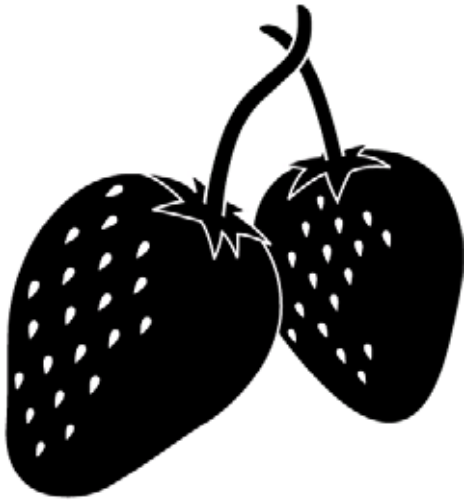
Oryza

ORYZA OIL & FAT CHEMICAL CO., LTD.

STRAWBERRY SEED EXTRACT

Food and cosmetic ingredients with moisture retention, whitening, antioxidation and antiobesity

- **STRAWBERRY SEED EXTRACT-P**
  (Water-soluble Powder, Food Grade)
- **STRAWBERRY SEED EXTRACT-PC**
  (Water-soluble Powder, Cosmetic Grade)
- **STRAWBERRY SEED EXTRACT-LC**
  (Liquid, Cosmetic Grade)
- **STRAWBERRY SEED OIL**
  (Oil, Food・Cosmetic Grade)



ORYZA OIL & FAT CHEMICAL CO., LTD.

Ver. 1.0 JT/HS

STRAWBERRY SEED EXTRACT

Health Ingredients with moisture-retention, whitening, antioxidation and antiobesitv effect

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) belongs to the roseceae family and is a widely consumed fruit (Fig. 1). As it's genus name –*Fragaria (*meaning flavor)- tells us, strawberry has a distinct flavor. Originating in middle Europe, strawberry has been brought to Japan by the Hollanders in the late Edo period. Now, strawberry is a popular fruit also in Japan with an annual harvest of around 200,000 tons. Strawberry is rich in vitamin C, polyphenols including anthocyanin, flavonoid, phenylpropanoid and ellagitannin. The health-promoting functions of strawberry have been broadly investigated. However, despite the fact that strawberry is eaten together with the small seeds inside, components and functions of the seeds have not well been studied.

Recently, we developed a panel of new products based on Strawberry Seed Extract, which are available as water-soluble powder, as solution or as oil. The Strawberry Seed Extract powders contain tiliroside and kaempferol-3-*O*-glucoside (Fig. 2). Our preliminary results revealed that both oral and local application of the Strawberry Seed Extract promote expression of aquaporins (AQP) and several other enzymes involved in ceramide- and hyaluronic acid-synthesis. AQP are membrane pore proteins known as "water channel". We further found that oral application of Strawberry Seed Extract to mice led to reduction of body weight, liver-fat, epididymis-fat and blood sugar. Oil extracted from strawberry seeds (Strawberry Seed Oil) contains more than 30% of α-linolenic acid, a essential fatty acid with anti-allergic effect. α-linolenic acid can not be synthesized in the body and thus needs external supply. Our Strawberry Seed Extract and Strawberry Seed Oil has other additional beauty and dietary effects by oral as well as local application, and thus can be used for various beauty-foods and cosmetics.

Fig1. Strawberry and seeds

2. Functional Components of Strawberry Seeds

Recently, we found that strawberry seeds contain functional components such as tiliroside (a flavonoid) and kaempferol-3-*O*-glucoside (Fig. 2). The polyphenol-content is much high in the seeds than in the flesh of strawberry while tiliroside and kaempferol-3-*O*-glucoside were almost exclusively found in the seeds (Fig. 3). Tiliroside is also contained in linden and in the seeds of rose hip, and has been reported to be liver-protective[1], anti-obesity[2] and anti-inflammatory[3]. Similar functionalities are expected for the other major component kaempferol-3-*O*-glucoside. All these data suggest that the extracts from strawberry seeds have more health-promoting effects that extracts from other parts of this fruit.

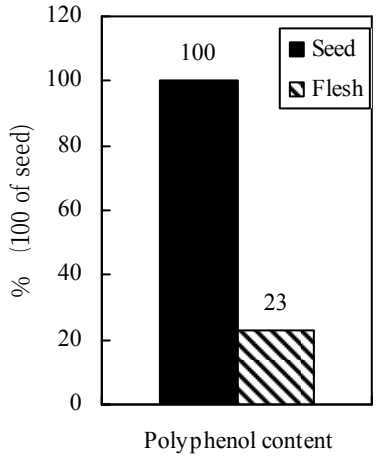
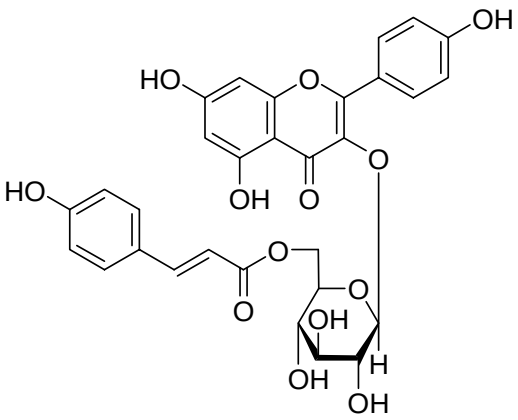
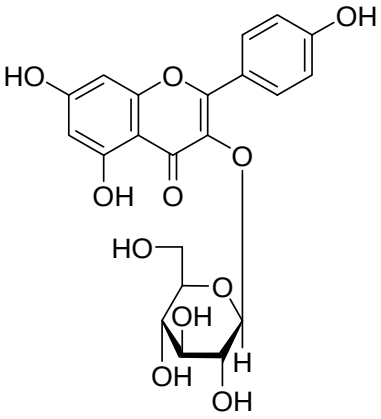


Fig 3. Polyphenol-content in strawberry seed and flesh



Tiliroside



Kaempferol-3-*O*-glucoside

Fig 2. Components of Strawberry Seed Extract

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3．Physiological Function of Strawberry Seed Extract

(1) Anti-obesity effect

To examine the anti-obesity effect of our Strawberry Seed Extract, mice were given free access to a high-fat diet and Strawberry Seed Extract was administered orally for 8 days at various doses. At the end of the test period, mice were starved for 18 hours before blood and organs were sampled for analysis. Significant suppression of body-weight-increase was observed at the dose 10 mg/kg (Fig. 4). Epididymis-fat-weight of mice given 10 mg/kg of the extract was decreased compared to those of mice in the control group. Liver-weight was also decreased in a dose-dependent manner. Furthermore, blood glucose was lowered (table 1).

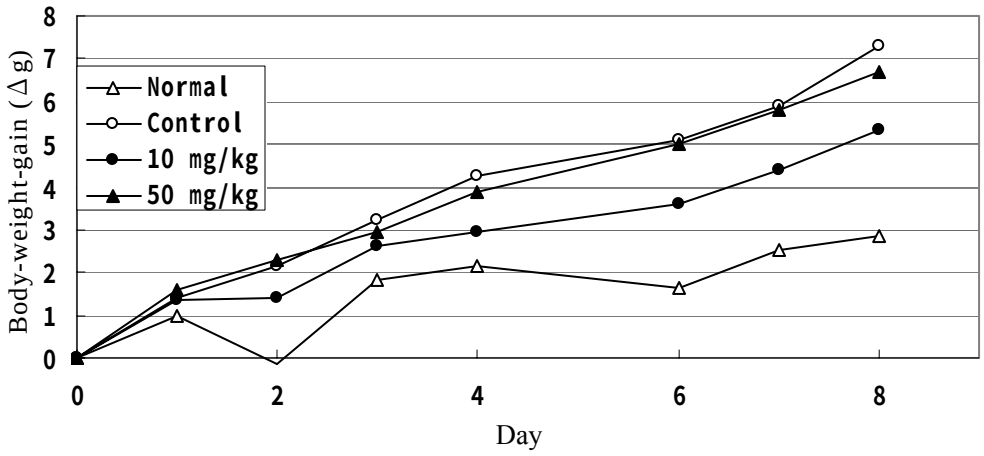
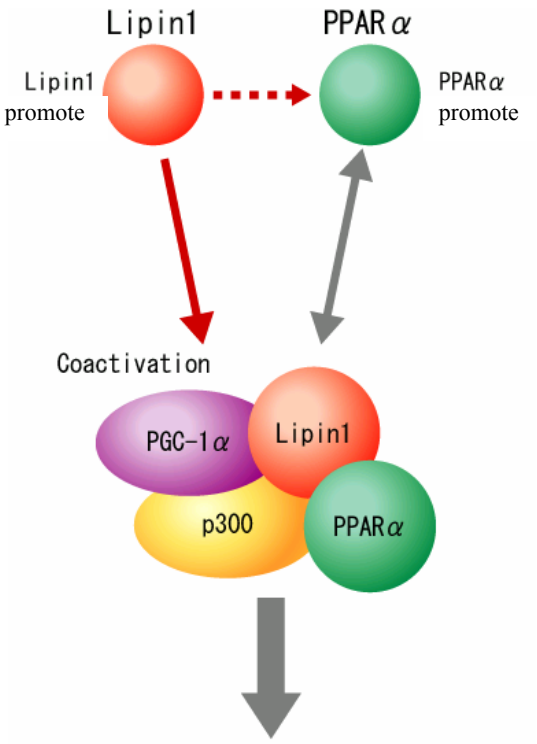


Fig 4. The effect of Strawberry Seed Extract on body-weight-gain in mice.

Table 1. The effect of Strawberry Seed Extract on epididymis fat weight, liver weight and blood glucose in mice. (N=5-6, Mean±SE).

	Dose	Epididymis fat		Liver		Glucose
	(mg/kg)	(g)	(mg/g weight)	(g)	(mg/g weight)	(mg/dL)
Normal	-	0.82±0.08	21.9±1.7	1.42±0.06	38.2±1.1	138.7±15.9
Control	-	1.69±0.15	39.5±2.8	1.68±0.07	38.4±1.0	219.4±9.8
Strawberry Seed Extract	10	**1.38±0.12**	**34.0±2.7**	**1.55±0.10**	38.0±1.9	**160.5±21.7**
	50	1.69±0.23	38.1±4.2	**1.48±0.08**	**33.7±1.2**	**178.4±15.8**

Expression of Lipin 1 was significantly increased upon administering of Strawberry Seed Extract (Fig. 5). Identified in 2005, Lipin 1 is known to promote lipid-metabolism in liver mitochondria via PPAR α [4]. Because of its key-role in the lipid-metabolism, diet-compounds enhancing the activity of PPAR α haven been developed. Our results demonstrated that Strawberry Seed Extract directly enhances expression of the terminal factor in the lipid-metabolism, and thus possesses a novel anti-obesity functionality.



Promote lipid-metabolism in liver mitochondria

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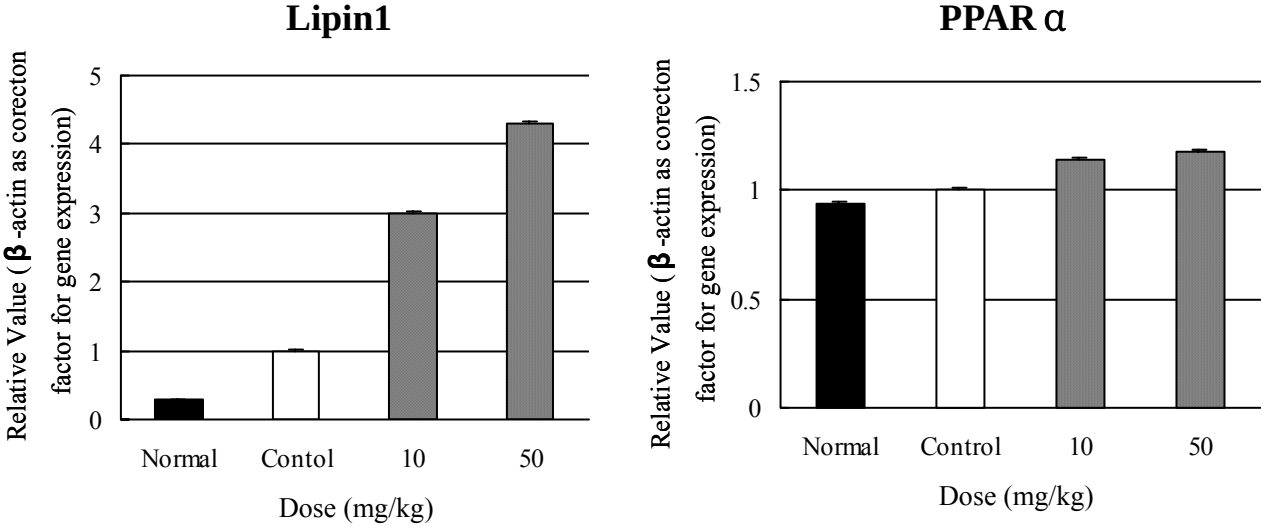

Fig 5. Effect of Strawberry Seed Extract on expression of genes involved in lipid-metabolism in mice. (N=5-6, Mean±SE).

Next, we evaluated the effect of Strawberry Seed Extract in expression of genes involved in diabetes-related pathways. We found that the extract promotes expression of Adipo (adiponetcin), PRAR γ , Insr (insulin receptor) and GLUT4 (Fig. 6). Adipo is an adipokine, which increases insulin sensitivity and stimulates fatty acid oxidation. The peroxisome proliferation-activated receptor PRAR γ regulates transcription of several genes involved in glucose and lipid metabolism and energy balance. On the other hand, oral application of 50 mg/kg of the extract did not have effect on epididymis-fat-weight in mice. These finding suggest that the functional mechanism of Strawberry Seed Extract in reducing fat-weight and blood glucose is mainly via activation of PPAR γ in adipocytes which leads to increased insulin-sensitivity and enhanced glucose-uptake. The elevated expression of insulin receptor and GLUT4 (a glucose transporter) upon oral application of 50 mg/kg Strawberry Seed Extract also promotes glucose uptake from serum to adipocytes.

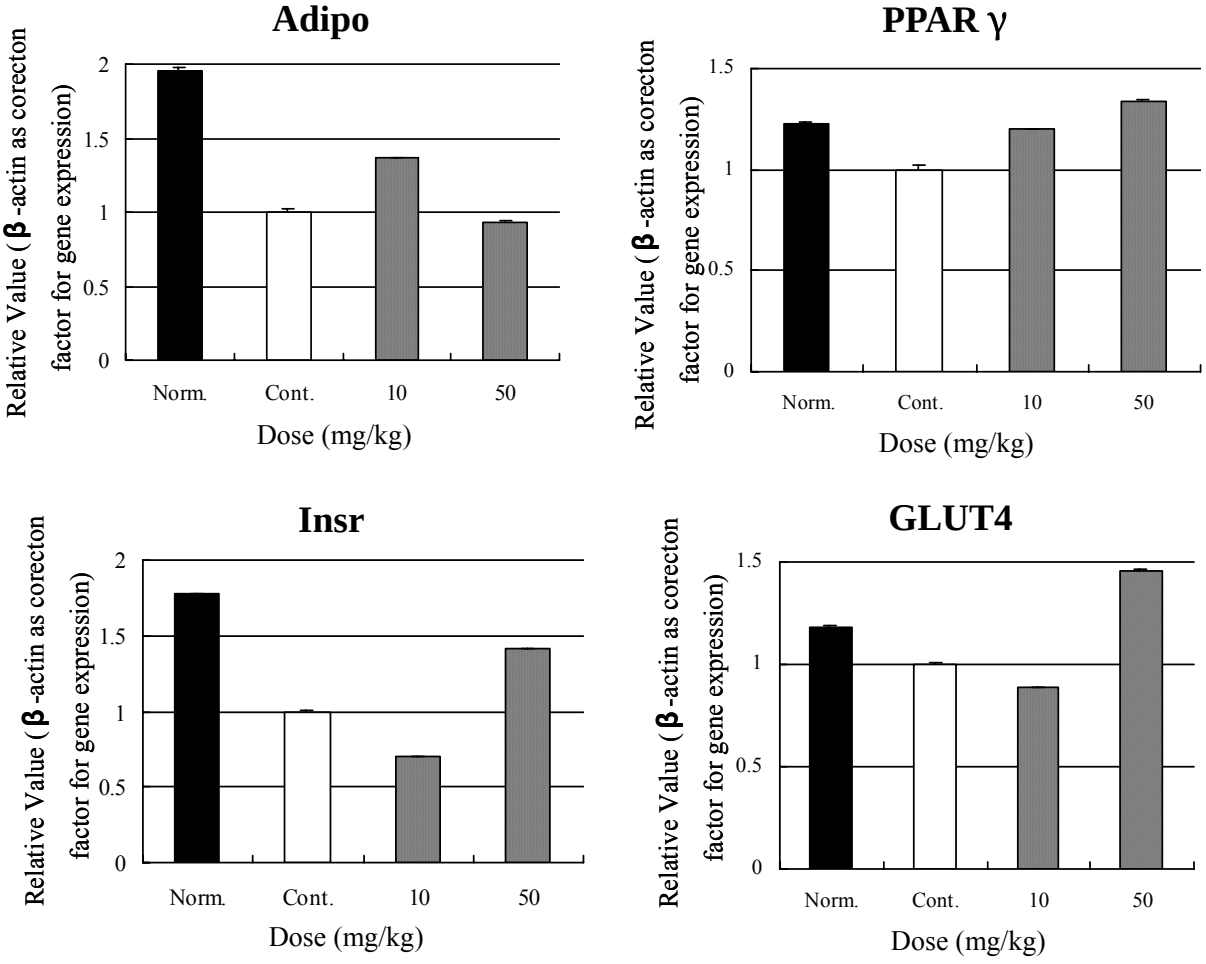

Fig 6. The effect of Strawberry Seed Extract on expression of diabetes-related genes in mice. (N=5-6, Mean±SE).

＜Function of lipid metabolism and diabetes related genes＞

Gene	Function
Lipin1	Regulating lipid metabolism
PPAR α	Regulating lipid metabolism, fatty acid oxidation, and glucose homeostasis
Adipo	Promoting fatty acid oxidation and glucose-uptake
PPAR γ	Enhancing insulin sensitivity
Insr	Inducing glucose-uptake
GLUT4	Glucose transporter

【Method of experiment】

Ten-week old male ddY mice were given free access to the High Fat Diet 32 for 8 days and Strawberry Seed Extract was administered orally at daily doses of 10 and 50 mg/kg for this period. At the end of the test period, the mice were starved for 18 hours before blood and organs were sampled for analysis. RNA was extracted from each organ and used for cDNA synthesis. Expression of genes was examined by means of RT-PCR.

(2) Beauty effect

1) Skin barrier function

PPARs are regulators in lipid and glucose metabolism. Recently, their roles in a number of skin diseases have been increasingly reported. PPAR α is involved in maturation of epidermis and in activation of sebaceous cells; PPAR β / δ promote differentiation of sebaceous cells and predifferentiation of keratinocytes; and finally, PPAR γ plays a role in differentiation of sebaceous gland cells[5]. Staumont-Sallé *et al*[6] reported improvement of atopic dermatitis in a mouse-model upon local embrocation of PPAR α -angonist. Using the same model, Dahten *et al*. [7, 8] found effect of local application of PPAR γ ligand. In their review, Sertznig *et al*[9] raised the issue of possible effect of thiazolidinedione derivative (a PPAR γ agonist) for psora. Man *et al*[10] reported effects of epidermic PPAR β / δ in promoting proliferation of keratinocytes, in improving skin inflammation and in regulating skin-barrier function.

All these findings suggest activities of PPARs in skin functions and in lipid metabolism. We thus studied the effect of Strawberry Seed Extract in regulating expression of the PPAR genes as well as expression of several other genes contributing to skin-moisture-retaining and skin-whitening. RNA was prepared from dorsal skin of mice fed with high-fat-diet. Subsequent RT-PCR revealed that our Strawberry Seed Extract enhances expression of PPAR α and PPAR γ in a dose-dependent manner (Fig. 7). Our results suggest that oral application of our Strawberry Seed Extract improves skin barrier function.

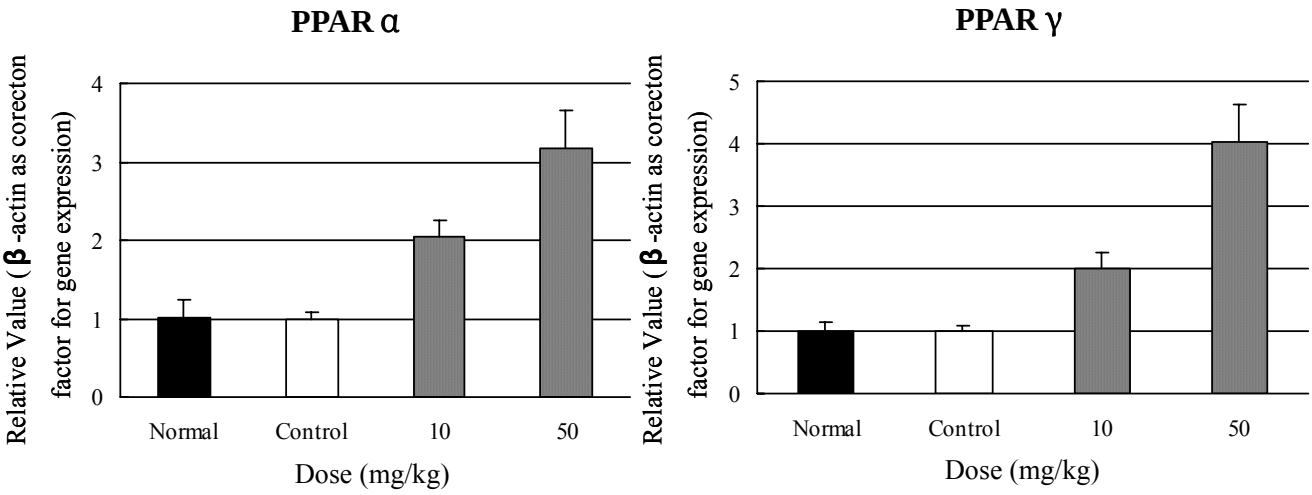

Fig 7. The effect of Strawberry Seed Extract on PPAR genes expression in mice skin. (N=5-6, Mean±SE).

＜Function of PPAR genes on skin＞

Gene	Function
PPAR α	Maturation of epidermis and activation of sebaceous cells
PPAR γ	Differentiation of sebaceous gland cells

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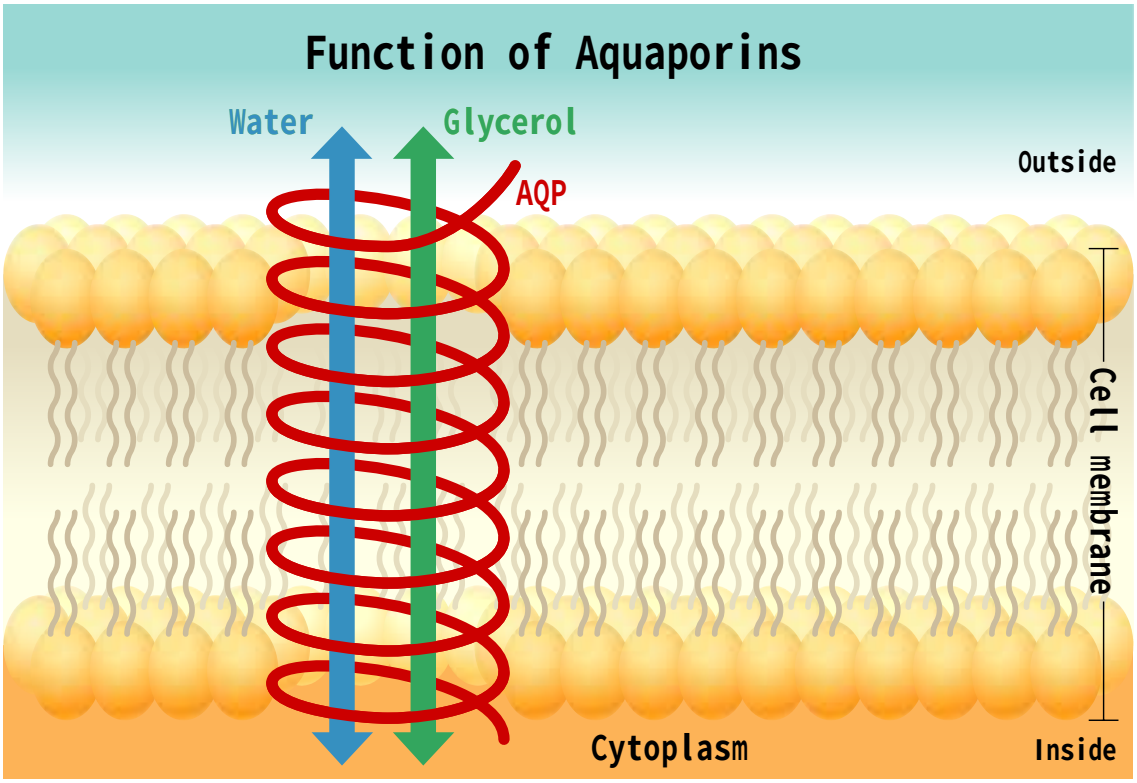
2) Moisture-retention effect

① Oral application

Similarly, expression of Sptlc1 and HAS2, 3 were enhanced by oral application of the Strawberry Seed Extract in a dose-dependent manner (Fig. 8). Sptlc1 codes for the serine palmitoyltransferase long chain base subunit 1 which is the rate-limiting enzyme in ceramide-synthesis, while HAS2, 3 are the genes for hyaluronic acid synthase 2 and 3, respectively. HAS2 is mainly expressed in dermal fibroblasts while HAS3 in epidermal cells. Strawberry Seed Extract thus enhances synthesis of hyaluronic acid in both of the two skin layers. Ceramides and hyaluronic acid as among the components essential for skin-moisture-retaining and skin-barrier function. Finally, we examined effect of Strawberry Seed Extract on expression of aquaporins (AQP) in skin. Located in the tight-junctions of cell membrane, aquaporins conduct water and glycerol in and out[11]. Especially AQP3 and AQP5 are predominantly expressed in skin. Expression of AQP3 was enhanced by 50 mg/kg oral application of Strawberry Seed Extract, while the expression of AQP5 was enhanced in a dose-dependent manner, starting from 10 mg/kg (Fig. 9). Our results suggest that oral application of our Strawberry Seed Extract helps skin moisture-retention.



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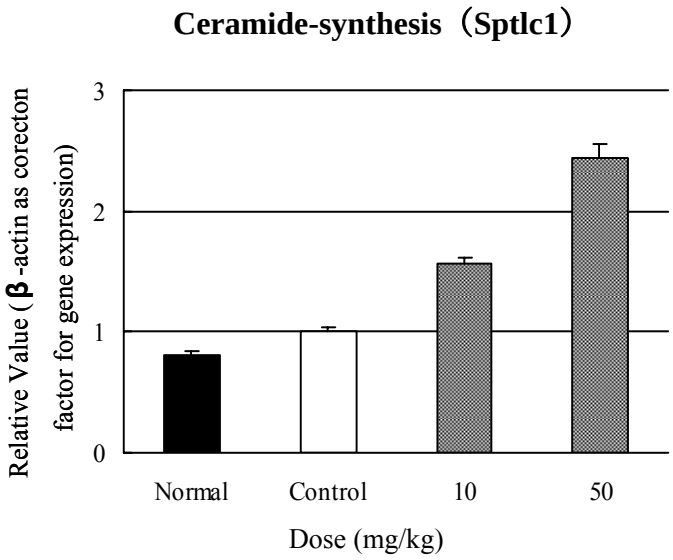

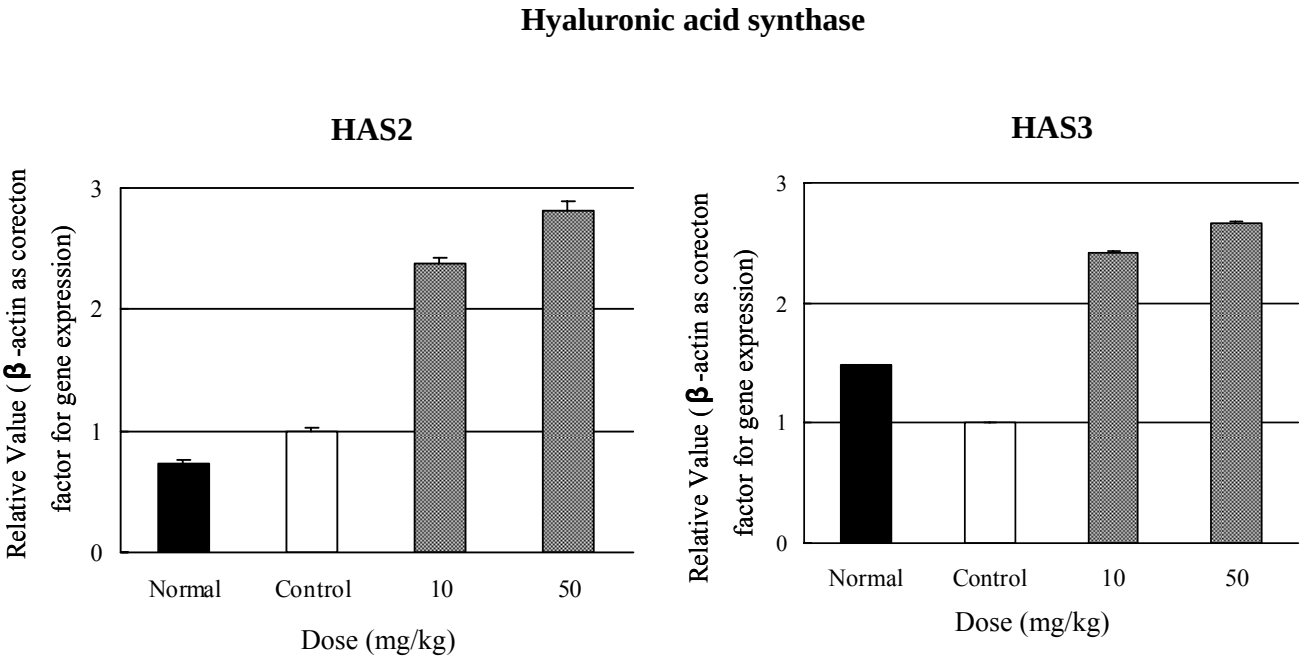

Fig 8. The effect of Strawberry Seed Extract on expression of ceramide synthase gene and hyaluronic acid synthase gene in mice skin. (N=5-6, Mean±SE).

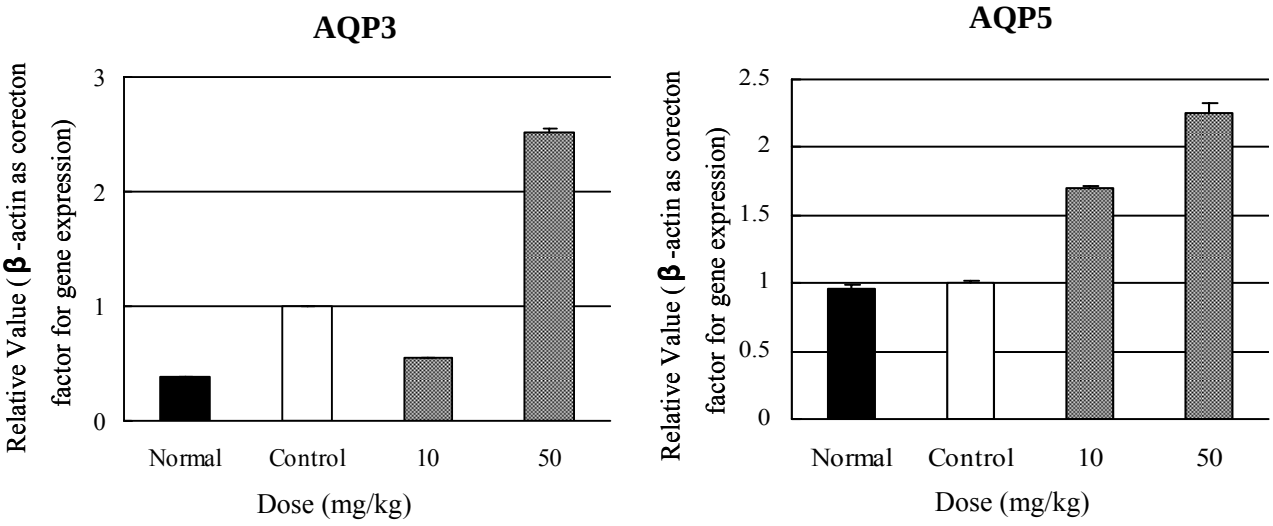

Fig 9. The effect of Strawberry Seed Extract on AQP gene expression in mice skin. (N=5-6, Mean±SE).

＜Function of moisture-retention related genes＞

Gene	Function
Sptlc1	Rate-limiting enzyme in ceramide synthesis
HAS2	Hyaluronic acid synthesis in dermis
HAS3	Hyaluronic acid synthesis in epidermis
AQP3	Water channel in skin
AQP5	Water channel in skin and buccal cells

② Local application

In another set of experiment, we examined the effect of local embrocated Strawberry Seed Extract on expression of genes involved in moisture-retention. Dorsal skin of hairless mice was embrocated with 0.5 and 2% Strawberry Seed Extract for 2 weeks. Water-content and expression of several relevant genes were examined in the treated area. Water-content in skin embrocated with 2% Strawberry Seed Extract was significantly increased when compared with that in the corresponding area of untreated mice (table 2). Expressions of Sptlc1, AQP3, AQP5, HAS2 and HAS3 were all found to be enhanced in skin areas treated with 2% Strawberry Seed Extract (table 3). These results demonstrate that both oral and local application of Strawberry Seed Extract improve skin-moisture-retention.

Table 2. The effect of Strawberry Seed Extract on moisture-retention in mice skin.

	% of initial value（100 of control ）	
	1 week (%)	2 weeks (%)
Control	100 ± 2	100 ± 3
Strawberry Seed Extract 0.5%	104 ± 2	104 ± 4
Strawberry Seed Extract 2.0%	118 ± 3**	117 ± 3**

N=6, Mean±SE, **: $p<0.01$

Table 3. The effect of Strawberry Seed Extract on moisture-retention related gene expression in mice skin.

	Sptlc1	AQP3	AQP5	HAS2	HAS3
Control	1.00 ± 0.04	1.00 ± 0.16	1.00 ± 0.10	1.00 ± 0.19	1.00 ± 0.23
Strawberry Seed Extract 0.5%	1.97 ± 0.30	0.95 ± 0.12	1.26 ± 0.12	1.06 ± 0.22	0.96 ± 0.19
Strawberry Seed Extract 2.0%	2.41 ± 0.31	1.78 ± 0.27	2.86 ± 0.16	2.28 ± 0.63	2.15 ± 0.59

β-actin as correction factor for gene expression (N=6, Mean±SE)

【Method of experiment】

Dorsal skin of 5-week old hairless female mice (Hos:HR-1) was embrocated with 0.5 and 2% Strawberry Seed Extract for 2 weeks. One and two weeks later, water-content of the embrocated skin areas was measured using a CORNEOMETER SM 825. After 2 weeks, embrocated skin was excised for preparing mRNA which was used for synthesize of cDNA. Expression of genes was evaluated by means of RT-PCR using these cDNA.

③ Tiliroside and kaempferol 3-*O*-glucoside

In order to elucidate the mechanism of the moisture-retaining effect of our Strawberry Seed Extract, we examined its components tiliroside and kaempferol 3-*O*-glucoside. In human newborn perisarc epidermal keratinocytes (NHEK), expression of the ceramide synthesis enzyme Sptlc1 was stimulated by 1-3 μg/mL tiliroside and 3 μg/mL kaempferol 3-*O*-glucoside (Fig. 10). In addition, kaempferol 3-*O*-glucoside promoted expression of HAS3 in a dose-dependent manner (Fig. 11). These findings suggest that tiliroside and kaempferol 3-*O*-glucoside are likely the principle components for the moisture-retaining effect of the Strawberry Seed Extract. Expression of HAS2, which is mainly in dermal fibroblasts, was not examined in this experiment as the cells used are of epidermal origin. Further studies addressing this issue as well as expression of AQP3, 5 using dermal fibroblasts are in progress.

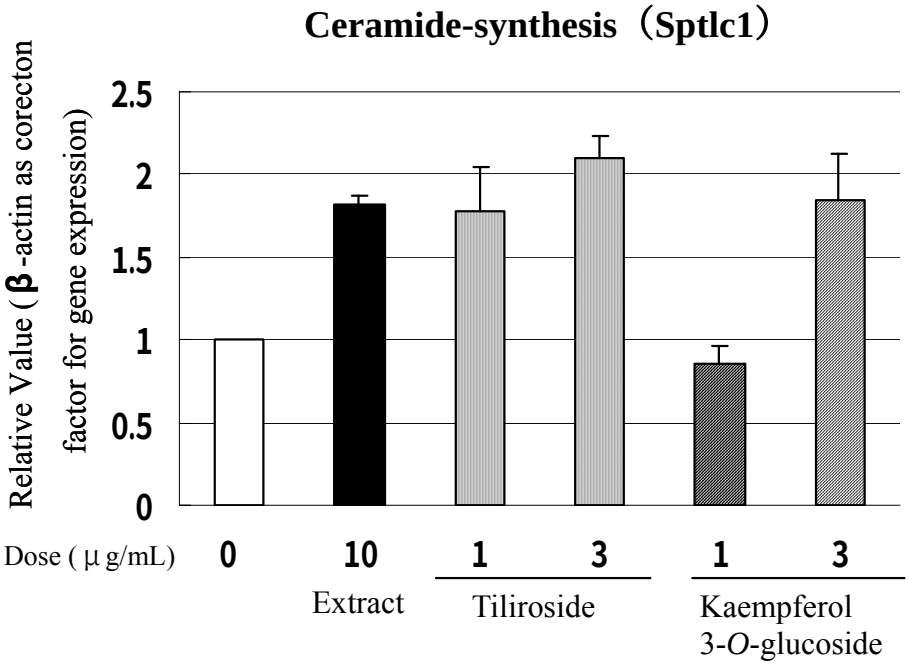

Fig 10. The effect of Strawberry Seed Extract, tiliroside and kaempferol 3-*O*-glucoside on ceramide-synthesis gene expression in NHEK. (N=4, Mean±SE).

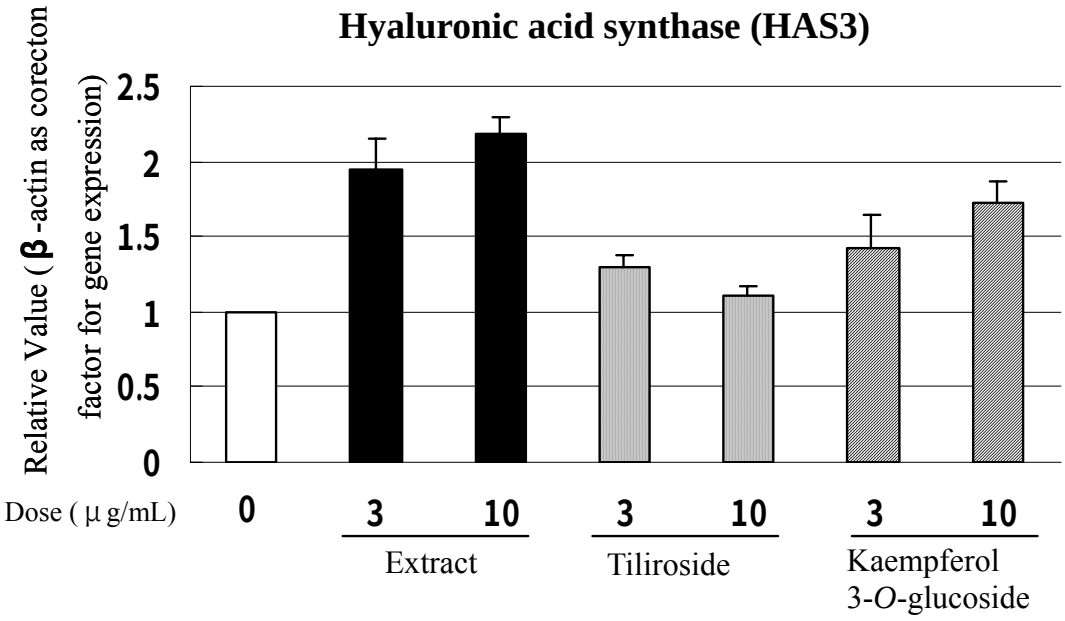

Fig 11. The effect of Strawberry Seed Extract, tiliroside and kaempferol 3-*O*-glucoside on hyaluronic acid synthase gene expression in NHEK. (N=4, Mean±SE)

【Method of experiment】

Perisarc epidermal keratinocytes of newborn (NHEK) were suspended in serum-free culturing medium at a density of 2×10^4 cells /mL. 500 μL of the suspension was placed into each well of a 24-well plate and cultured for 3 days before test-samples were added. After 24-treatment with the test-samples, cells were harvested for preparation of mRNA. Subsequent cDNA synthesis and RT-PCR for evaluation of expression of genes were carried out following standard protocols.

3) Skin-whitening effect

① Oral application

Melanin is the cause for somberness, pigments and mottle of the skin. In the body, tyrosin is converted to dopaquinone, which is then oxidized to dopachrom and finally to melanin. Tyrosinase (Tyr) and Tyrosinase related protein (TRP1) are the key enzymes in this cascade. Suppression of these enzymes will thus lead to reduced melanin production with a skin-whitening effect. To examine such effect in the Strawberry Seed Extract, we irradiated dorsal skin of hairless mice with UV for 8 days and orally administered the extract to mice in the test group. At the end of test period, RNA was prepared from the irradiated skin and used for RT-PCR. Oral application of 50 mg/kg Strawberry Seed Extract suppressed expression of Tyr, though not that of TRP1 (table 4). Expression of Neurotrophin (NT-3) in kerytinocytes was also suppressed. NT-3 is known to bind receptors on the surface of melanocytes and initiate melanin-synthesis[12]. These results demonstrate a skin-whitening effect of our Strawberry Seed Extract by oral application.

Table 4. The effect of Strawberry Seed Extract on melanin production gene expression in mice.

	Tyr	TRP1	NT-3
Normal	2.37±0.35	3.42±0.44	0.31±0.23
Control	1.00±0.13	1.00±0.11	1.00±0.87
Strawberry Seed Extract 10 mg/kg	2.29±0.21	3.06±0.44	1.12±0.06
Strawberry Seed Extract 50 mg/kg	**0.56±0.16**	0.96±0.30	**0.28±0.25**

β-actin as correction factor for gene expression (N=4, Mean±SE)

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【Method of experiment】

Strawberry Seed Extract was administered to six-week old female hairless mice (Hos:HR-1) orally for 8 days. During this period, dorsal skin was irradiated with a solar simulator (Usio, Inc) using UV-B at 100 mJ/cm^2. At the end of the test period, irradiated skin was excised and used for extracting RNA. Subsequent cDNA-synthesis and RT-PCR for evaluation of gene-expression was carried out following standard protocols.

② Local application

We also examined effect of Strawberry Seed Extract by local embrocation. Dorsal skin of hairless mice were embrocated with 0.5 to 2% Strawberry Seed Extract and irradiated with UV for 8 days. Expressions of Tyr, TRP1 and NT-3 were suppressed by the extract in a dose-dependent manner (table 5). This result shows that also external local application of the Strawberry Seed Extract is effective in suppressing expression of enzymes for melanin synthesis.

Table 5. The effect of Strawberry Seed Extract on melanin production gene expression in mice.

	Tyr	TRP1	NT-3
Normal	0.84±0.13	0.78±0.12	0.35±0.26
Control	1.00±0.04	1.00±0.02	1.00±0.61
Strawberry Seed Extract 0.5%	**0.48±0.08**	**0.55±0.11**	**0.24±0.21**
Strawberry Seed Extract 2.0%	**0.40±0.03**	**0.54±0.03**	**0.11±0.07**

β -actin as correction factor for gene expression (N=4, Mean±SE)

【Method of experiment】

Dorsal skin of 6-week old hairless female mice (Hos:HR-1) was embrocated with 0.5 and 2% Strawberry Seed Extract for 8 days. During this period, dorsal skin was irradiated with a solar simulator (Usio, Inc) using UV-B at 100 mJ/cm^2. At the end of the test period, irradiated skin was excised and used for extracting RNA. Subsequent cDNA-synthesis and RT-PCR for evaluation of gene-expression was carried out following standard protocols.

＜Function of whitening related genes＞

Gene	Function
Tyr	Rate-limiting enzyme in melanin synthesis
TRP1	Melanin synthase
NT-3	Initiating in melanin synthesis

4) Antioxidant activities

① *In vitro*

Free radicals are generated in our body in response to various endogenous metabolic reactions (e.g. stress & medications). Free radicals such as reactive oxygen species (ROS) activates series of cells oxidation process leading to cells death and various degenerative diseases. Meanwhile, ageing process is accelerated by increased endogenous free radicals. The antioxidative effect of Strawberry Seed Extract is evaluated using superoxide dismutase (SOD) model and 1,1-diphenyl 2-picryl-hyrazil (DPPH) radical scavenging model. As illustrated in Fig. 12, Strawberry Seed Extract with high content of plant polyphenols demonstrated a dose-dependent antioxidative effect in SOD & DPPH radical scavenging models.

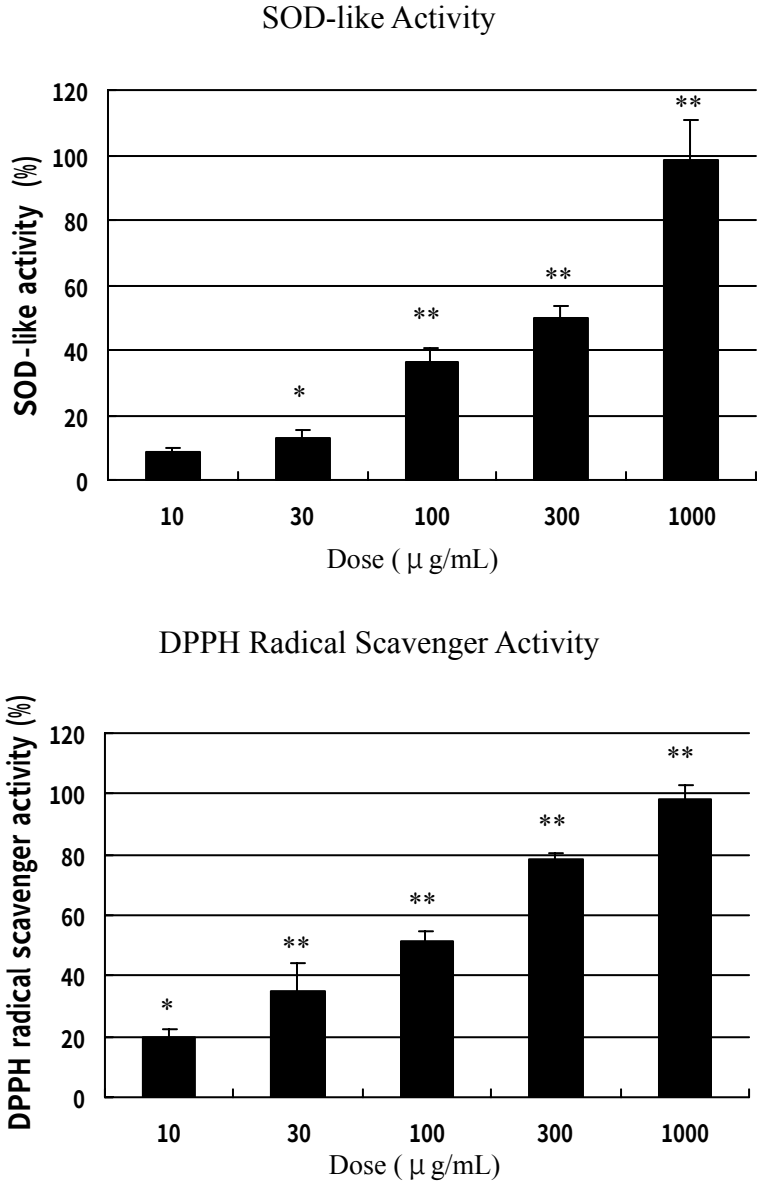

Fig. 12. Antioxidative activity of Strawberry Seed Extract（N=5, Mean±SD, *: p<0.05, **: p<0.01）

② Oral application

To examine the anti-aging and anti-oxidative effects of Strawberry Seed Extract *in vivo*, we irradiated dorsal skin of hairless mice for 8 days and administered the Strawberry Seed Extract during this period. At the end of the test period, irradiated skin was excised and used for RNA extraction. RT-PCR was carried out to examine expression of heat-shock protein (HSP) related genes. Heat-shock proteins play a key role in repairing of skin cells damaged by heat and UV. We found that expression of HSP72 was enhanced by 10-50 mg/kg Strawberry Seed Extract in a dose-dependent manner (Fig. 13). Expression of HSP72 is known to be triggered by heat-shock in a number of cells and organisms. HSP 72 is a chaperone protein which binds to newly synthesized proteins and assists their folding and assembly during their maturation. Enhanced expression of HSP72 is thus expected to help preventing skin-aging. This suggests another beauty-effect of the Strawberry Seed Extract.

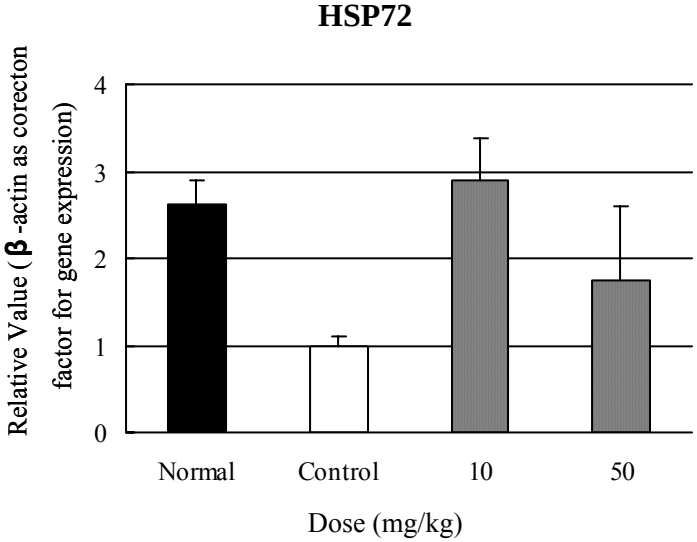

Fig 13. The effect of Strawberry Seed Extract on HSP gene expression in mice. (N=4, Mean±SE).

③ Local application

Next, we examined the effect of local embrocation of the Strawberry Seed Extract. Dorsal skin of hairless mice were embrocated with the extract and irradiated with UV for 8 days. Expression of HSP72 was increased in skin embrocated with 2.0% Strawberry Seed Extract (Fig. 14). These findings suggest that both oral and local application of Strawberry Seed Extract enhance expression of HSP-related genes and thus have anti-aging and anti-oxidation effects for the skin.

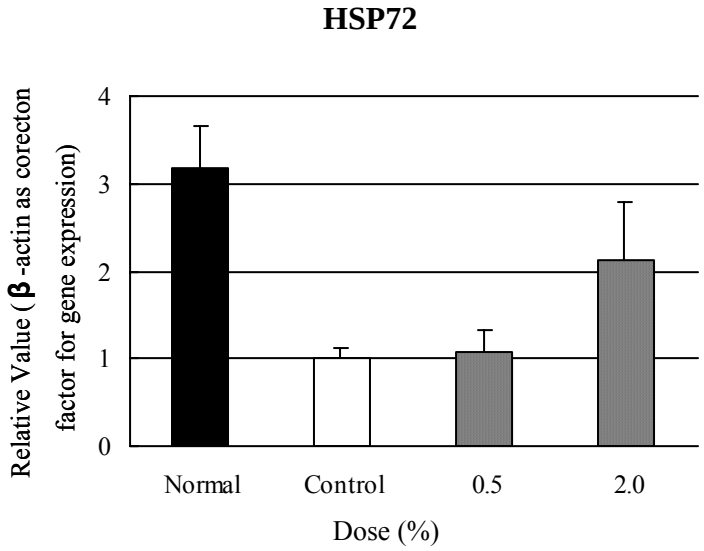

Fig 14. The effect of Strawberry Seed Extract on HSP gene expression in mice. (N=4, Mean±SE).

【Method of experiment】

Strawberry Seed Extract was administered to 6-week old female hairless mice (Hos:HR-1) orally for 8 days. Or, dorsal skin of 6-week old hairless female mice was embrocated with 0.5 and 2% Strawberry Seed Extract for 8 days. During this period, dorsal skin was irradiated with a solar simulator (Usio, Inc) using UV-B at 100 mJ/cm^2. At the end of the test period, irradiated skin was excised and used for extracting RNA. Subsequent cDNA-synthesis and RT-PCR for evaluation of gene-expression was carried out following standard protocols.

＜Function of HSP gene＞

Gene	Function
HSP72	Repairing damaged skin cells

(3) Human trial

Eight healthy females aged between 27 and 42 years have intaken Strawberry Seed Extract-P for 2 weeks at a daily dose of 40 mg. Skin-parameters were examined and compared before and after the intake-period. Increased water-content was found in the skin under the left eyes for 6, and in the skin of the inside left arm for 5 out of the 8 test subjects (Fig. 15). In average, water-content was increased in both areas. Elasticity, sebum, pH and brightness of skin were measured in the area under the left eye of each test subject. Elasticity was increased in 5 (Fig. 16), sebum decreased in 6 (Fig. 17), and brightness (L* value) increased in 6 test subjects (Fig. 19). pH moved toward the healthy range of 5.0 - 6.0 (Fig. 18). When averaged for all 8 test subjects, improvement was found for all these skin parameters. In addition, a survey after the test period revealed a slight improvement in dry and rough skin including face area (Fig. 20). These results demonstrate the beauty effect of Strawberry Seed Extract such as moisture-retention, skin-softening and skin-whitening.

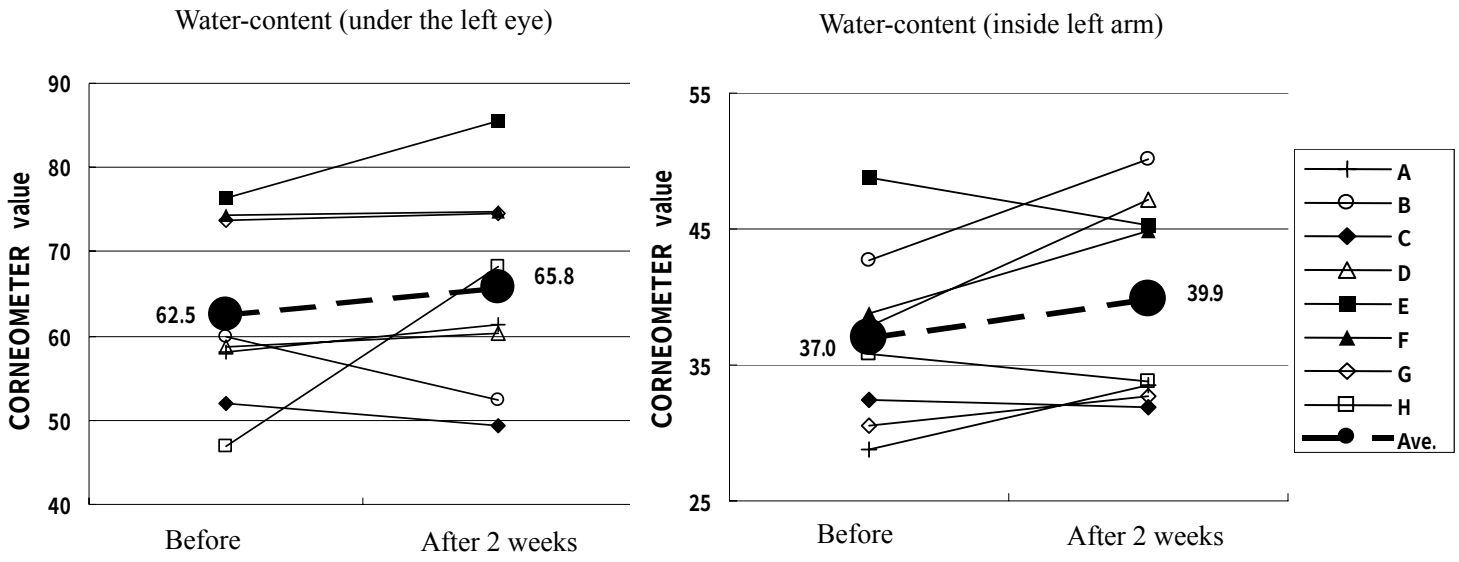

Fig 15. The effect of Strawberry Seed Extract-P on water-content in human (left：under the left eye, right：inside left arm)

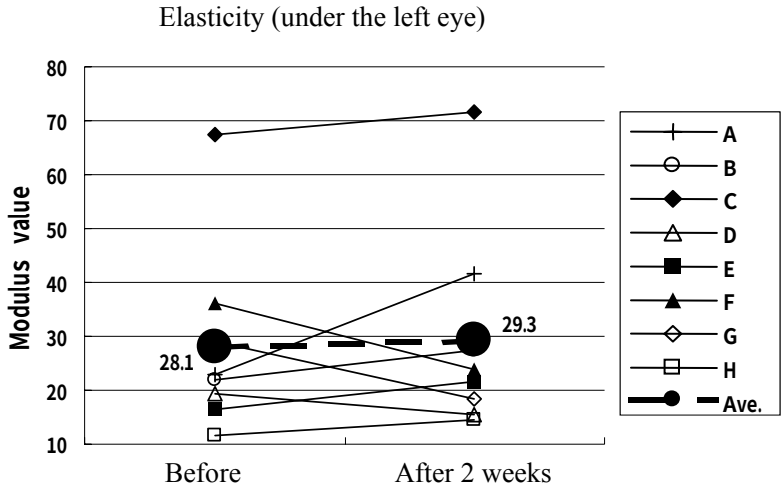

Fig 16. The effect of Strawberry Seed Extract-P on elasticity in human

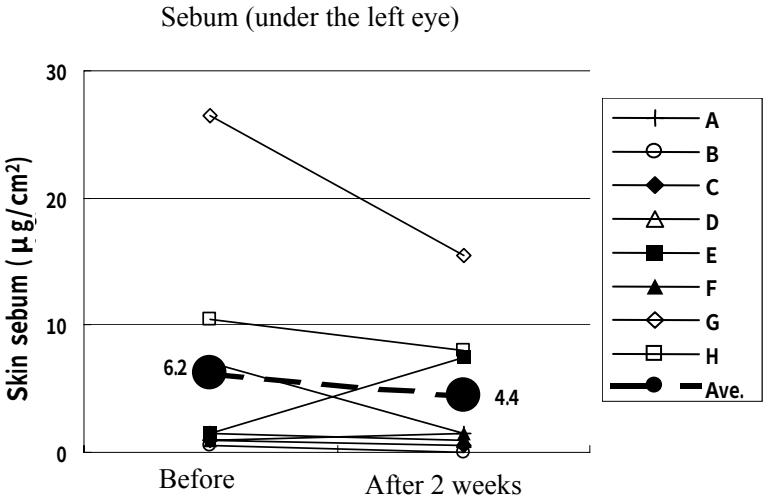

Fig 17. The effect of Strawberry Seed Extract-P on sebum in human

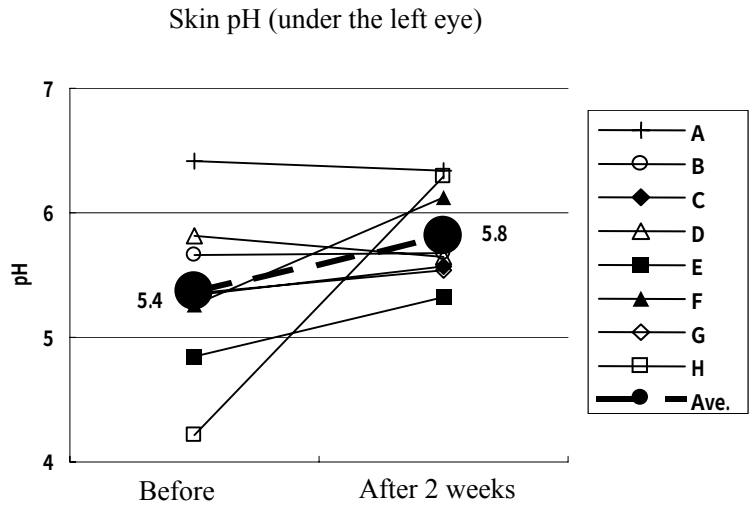

Fig 18. The effect of Strawberry Seed Extract-P on pH of skin in human

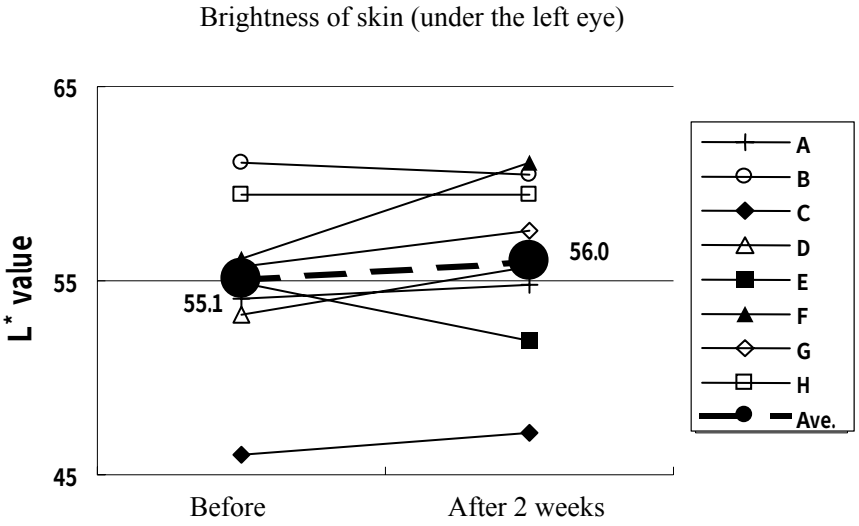

Fig 19. The effect of Strawberry Seed Extract-P on brightness of skin in human

Cosmetic rash	3.0
Dryness of the face	3.4
Flushing of the face	3.0
Fitness in makeup	3.1
Smoothness of the skin	3.1
Feeling of wetness	3.5
Skin elasticity	3.0
Degree of dryness	3.4
Itching	3.0
Skin roughness	3.4
Improvement in crease	3.0
Dullness of the skin	3.0

＊Each score is average value of 8

＜Score of 5-point scale＞
1 In obvious worsened
2 Slightly worsened
3 No change
4 Slightly improved
5 In obvious improved

Fig. 20. Subjective response on the effect of ingestion of Strawberry Seed Extract-P

【Method of experiment】

Eight healthy female aged between 27 and 42 years were given 40 mg Strawberry Seed Extract-P daily for 2 weeks. Water-content in the skin under the left eye and of the inside left arm was measured using a CORNEOMETER SM825 before and after the intaking period. Elasticity, sebum level, pH and brightness of the skin under the left eye of each test subject were also measured before and after the intaking period using a Modulus, a SEBUMETER SM810, a SKIN-pH-METER PH900, and a Spectro Color Meter SE 2000, respectively.

4. Stability of Strawberry Seed Extract

(1) Thermostability

As illustrated in Fig. 21, polyphenols and tiliroside content of Strawberry Seed Extract-P is highly stable at 110°C for 1 hour. It is stable at temperatures for processing food.

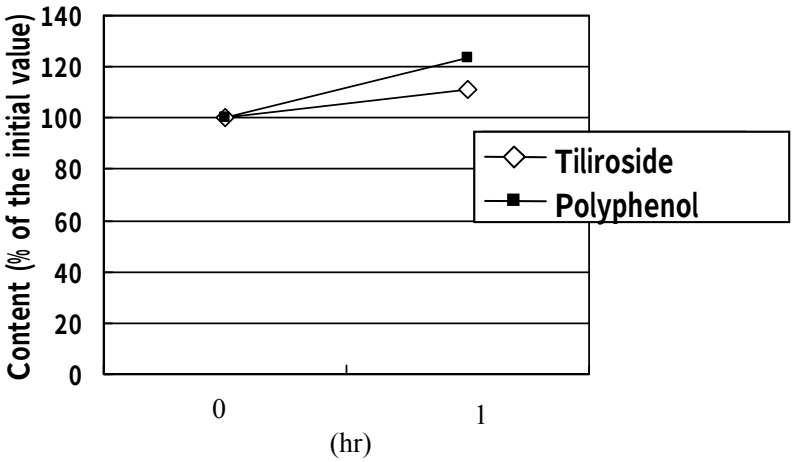

Fig. 21. Thermostability of Strawberry Seed Extract

(2) pH stability

Strawberry Seed Extract-P was dissolved in distilled water, adjusted to its pH and stored at room temperature for 1 week. Polyphenols content of Strawberry Seed Extract was measured and results showed (Fig. 22) that polyphenols content remained stable at acidic condition but reduced by about 10% in alkaline condition after 1 week.

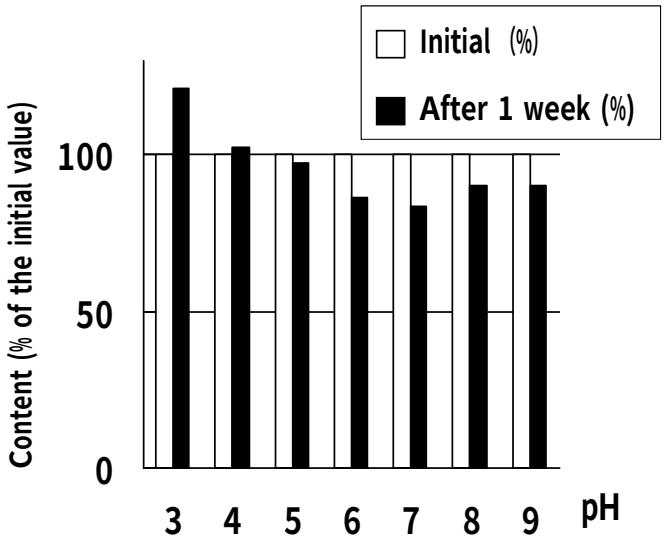

Fig. 22. pH stability of Strawberry Seed Extract（100% as initial value）

(3) Stability in Aqueous Solution

A 0.04% solution (pH 3.5) of Strawberry Seed Extract-P (powder, water soluble) was prepared and stored at room temperature (with and without light), 40°C (without light) & 5°C (without light) for 2 weeks. Visual observation on precipitation, turbidity and color change was conducted. As tabulated below, Strawberry Seed Extract-P is highly stable in aqueous condition.

	Liquid stability (0.04% solution、pH 3.5)			
	Room temperature (light shielding)	25°C (without light shielding)	40°C (without light shielding)	5°C (without light shielding)
Precipitation, turbidity	Negative	Negative	Negative	Negative
Color changes	Negative	Negative	Negative	Negative

5. Nutrition Information (Strawberry Seed Extract-P)

Description	Amount	Note	Analytical Method
Moisture	2.5g/100g		Heat-drying at atmospheric pressure
Protein	4.7g/100g	1	Combustion Method
Fat	3.9g/100g		Acid degradation
Ash	0.8g/100g		Direct Incineration
Carbohydrate	88.1g/100g	2	
Energy	406kcal/100g	3	Atwater Method (Revised)
Dietary fiber	0.0g/100g		Prosky Method
Sodium	36mg/100g		Atomic absorption spectrophotometory
Sodium	0.1g/100g		Sodium Equiv. value

1. Nitrogen, protein conversion factor: 6.25
2. Carbohydrate expression standard (Ministry of Health and Welfare's announcement No. 176)
   Calculation: 50 – (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

Test trustee: SRL, Inc
Date of analysis: April 24, 2008
Test No.: 200804110033

6. Strawberry Seed Extract (in non-excipient form) – Product Safety Profile

(1) Residual Agricultural Chemicals

Strawberry Seed Extract (without binder) is conformed to regulation stipulated for 497 residual agricultural chemical compounds. No residual agricultural chemicals detected as confirm by test trustee.

Test trustee：Masis Co. Ltd.
Data：April 18, 2008
Report No.：20513

(2) Acute Toxicity (LD_{50})

Acute Toxicity test was conducted accordingly to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products. Strawberry Seed Extract was orally administered to male and female ddY mice (aged 5 weeks old) at 2,000 mg/kg and kept for 14 days. No abnormalities and fatal event observed at 2,000 mg/kg. Upon autopsy performance, no abnormalities observed under macroscopic examinations. Thus, LD_{50} of Strawberry Seed Extract is deduced to be >2,000 mg/kg in both male and female mice.

(3) Mutagenicity (Ames test)

Ames test was conducted and finding was Negative. Strawberry Seed Extract is non-mutagenic.

(4) Drug interactions

Strawberry Seed Extract contains tiliroside, which has been reported for similar effect as grape-fruit juice in inhibiting cytochrome P450 (CYP3A4) with an IC_{50} of 0.7 μM[*)]. Cytochrome P450 is a hepatic detoxicating enzyme which also exists in the small intestine. Intake of Strawberry Seed Extract may thus lead to delayed breakdown and subsequent rapid increase of level of drugs which depend on CYP3A4 for metabolism in the body. We thus alert consumers who orally intake the following drugs:

Bioavailability of the following drugs can be increased by grape-fruit-juice:

Class of beneficial effect	Drug medicine
Heart and circulatory organ drug	Dihydropyridine calcium channel blocker (felodipine, nisoldipine, amlodipine, nimodipine, nifedipine, nitrendipine), diltiazem, quinidine
Immune suppressor	cyclosporine, tacrolimus
HMGCoA reductase inhibitory drug	simvastatin, lovastatin, atorvastatin, pravastatin
Antihistamine drug	terfenadine etc.
Bronchodilating agent	theophylline
Anticoagulant	warfarin

Adrenal cortical steroid	estradiol, cortisol
Antianxiety agent	triazolam, midazolam, diazepam
Antibacterial drug	quinine, clarithromycin
etc.	haloperidol, buspirone, cisapride, carbamazepine

By Japan Health Food & Nutrition Food Association advisory staff text book.

*: Tsukamoto Sachiko, Tomise kyoko, Aburatani Maki, Onuki Hiroyuki,Hirota Hiroshi, Ishiharajima Eiji, Ohta Tomihisa, Isolation of cytochrome P450 inhibitors from strawberry fruit, *Fragaria ananasa*. *J. Nat. Prod*., **67**, 1839-41 (2004).

7. Strawberry Seed Extract – Recommended Daily Dosage

The recommended daily dosage for Strawberry Seed Extract–P is 40-80 mg/day.

8. Applications

	Applications	Claims	Examples
Foods	Beauty food, Anti-obesity food	1) Moisture-retention 2) Skin whitening 3) Anti-aging 4) Antioxidation 5) Anti-obesity	Beverages, hard & soft capsules, tablets, candies, chewing gums, chocolates, wafers, jellies etc
Cosmetics	Beauty cosmetic		Body lotions, body gel etc.

9. Packaging

STRAWBERRY SEED EXTRACT-P（Water-soluble Powder, for food）
STRAWBERRY SEED EXTRACT-PC（Water-soluble Powder, for cosmetic）
5kg Interior packaging：Aluminum bag
Exterior packaging：Cardboard

STRAWBERRY SEED EXTRACT-LC（Liquid, for cosmetic）
5kg Interior packaging：Cubic polyethylene container
Exterior packaging：Cardboard

STRAWBERRY SEED OIL（Oil, for food and cosmetic）
16kg Interior packaging：Tin can
Exterior packaging：Cardboard

10. Storage

Store in cool, dry dark place.

11. Expression

＜Food＞

STRAWBERRY SEED EXTRACT-P

Expression：Strawberry Seed Extract

STRAWBERRY SEED OIL

Expression：Strawberry Oil, Strawberry Seed Oil

＜Cosmetic＞

STRAWBERRY SEED EXTRACT-PC

INCI Name: Dextrin (and) Fragaria Ananassa (Strawberry) Seed Extract

STRAWBERRY SEED EXTRACT-LC

INCI Name: Butylene Glycol (and) Water (and) Fragaria Ananassa (Strawberry) Seed Extract

STRAWBERRY SEED OIL

INCI Name: Fragaria Ananassa (Strawberry) Seed Oil

PRODUCT STANDARD
PRODUCT NAME

STRAWBERRY SEED EXTRACT-P

FOOD

This product is extracted from strawberry (*Fragaria* × *ananassa* Duch.) seeds with aqueous ethanol. It guarantees minimum of 0.5 % tiliroside and 2.0 % polyphenols. This product is water-soluble.

Appearance	Pale yellowish brown powder with light unique smell.	
Polyphenols	Min. 2.0 %	(Folin-Denis method)
Tiliroside	Min. 0.5 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105℃, 2 h)
Purity Test		
(1) Heavy Metals	Max. 20 ppm	(The Japanese Standards for 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)

Composition

Ingredients	Contents
Dextrin	75 %
Strawberry Seed Extract	25 %
Total	100 %

PRODUCT STANDARD
PRODUCT NAME

STRAWBERRY SEED EXTRACT-PC

COSMETIC

This product is extracted from strawberry (*Fragaria* × *ananassa* Duch.) seeds with aqueous ethanol. It guarantees minimum of 0.5 % tiliroside and 2.0 % polyphenols. This product is water-soluble.

Appearance	Pale yellowish brown powder with light unique smell.	
Polyphenols	Min. 2.0 %	(Folin-Denis method)
Tiliroside	Min. 0.5 %	(HPLC)
Loss on Drying	Max. 10.0 %	(1 g, 105℃, 2 h)
Purity Test		
(1) Heavy Metals	Max. 20 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)

Composition

Ingredients	Contents
Dextrin	75 %
Fragaria Ananassa (Strawberry) Seed Extract	25 %
Total	100 %

Ref: The Japanese Standards of Quasi-Drug Ingredients.

PRODUCT STANDARD
PRODUCT NAME

STRAWBERRY SEED EXTRACT-LC

COSMETIC

This product is extracted from strawberry (*Fragaria* × *ananassa* Duch.) seeds with aqueous 1,3-butylene glycol.

Appearance Brown liquid. Odorless or light unique smell.

Certification Test
Polyphenols — Dissolve 30 μl of this product in 3.5 ml water. Add 0.2 ml Folin-Denis reagent into the solution followed by 0.4 ml saturated Na_2CO_3. The solution will turn into blue color.

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)

Composition

Ingredients	Contents
Butylene Glycol	69 %
Water	30 %
Fragaria Ananassa (Strawberry) Seed Extract	1 %
Total	100 %

Ref: The Japanese Standards of Quasi-Drug Ingredients.

PRODUCT STANDARD
PRODUCT NAME

STRAWBERRY SEED OIL

FOOD

This oil is extracted and refined from strawberry（*Fragaria* × *ananassa* Duch.）seeds.

Appearance	Light yellowish liquid oil with light unique smell.	
Acid Value	Max. 0.5	
Color	Max. 3	(Gardner Method)
α-Linolenic Acid	Min. 30.0 %	(GC)
Purity Test		
(1)Heavy Metals	Max. 10 ppm	(The Japanese Standards for Food Additives)
(2)Arsenic	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Negative	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Strawberry Seed Oil	100 %

Ref: Japan Oil Chemists` Society.

PRODUCT STANDARD
PRODUCT NAME

STRAWBERRY SEED OIL

COSMETIC

This oil is extracted and refined from strawberry（*Fragaria* × *ananassa* Duch.）seeds.

Appearance	Light yellowish liquid oil with light unique smell.	
Acid Value	Max. 0.5	(The First method, 10g)
Color	Max. 3	(Gardner Method)
α-Linolenic Acid	Min. 30.0 %	(GC)
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	Negative	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition	Ingredient	Content
	Fragaria Ananassa (Strawberry) Seed Oil	100 %

Ref: The Japanese Standards of Quasi-Drug Ingredients.

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：

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Established Date: September 12, 2008

ORYZA OIL & FAT CHEMICAL CO., LTD.