.1, Numata Kitagata-cho, Ichinomiya-city, Aichi-pref.,
493-8001 JAPAN
TEL : +81 (0) 586 86 5141
FAX : +81 (0) 586 86 6191
URL/http : //www.oryza.co.jp/
E-mail : info@oryza.co.jp

Tokyo sales office:

5F Diamant-building 1-5 Kanda-suda-cho
Chiyoda-ku, Tokyo, 101-0041 Japan
TEL :+81-3-5209-9150 FAX :+81-3-5209-9151
E-mail: tokyo@oryza.co.jp

“The catalog was created based on academic data. For expressions of consumer products containing this product, follow the Health Promotion Law, Pharmaceutical Low, and other related laws and regulations.”

Revision:
*New evidence data were added (p13-15, 22-23).
*Specification format was changed (p30-37).

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ORYZA OIL & FAT CHEMICAL CO., LTD.