



ORYZA OIL & FAT CHEMICAL CO., LTD.

RESVERATROL

For Anti-ageing,
Anti-oxidation, Neuron Protection
& Metabolic Syndrome

- Resveratrol – P5
(Food, Powder)
- Resveratrol – WSP0.5
(Food, Water-Soluble Powder)
- Resveratrol – PC5
(Cosmetic, Powder)
- Resveratrol – WSPC0.5
(Cosmetic, Water-Soluble Powder)

ORYZA OIL & FAT CHEMICAL CO., LTD.

Ver. 1.5 YF

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RESVERATROL

1. Introduction

The **grape** is the most-widely cultivated fruit in the world. People have been eating grapes for over eight thousands years and we cannot speak about the history of mankind without mention of grape. Ancient Egyptians made offerings of red wine to their gods and Christians use it to represent the blood of Jesus Christ in ceremonies. During the Tang dynasty in China, wine stimulated people's interest about the west (Persia and other countries). Currently, eighty percent of the grapes cultivated in the world are used to make wine.

Grape skin contains **polyphenol** known for its high antioxidant activity. The purple color of grape skin is of anthocyanin-family polyphenol, the same family of blueberry, believed to maintain good eyesight. A growing awareness about **Resveratrol** as a **characteristic component** of grape has caught people's attention.

Resveratrol was discovered in 1940 and reported to be contained in red wine in 1992. It has been enthusiastically studied as its cancer prevention activity was first reported in 1997. Every year over 1,000 **academic papers** are cited about it.

Resveratrol is now believed to perform various activities including life extension by controlling energy generation, cell division, *etc.* Increased studies about resveratrol will continue to uncover new findings.

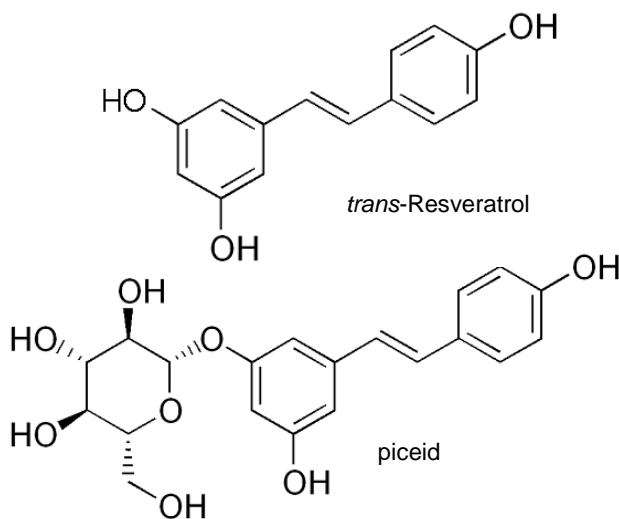
Oryza Oil&Fat Chemical has released the natural ingredient "Resveratrol" developed by concentrating resveratrol with the pigment compositions in grape. New data is also available. Please use Resveratrol to maintain your beauty and health.



Chemical Features

Grape and red wine are rich in polyphenols such as catechin, gallic acid, tannins and anthocyanins (pigment). Resveratrol is contained in grape, cowberry or *Polygonum* plants, and presents mainly as *trans* form in nature (Scheme 1). A glycoside called piceid dissolve in water, and is found in red wine.

Resveratrol-P5 (PC5) is made from grape, being proven by fingerprints (Fig. 1). Polyphenols other than resveratrol present in it and play an important role together with *trans*-resveratrol.



Scheme 1. Chemical Structures of *trans*-resveratrol and piceid.

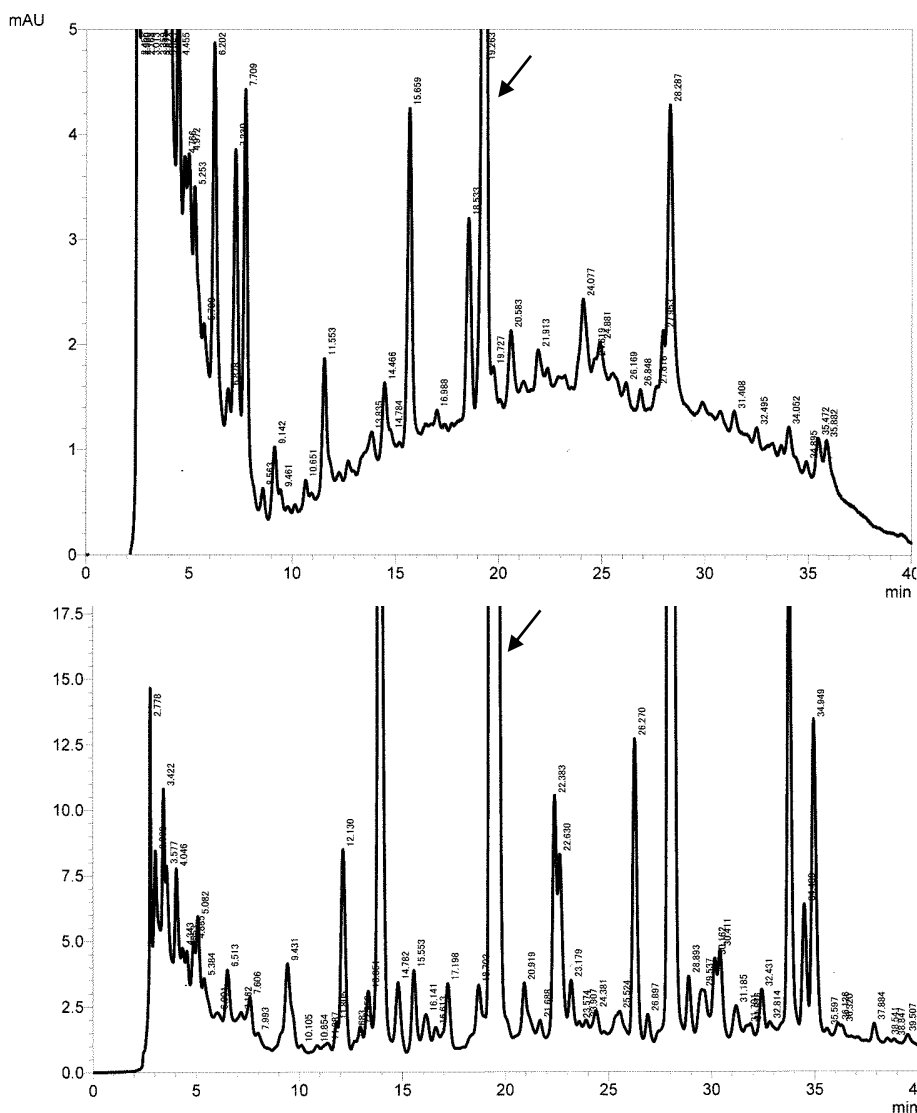


Fig. 1. Polyphenolic Fingerprints of grape extract (top) and Resveratrol-P5 (bottom)

HPLC charts of detection wavenumbers of 306 nm. Arrows show *trans*-resveratrol.

2. Anti-ageing

2-1. What is it Called Ageing?

In general, over sixty percent of women regard ageing as something that they want to avoid or something threatening. For most women, ageing means **ageing of skin**.

Ageing also means higher risk of **diabetes** or **cancer**. Do you feel that you **gain weight easier** as compared to when you were younger? This is because of poor metabolism, unbalanced **hormones**, and dull hormone sensitivity. The risk of circulatory system diseases such as **cardiac arrest** also increases as you age. Brain (nerve) disorders such as **Alzheimer's disease** are also things to be concerned about while **brain atrophy** causes a decline in memory.

These ageing phenomena are closely related to the decline in bodily functions. The key to anti-ageing is the activation of the body's functions.

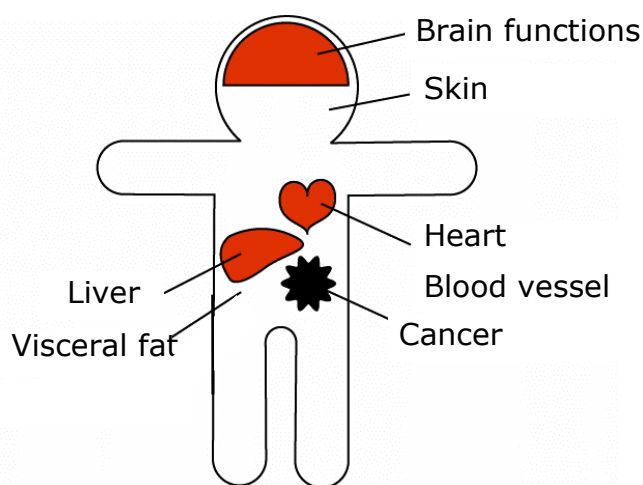


Fig. 2 Various Problems that Appear Due to Ageing

2-2. Extension of Lifespan

In 2006, a revolutionary thesis on resveratrol's life extension was released. According to the thesis, resveratrol's life extension was first confirmed on a mammal (mice). (Baur JA *et al.*, *Nature*, **444** (7117), 337-342, 2006)

The author of the thesis stated that he had conducted a preliminary test on over 20,000 types of compounds and **resveratrol showed the most promise** for the ability to extend life. He conducted tests and proved its life extension on mice.

"Life-span factor" or "life-span gene" on higher animals has not been discovered yet. However, if the **actions of enzymes can be re-activated after their functions decline** due to ageing, the functions of a young body can be regained. This is because actions of enzymes are the source of bodily functions.

The human body has a protein called SIRT-1 that activates another protein called PGC-1alpha which is activated when the body lacks energy. The activation increases the sensitivity to hormones such as insulin and a gene induces the absorption of sugar and lipids in blood into cells (Fig. 3). Namely, PGC-1alpha helps the body to be ready for ingestion of meals and promotes the efficient use of remaining energy to be ready for hunger (maintenance of homeostasis, Fig. 4). However, a continuous high-calorie diet inhibits this regulating function and affects homeostasis of the body (Fig. 4). Although only a small percentage of people have hyperlipemia or diabetes, many people today seem to ingest too many calories.

A unique feature of resveratrol is its function to activate the metabolic pathway through SIRT-1 even in people with a high-calorie diet. In other words, it **activates metabolism through the same pathway utilized during fasting**. No other known material performs like this.

SIRT-1-homologous protein exists in lower creatures like nematode worms and works actively under low nutrient conditions. As a result, nematode worms, cultivated under the conditions live 1.5 times longer than the ones cultivated under regular nutrient conditions. It has been confirmed that adding resveratrol to yeast, nematode worms, and fruit flies extends their life. Many people in throughout the world are interested in resveratrol and want to know if it can extend mammalian life as well.

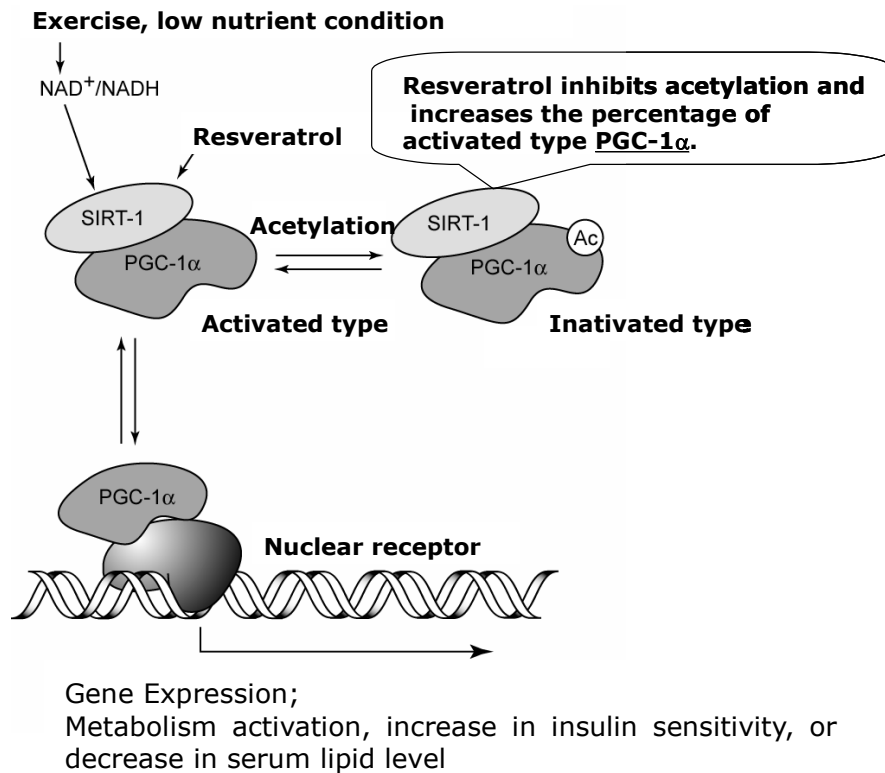


Fig. 3 Example of Actions of the SIRT-1 Gene

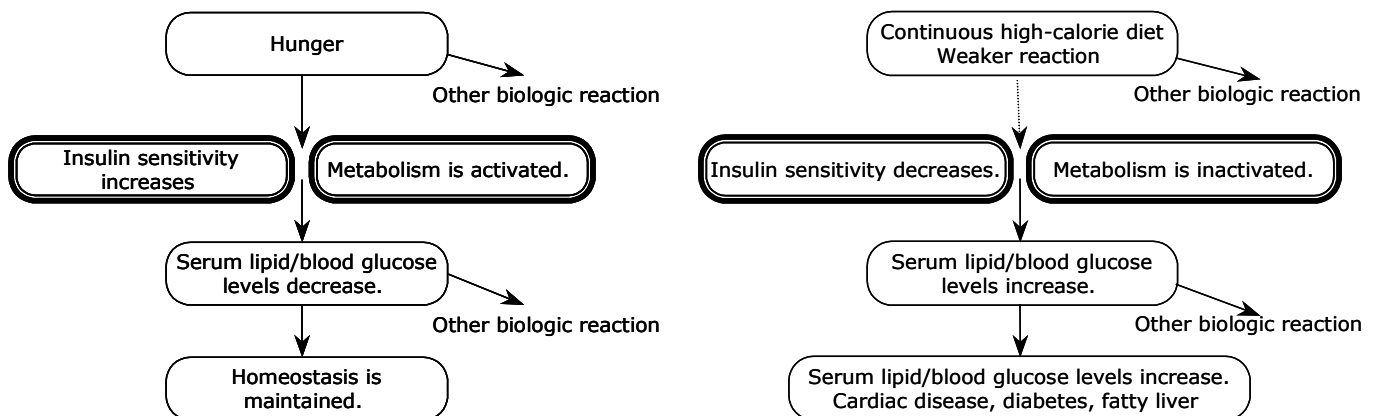


Fig. 4 Continuous High-calorie Diet Inhibits the Maintenance of Metabolic Homeostasis.

3. Anti-oxidation

3-1. UV and Mental Stresses Accelerate Ageing

UV ray is known to cause tan and dark spots and is deeply related to skin's ageing. It produces **reactive oxygen species** to make the skin coarse and less elastic. Reactive oxygen species are also produced by **stress**. This is why stress is often thought of as an archenemy of the skin.

Studies on animals confirmed that resveratrol prevents damage on DNA caused by UV ray, increases the **anti-oxidative potency** in serum, decreases lipoperoxide (lipids damaged by reactive oxygen species), and also **controls the activity of the inflammation transcription factor NF-κB** (chapters 4 and 5).

3-2. SOD-like Activity, DPPH Free Radical Quenching Activity

Resveratrol has been confirmed to perform extremely strong SOD-like activity (Fig. 5) and DPPH radical scavenging activity (Fig. 6) that are indexes of **anti-oxidative potency**. This indicates that resveratrol can protect living bodies from reactive oxygen species.

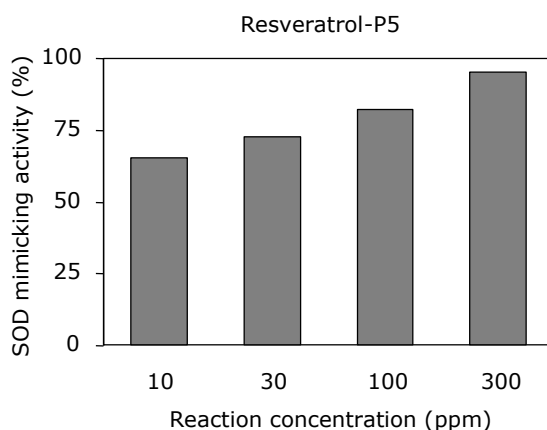


Fig. 5. SOD-like activity

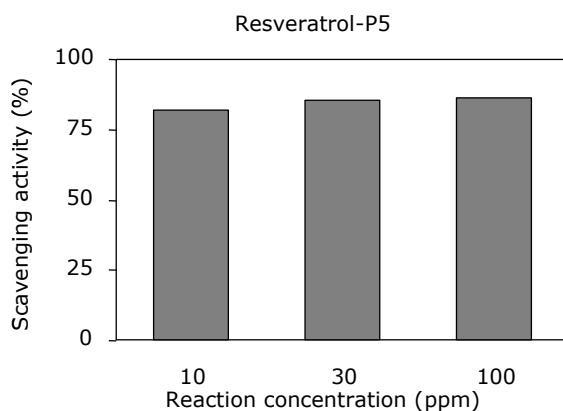


Fig. 6. DPPH radical quenching activity

4. Beauty Stuffs

4-1. Tyrosinase Inhibition (*in vitro*)

Melanin is a cause of dull skin and dark spots and is produced by the enzyme tyrosinase in the body. Resveratrol is expected to inhibit the activity of the enzyme to control **melanin** production and perform brightening effect.

The inhibitory effect was studied resveratrol showed concentration-dependent inhibition on tyrosinase (Fig. 7).

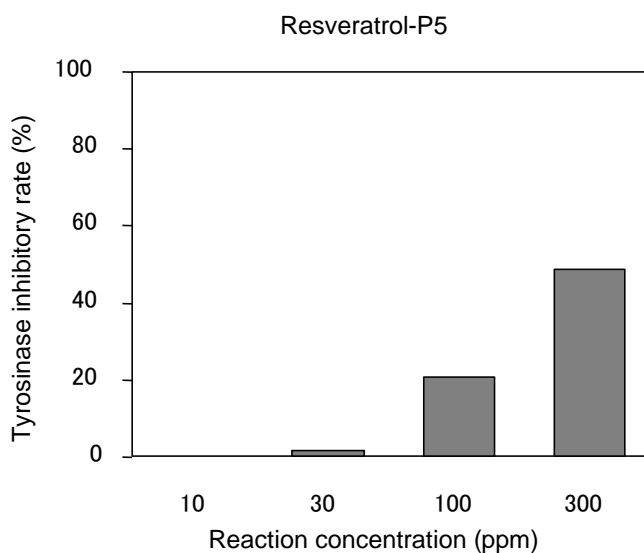
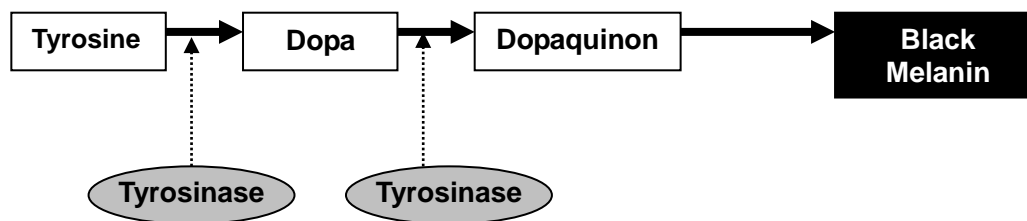


Fig. 7. Tyrosinase Inhibition

Table 1. Tyrosinase Inhibition at 100 mM of either resveratrol (pure substance) or Kojic acid

	Tyrosinase Inhibition (%)	
	Mushroom	Mouse B16 Melanoma
Resveratrol (pure substance)	63.8±3.8	32.7±5.1
Kojic acid	76.7±1.1	43.0±2.5

Kim *et al.*, Oxyresveratrol and hydroxystilbene compounds. Inhibitory effect on tyrosinase and mechanism of action. *J. Biol. Chem.*, **277**(18), 16340-16344, 2002.

4-2. Hyaluronidase Inhibition (*in vitro*)

Hyaluronic acid is a **water-retentive** high polymer widely distributed in the skin, joint fluids, vitreous bodies, and ligaments of living organisms. In the skin, hyaluronic acid is involved in bonding and protecting cells and also maintaining the skin's moisture content and elasticity.

Hyaluronic acid content is believed to **decrease according to age**. When inflammation occurs on the skin due to **UV ray** exposure or other causes, hyaluronidase (degradative enzyme) is activated to accelerate the degradation of hyaluronic acid. Lack of hyaluronic acid results in less moisture and firmness on the skin and eventually dark spots and sagging.

Resveratrol's hyaluronidase activity inhibitory effect was studied and the results showed its concentration-dependent effect apparently. (Fig. 8)

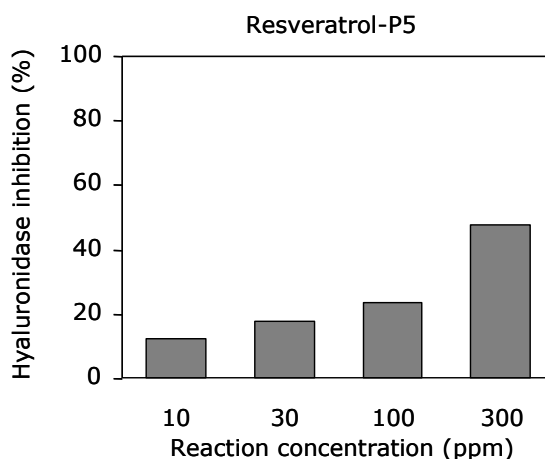


Fig. 8. Hyaluronidase Inhibition

4-3. Collagenase Inhibition (*in vitro*)

Seventy percent of the dermis of the skin is collagen which is distributed throughout the entire dermis. Namely, collagen is the **architecture** of skin tissue and maintains the skin's suppleness and strength at an adequate level. However, when inflammation occurs on the skin due to **UV ray** exposure or other causes, collagenase (degradative enzyme) is activated to accelerate the degradation of collagen. Lack of collagen causes skin ageing signs such as wrinkles and sagging.

Resveratrol's collagenase activity inhibitory effect was studied and the results showed its concentration-dependent effect apparently. (Fig. 9)

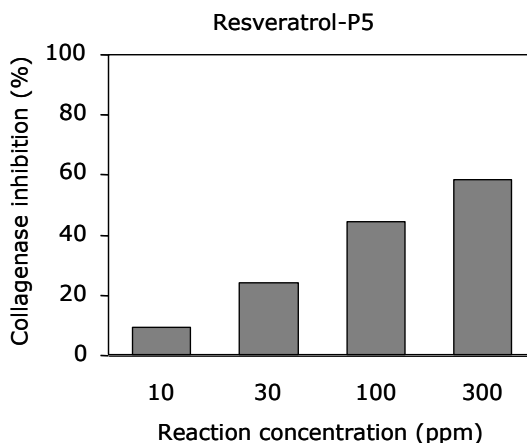


Fig. 9. Collagenase Inhibition

4-4. Elastase Inhibition (*in vitro*)

Elastin is a protein contained in the skin like collagen and is linked to the skin's elasticity. However, when inflammation occurs on the skin due to **UV ray** exposure or other causes, elastase (degradative enzyme) is activated to accelerate the degradation of elastin. Lack of elastin causes skin ageing such as wrinkles and sagging.

Resveratrol has been proven to inhibit elastase at low concentration and control the degradation of elastin. (Fig. 10)

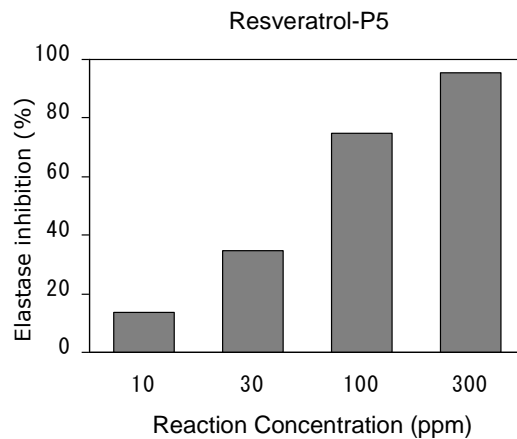


Fig. 10. Elastase Inhibition

4-5. Anti-inflammation

Resveratrol performs its anti-inflammatory activity by controlling the action of transcription factor NF-κB. NF-κB is involved in inflammatory reactions and the increase of cancer cells.

Normally, NF-κB is inactivated by a suppressor. However, it is activated when the suppressor is dissociated from NF-κB by an external factor such as UV ray and bacterial infection. Studies have proved that resveratrol inhibits the suppressor dissociation stage (to maintain NF-κB inactivated).

Resveratrol seems to **control redness, progression of pimples, and itch** by inhibiting the production of inflammatory factor.

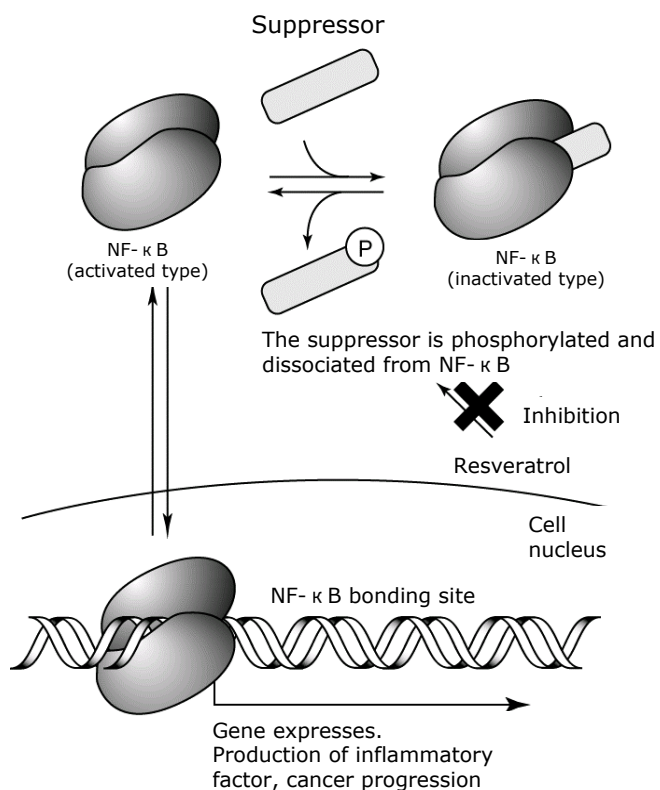


Fig. 11. Schematic Illustration of Anti-inflammation Mechanism

5. Anti-bacteria

5-1. *P. acnes* (*in vitro*)

Acne is considered to occur and progress due to *P. acnes*, which is resident microbiota in hair follicles of human skin.

P. acnes multiplies using sebum as the source of nutrient and produces lipase. Lipase degrades triglyceride in sebum, frees fatty acid, and induces a series of **inflammatory reactions**; white blood cells' go astray and infiltration to the dermis and release of inflammatory factor. The inflammatory factor irritates the skin and accelerates the cornification of the epidermis in addition to inflammation. A test, where resveratrol was added to an agar medium, proved that it controls the multiplication of *P. acnes* (antibacterial activity) at the concentration of 300 µg/ml or higher (Fig. 12).

Since resveratrol has a strong anti-inflammatory activity, it is expected to control acne and its progression by performing its antibacterial activity as well.

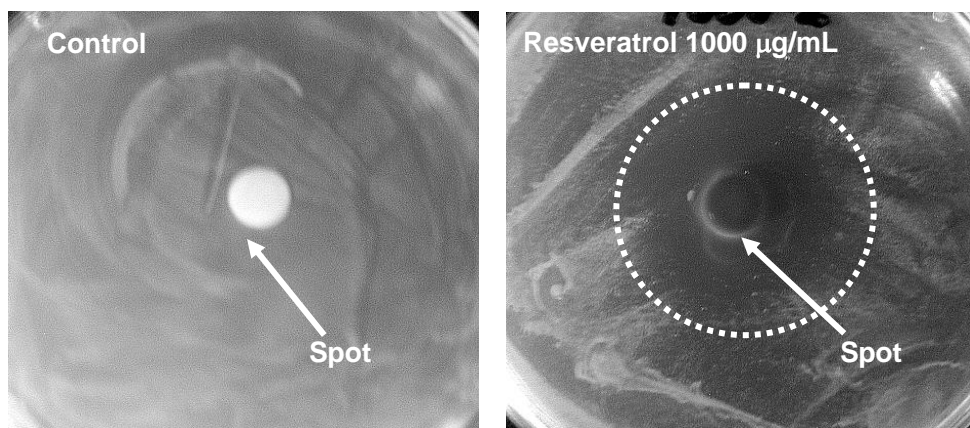


Fig. 12 Resveratrol's Anti *P. acnes* Activity
DMSO or resveratrol (1,000 µg/ml) was dropped on the spot at the center, and cultivated. The entire photo in the left looks whitish because *P. acnes* spread all over the area. In the photo at right, resveratrol inhibited the multiplication of *P. acnes* around the spot (area inside the white dotted line). $n=2$.

5-2. *H. pylori* (*in vitro*)

H. pylori is a bacterium considered to cause **gastric ulcer** and **stomach cancer** and lives in the stomach. Japanese people have a high *H. pylori*-carrying rate. Over fifty percent of people in their sixties or older have the bacterium. Prompt elimination is recommended when *H. pylori* is found.

Resveratrol's antibacterial activity is effective on *H. pylori* and the effect has been proven by *in vitro* tests. CLO test is a method to determine the quantity of ammonia produced by *H. pylori*.

Chatterjee A, et, al. The bactericidal effects of Lactobacillus acidophilus, garcinol and Protargin compared to clarithromycin, on *Helicobacter pylori*. *Mol. Cell. Biochem.*, **243** (1-2), 29-35, 2003

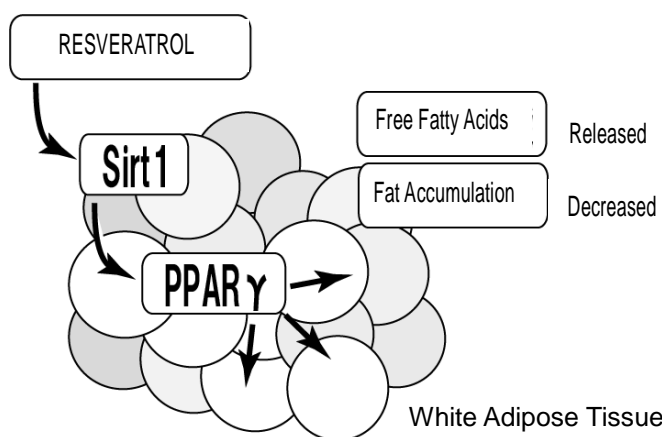
6. Metabolic Syndrome

6-1. Lipid Metabolism (*in vitro*)

As Japanese people now consume more fat because their dietary habits have become more westernized. Excessive fat intake causes obesity, fatty liver, and hardened arteries.

However, in some regions of the world, the incidence of vascular disease is low even though people in the regions have high-fat diets. This is known as the French paradox and many researchers are interested in it. Research results indicate that the French paradox is related to the consumption of red wine.

Molecular mechanisms are actively investigated. Picard *et al.*, demonstrated that Sirt1 protein (chapter 2-2.) suppressed activity of PPAR γ nuclear receptor (*Nature*, 429, 771-776, 2004.) in 3T3-L1 adipose progenitor cells. PPAR γ nuclear receptor works for adipose differentiation or fat accumulation in adipocytes. They cultured adipocytes with resveratrol, and found decreased fat accumulation, enhanced free fatty acids release, *etc.* These results suggest that resveratrol moderate fat metabolism. Please refer to chapter 6-4.



6-2. Prevention of Cardiac Diseases

Resveratrol has been reported to have an effect to prevent hardened arteries. It has been also reported to lower the risk of cardiac diseases by controlling platelet aggregation. Resveratrol's activity to prevent cardiac diseases is also widely known.

6-3. Diabetes

Resveratrol has been reported to have activities to increase insulin sensitivity of high-calorie diet mice, increase lipid metabolism of diabetes model mice, reduce nerve pain, and protect neurons.



6-4. Lipid Accumulation (*in vitro*)

(1) 3T3-L1 Progenitor Cells

Resveratrol-P5' activity to control fat accumulation was evaluated on fat cells where 3T3-L1 was differentiation-induced.

In visual observation on photomicrograph, the number and size of fat droplets were confirmed to be reduced in area in a dose dependent manner. An absorbance measurement of oil red O stained extract also clarified that resveratrol controls fat accumulation concentration dependently. These results indicate that resveratrol could control fat accumulation.

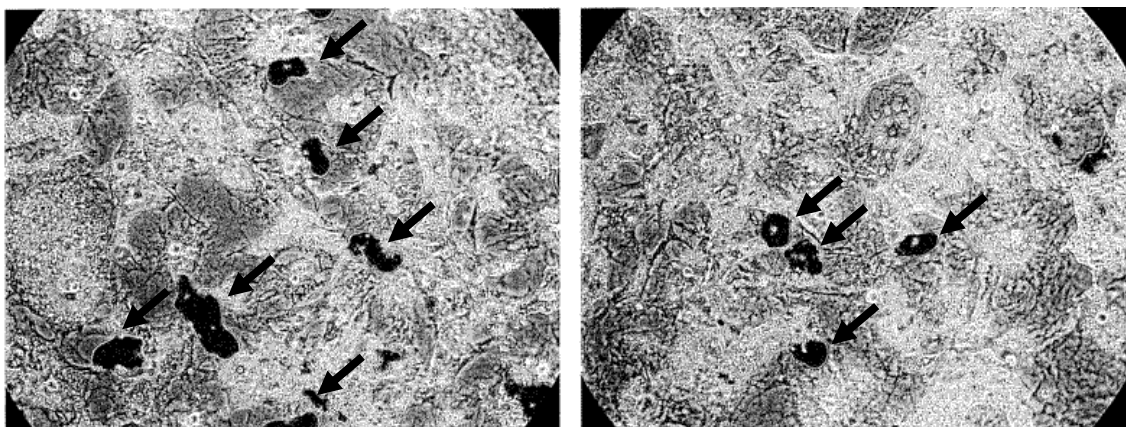


Fig. 13 Fat droplets (arrows) accumulated on 3T3-L1 differentiated cells
Left: Control (DMSO), Right: Resveratrol (1 µg/mL) oil red O staining

(2) VAC Visceral Fat Cells

Resveratrol-P5' activity to control fat accumulation was evaluated on VAC visceral fat cells (rat).

In visual observation on photomicrograph, the number and size of fat droplets were confirmed to be reduced in a dose dependent manner. An absorbance measurement of oil red O stained extract also clarified that resveratrol controls fat accumulation concentration dependently. These test results indicate that resveratrol has a strong fat accumulation control activity.

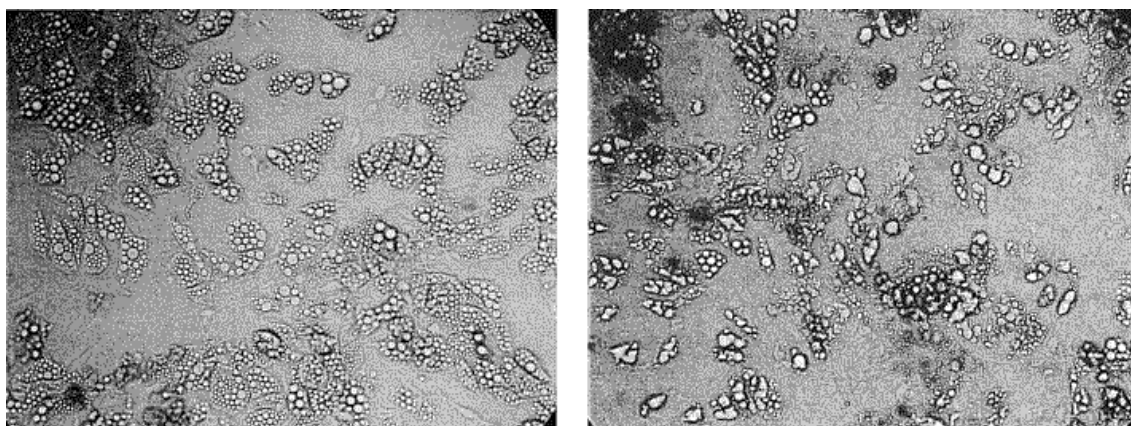


Fig. 14. Fat droplets accumulated on VAC cells
Left: Control (DMSO), Right: Resveratrol (100 µg/mL)

7. Neuron Protection

7-1. Protection against Cellular Damage Caused by Amyloid-beta Peptide (*in vivo*)

In Alzheimer's disease, a substance called amyloid-beta peptide accumulates in the brain, inducing cognitive impairment. Although detailed pathogenic mechanism is still unknown, neuron damage induced by amyloid-beta peptide is believed to be involved. Resveratrol has been proven to reduce cell damage induced by amyloid-beta peptide.

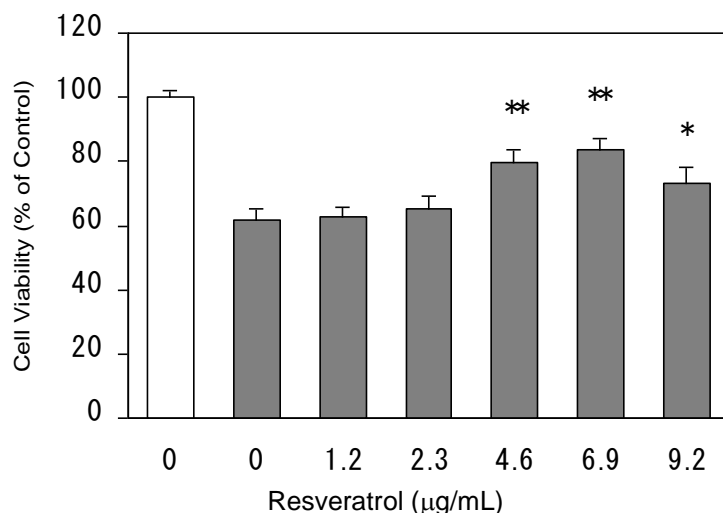


Fig. 15 Reduction of Neurotoxicity Induced by Amyloid Beta-peptide ($A\beta$)

Resveratrol was added two hours after $A\beta$ cell damage. When 20 μ M of $A\beta$ was added to rats' hippocampal neurons (gray bars) and cultivated together, nerves are damaged and the cell viability lowers. Resveratrol reduces cell damage.

* $p < 0.05$ as compared to $A\beta$ cell damage only. ** $p < 0.01$ as compared to $A\beta$ cell damage only. $n=3$

Han YS *et al.*, Neuroprotective effects of resveratrol against beta-amyloid neurotoxicity in rat hippocampal neurons: involvement of protein kinase C. *Br. J. Pharmacol.* **141** (6), 997-1005, 2004

7-2. Protection against Oxidative Stresses (1) (*in vivo*)

The central excitotoxin kainic acid bonds with glutamic acid receptor and inhibits the normal function of neurons. Abnormal excitement of neurons induces the production of **reactive oxygen species** and eventually irreversible neurodegeneration. For this action mechanism, kainic acid is often used to study neurodegeneration induced by reactive oxygen species.

For factors of the generation of reactive oxygen species in the brain, **ageing** is pointed out in addition to Alzheimer's disease, Parkinson's disease, and cerebral ischemia. Normal saline solution and kainic acid (8 mg/kg/day) or kainic acid + resveratrol (30 mg/kg/day) were orally given to rats for five days. Three hours after the last administration, their brains were removed and nerve damage was evaluated by immuno-staining.

The result showed that resveratrol significantly controlled the decrease of living cell count induced by kainic acid in hippocampal regions CA1 and CA3 and also in dentate gyrus. It also significantly controlled the increase of oxidative lesion markers GFAP and isolectin B4. Thus, resveratrol was proved to control reactive oxygen species generated in the brain and protect neurons.

Wang Q *et al.*, *Neurochem Res.*, **29**(11), 2105-2112, 2004.

7-3. Protection against Oxidative Stresses (2) (*in vivo*)

In elderly people, cerebral ischemia is the most common neuron damage cause. Cortex and hippocampus are brain tissues responsible for judgment and memory. They are susceptible to the influence of temporary cerebral ischemia. When cerebral ischemia occurs, excitatory neurotransmitter is released, active oxygen is generated, and neurons are eventually destroyed.

In a test, both common carotid arteries of rats were pinched to stop blood flow and create ischemic condition (reference: Fig. 16). Resveratrol of 20 mg/kg was administrated through the tail vein immediately after the operation to create an ischemic condition.

Fig. 17 is the graph of monitoring blood pressure, cerebral blood flow, and heart rate. Blood pressure lowered by approximately 20% in the resveratrol administration group. This seems to be caused by resveratrol's vasodilatory activity. Cerebral blood flow was significantly higher than the ischemia control group ($p < 0.01$). The results indicate that resveratrol has an activity to reduce cerebral ischemia. There was no significant difference of heart rate between the groups.

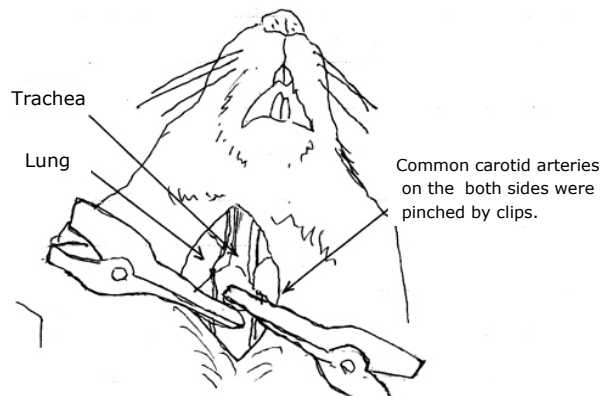


Fig. 16 Outline of the Operation to Pinch Rats' Both Common Carotid Arteries

Fig. 17 (in the right) Change of Blood Pressure (A), Cerebral Blood Flow (B), and Heart Rate (C) before and after Creating an Ischemic Condition by Pinching Both Carotid Arteries over Time

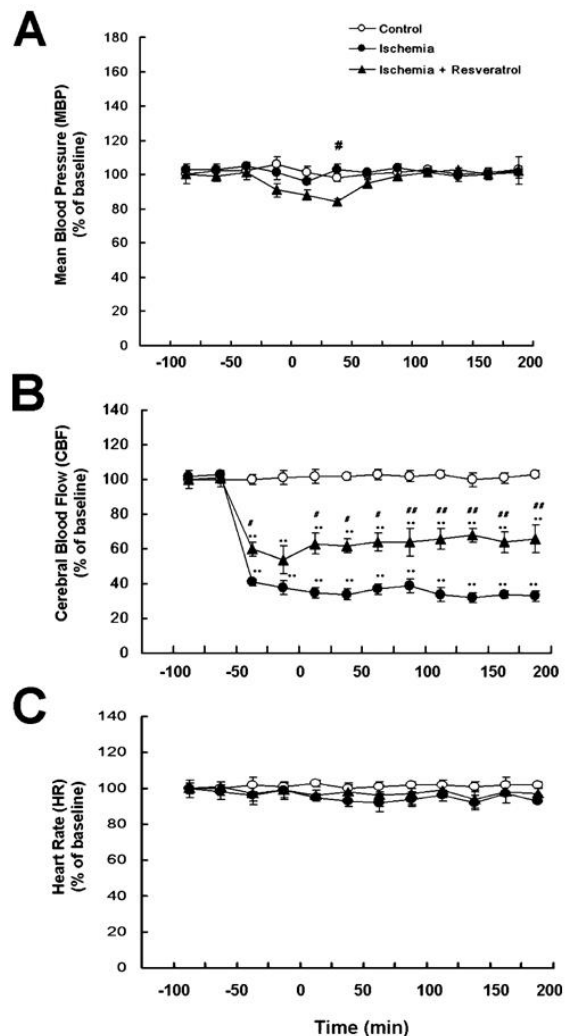
$n=12$, mean \pm s.e.

$p < 0.05$ as compared to the ischemia group

$p < 0.01$ as compared to the ischemia group

Cerebral blood flow was measured by inserting a needle sensor of a laser Doppler flowmeter (Moor Instrument) into hippocampus.

Quoted from Lu KT et al., Neuroprotective effects of resveratrol on cerebral ischemia-induced neuron loss mediated by free radical scavenging and cerebral blood flow elevation. *J. Agric. Food Chem.*, **54** (8), 3126-3131, 2006



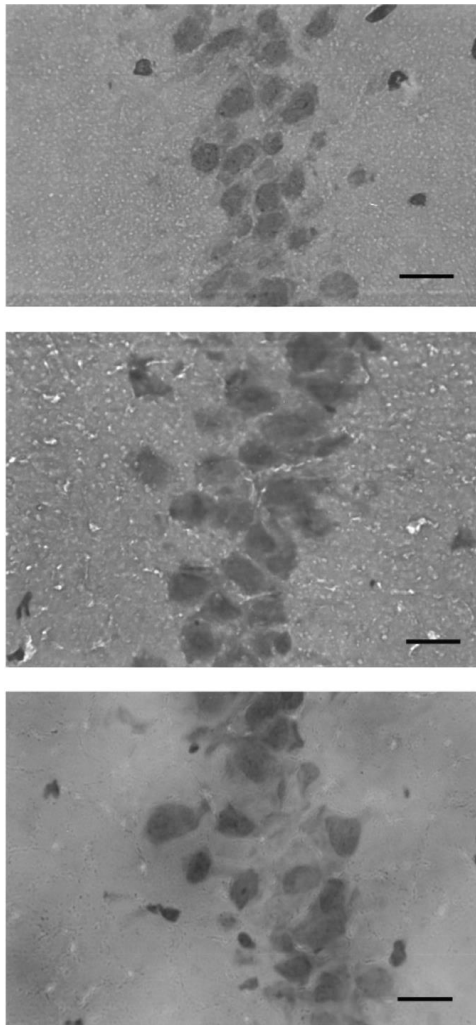


Fig. 18 photomicrograph of Hippocampus Section

Top: Normal brain tissue
 Middle: Brain tissue of the ischemia control group
 Bottom: Brain tissue of the ischemia + resveratrol administration group

Table 2. Nerve Damage Score

Group	Score
Sham surgery control group	0.5±0.3
Ischemia control group	2.6±0.5
Ischemia + resveratrol administration group	1.2±0.4*

Score chart: mean±s.e., n=12,
 * p<0.05 vs. ischemia control group

<Scores>
 0: normal
 1: One-third of area damaged
 2: Two-third of area damaged
 3: Entire area damaged

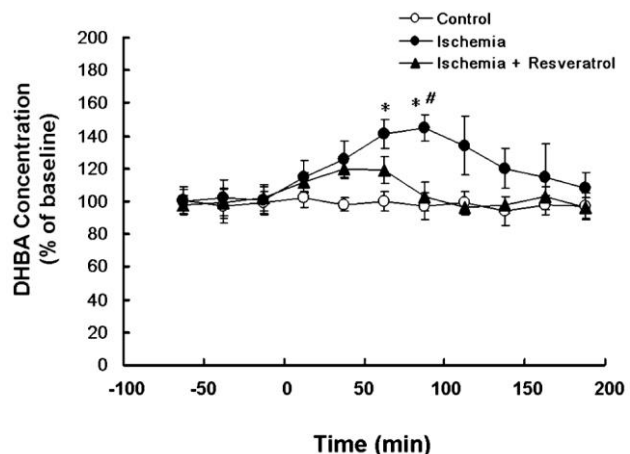


Fig. 19 Change of Cerebral DHBA Concentration over Time n=12, mean±s.e.

* p<0.05 vs. sham operation group
 # p<0.05 vs. ischemia control group

Lu KT *et al.*, Neuroprotective effects of resveratrol on cerebral ischemia-induced neuron loss mediated by free radical scavenging and cerebral blood flow elevation. *J. Agric. Food Chem.*, **54**(8), 3126-3131, 2006

The test results showed that improvement of cerebral blood flow helped to reduce the brain atrophy in the resveratrol administration group. Brain section was studied using a microscope six hours after the ischemia operation. There were gaps in the brain of the ischemia control group due to brain atrophy. In the resveratrol administration group however, brain atrophy was minimal (Fig. 18). When damage to nerves was indicated by score for comparison, the value in the resveratrol administration group was significantly lower (improvement). (Table 2)

Reactive oxygen species in the brain generates a substance called DHBA as a product of oxidation reaction. The DHBA concentration was significantly lower in the resveratrol administration group (Fig. 19), clarifying that resveratrol controls damage induced by reactive oxygen species in the brain.

8. Monitor Tests

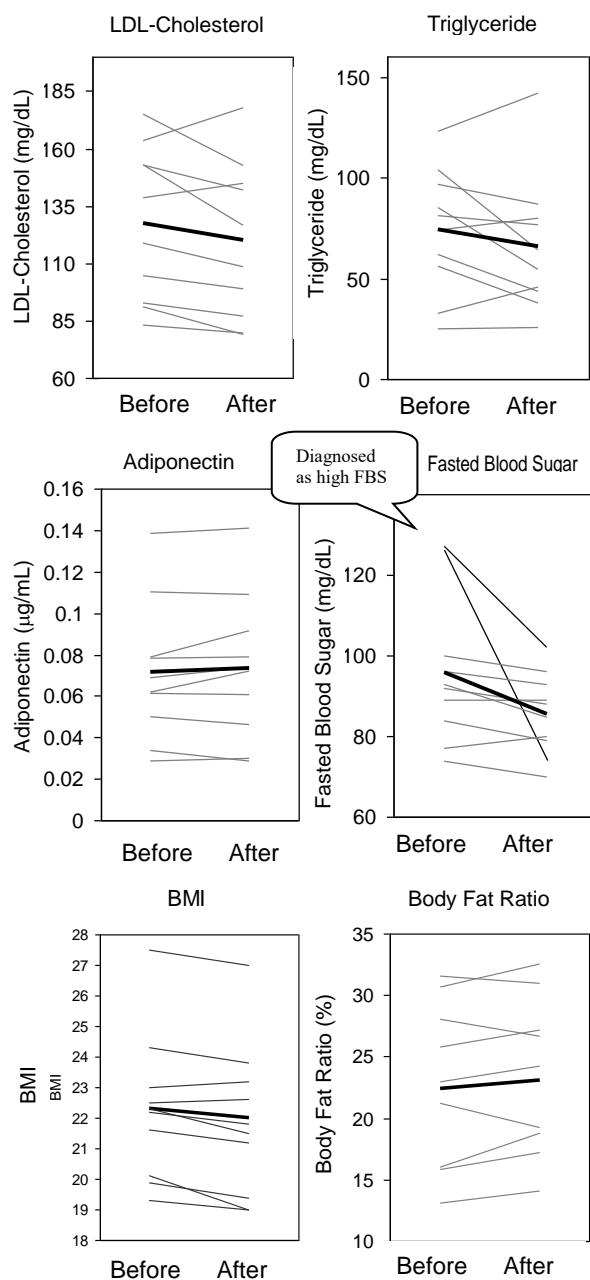
Four-week, successive intake monitor test was performed with subjects of healthy volunteers. Test outline is as follows.

Subjects	Six males aged 42-63, four females aged 25-35. With no chronic syndromes.
Period	4 weeks Take one capsule a day in the evening.
Dose	Resveratrol-P5, 40 mg/day

8-1. Serum Contents (Serum Lipids)

Total cholesterol (mg/dL)	Before	209.7±38.1
	After	200.8±37.4
LDL-cholesterol (mg/dL)	Before	127.5±33.5
	After	103.5±20.0
HDL-cholesterol (mg/dL)	Before	66.2±18.4
	After	66.4±20.9
Triglyceride (mg/dL)	Before	74.0±30.8
	After	65.9±33.2
Free Fatty Acid (mg/dL)	Before	0.44±0.31
	After	0.45±0.17
Fasted Blood Sugar Level (mg/dL)	Before	95.8±18.1
	After	85.6±10.0
Adiponectin (μg/mL)	Before	7.13±3.34
	After	7.33±3.35

mean±s.d. (N=10)



8-2. Body Weight, BMI, Body Fat Ratio

Body Weight (kg)	Before	58.6±10.9
	After	62.5±13.16
BMI	Before	22.3±2.4
	After	22.0±6.7
Body Fat Ratio (%)	Before	22.4±6.5
	After	23.1±6.2

mean±s.d. (N=10)

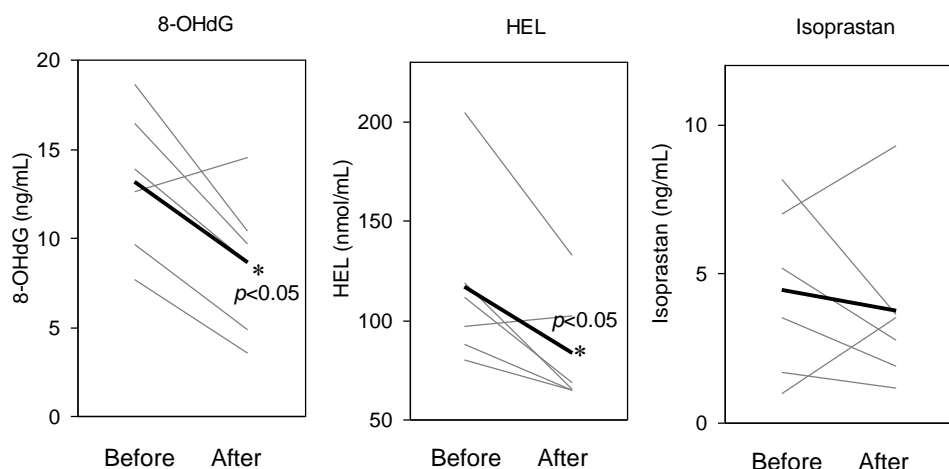
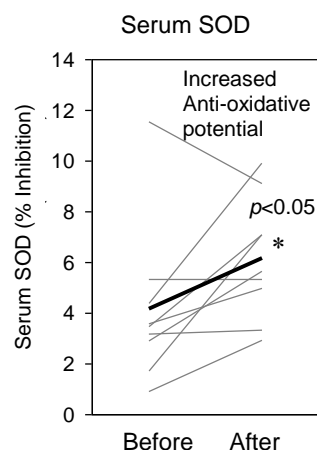
[Graph Readings]

Thin lines show values of individual subjects, before and after the test period. Bold line shows the average.

8-3. Oxidative Stress Markers

Serum SOD (% Inhibition)	Before	4.1 ± 3.1
	After	6.2 ± 2.4 $p < 0.05$
Urinary 8-OHdG (ng/mL)	Before	13.1 ± 4.1
	After	8.5 ± 4.0 $p < 0.05$
Urinary HEL (nmol/mL)	Before	116.5 ± 45.4
	After	83.1 ± 28.2 $p < 0.05$
Urinary Isoprostan (ng/mL)	Before	4.4 ± 2.9
	After	3.7 ± 2.9 $p = 0.056$

mean ± s.d. (N=6) except for serum SOD (N=10)



8-4. Discussion

A clinical test of 4-week successive intake with 10 healthy subjects was conducted, in which the subjects were obliged to take a capsule with 40 mg of Resveratrol-P5 in the evening. We examined biological parameters of serum and urine, before and after the test period.

During the test period, no unfavorable symptom was found in either physical or mental. In addition to that, no change in index implying sickness was found.

In serum lipid contents, total cholesterol, LDL-cholesterol and triglyceride showed trend to decrease. Fasted blood sugar level also showed trend to decrease. Notably, high fasted blood sugar (> 110 mg/dL) of two subjects dropped to normal, but not too low. These results suggest that resveratrol intake **decrease serum lipids and moderate fasted blood sugar level**.

Serum SOD activity significantly increased, which means the strengthened guard potential against active oxygen species in serum. At the same time, urinary 8-OHdG (DNA oxidation), HEL (lipid peroxidation) significantly ($p < 0.05$) decreased, and isoprostan (lipid peroxidation) showed trend to decrease. These results suggest that resveratrol intake **decreased oxidative stresses, and strengthened guard potential**.

9. Experimental Methods and Citations

Fig. 5	Measured by SOD Test Wako (Wako Pure Chemical) kit.
Fig. 6	Samples were added DPPH (1,1-diphenyl-2-picrylhydrazyl) solution, and then the optical density were measured.
Fig. 7	Samples, dissolved in DMSO, were added to tyrosinase (mushroom origin) solution, and then the optical density, accompanies with the conversion of L-tyrosin to dopa-quinone, were measured.
Table 1	Kim <i>et al.</i> , <i>J. Biol. Chem.</i> , 277 , 16340-16344, 2002.
Fig. 8	Samples, dissolved in DMSO, were added to hyaluronic acid and hyaluronidase solution, and then the optical density, accompanies with the conversion of paradimethylaminobenzaldehyde, were measured.
Fig. 9	Samples, dissolved in DMSO, were added to PZ-peptide and collagenase solution, and then the optical density was measured.
Fig. 10	Samples, dissolved in DMSO, were added to DQ-elastin and elastase solution, and then the fluorescence was measured.
Fig. 12	<i>P. acnes</i> was pre-cultured in GAM liquid medium (Nissui Pharm.). The culture was uniformly spread over GAM agar plates (Nissui Pharm.). At the center of the plate, a paper disk (8 mm in diameter) dropped by 80 μ L of sample solution. The disk was cultured for 48 hours.
Fig. 13	3T3-L1 adipocytes (4.0×10^4 cells/mL/well) were seeded in 24-well plates, and cultured for two days in DMEM containing 10% FBS, insulin (167 μ M) isobutylmethylxanthin (0.5 μ M), dexamethazon (1 μ M). Then cells were cultured in DMEM containing 10% FBS, and the medium was exchanged in every three days. Stained by oil red O.
Fig. 14	VAC adipocytes (3.0×10^6 cells/mL/well) were seeded in 24-well plates, and cultured for two days in differentiation medium (Cell Garage Inc.). Medium was exchanged in every two days. In day8, medium containing norepinephrin (2.0×10^{-7} M) was added, and cultured for 6 hours. Cells were fixed by formaldehyde.
Fig. 15	Han YS <i>et al.</i> , <i>Br. J. Pharmacol.</i> 141 (6), 997-1005, 2004.
Figs. 16-19 Table 2	Lu KT <i>et al.</i> , <i>J. Agric. Food Chem.</i> , 54 (8), 3126-3131, 2006.

10. Stability

(1) Thermal Stability

Heating of Resveratrol-P5 at 120°C for an hour did not reduce resveratrol nor polyphenol contents therein.

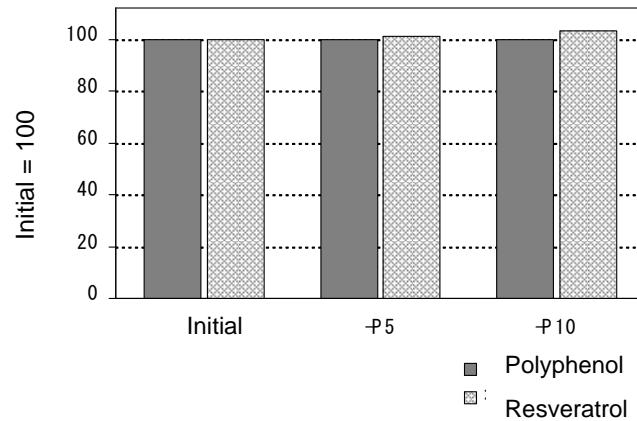


Fig. 20. Thermal Stability

Heating of Resveratrol-WSP solution (10% sucrose, pH4 with citric acid) at concentrations of 0.1 or 0.03% at 80°C for minutes did not reduce resveratrol nor polyphenol contents therein.

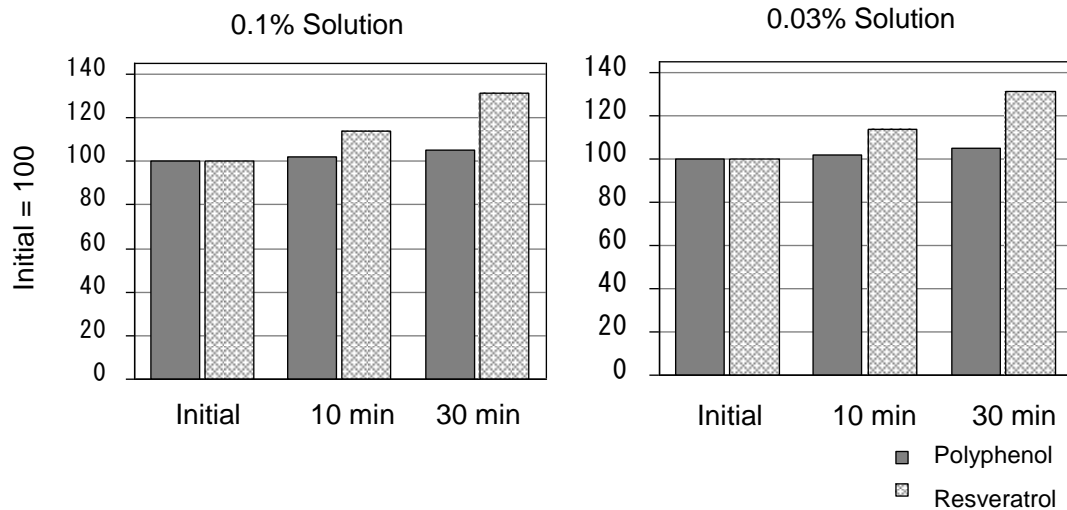


Fig. 21. Solution Stability against Heat

(2) pH

Resveratrol-WSP0.5 was dissolved in water of various pHs (pH3 to 7), and the solutions were stored at room temperature and under natural lightning. One-week storage did not reduce resveratrol nor polyphenol contents.

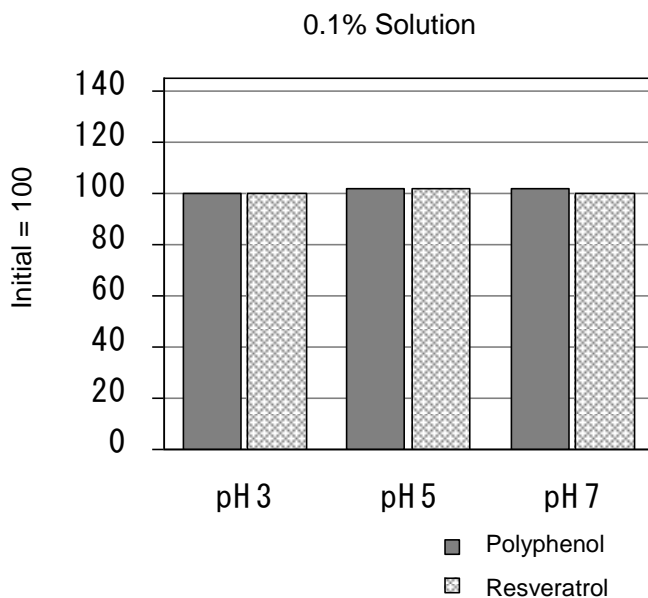


Fig. 22. Solution Stability against pHs

(3) Solubility and Color

One-week storage of Resveratrol-WSP solution (10% sucrose, pH4 with citric acid) at concentrations of 0.1% at 80°C for minutes did not cause cloudiness nor coloring.

	Stability (0.1% solution, pH4)		
	25°C (dark place)	40°C (dark place)	5°C (dark place)
Cloudi-ness	None	None	None
Coloring	None	None	None

11. Safety Data

(1) Residual agricultural chemicals

None of the residual agricultural chemical (498 items) was detected in Resveratrol-P5.

Test trustee: MASIS Co. Ltd
 Date of analysis: July 2, 2007
 Test No. 12590

(2) Acute Toxicity (LD₅₀)

Safety of Resveratrol-P10 was conducted according to the Pharmaceuticals Guidelines on Single-dose Toxicity Test. 2,000 mg/kg of Resveratrol-P10 (maximum dosage without burden on animals) was given to fasting ddY male/female mice of 5-week old followed by close observation for 14 days. No fatal event and no abnormal changes observed in the weight of mice (compared to control). Similarly, no abnormal changes detected in organs of mice upon partial inspection conducted after the test. The LD₅₀ (oral) of Resveratrol-P10 on male/female mice is deduced to be >2,000 mg/kg.

(3) Sub-acute Toxicity

Toxicity on 28-day repeated dose was conducted on Sprague-Dawley male rats. Twenty mg/kg of pure resveratrol (equivalent to 400 mg/kg of Resveratrol-P5) was given to rats without any restrictions on weight and test condition during the 28-day period. No abnormal changes was reported in organs, weight and blood profile of rats at end of test.

Ref: Juan ME, Vinardell MP, Planas JM.

The daily oral administration of high doses of trans-resveratrol to rats for 28 days is not harmful.

J. Nutr., **132**(2), 257-260, 2002.

(4) Mutagenicity

Micronucleus Test was conducted and finding was negative. Resveratrol-P5 is conducted to be non-mutagenic.

12. Nutrition Fact

Description	Resveratrol-P5	Resveratrol -WSP0.5*	Note	Analytical method
Water	3.0g/100g	0.3g/100g		Distillation method
Protein	2.4g/100g	0.2g/100g	1	Kjeldahl method
Fat	1.8g/100g	0.2g/100g		Soxhlet extraction method
Ash	5.1g/100g	0.5g/100g		Direct incineration
Carbohydrate	87.7g/100g	98.8g/100g	2	
Energy	377kcal/100g	398kcal/100g	3	Revised Atwater method
Dietary fiber	5.1g/100g	0.5g/100g		Enzymatic weight method
Sodium	150mg/100g	15mg/100g		Atomic absorption spectrophotometry

1. Nitrogen, protein, conversion factor: 6.25

2. Calculation: 100 – (water+protein+fat+ash+dietary fiber)

3. Energy expression standard: protein 4, fat 9, sugar 4, dietary fiber 2

Test trustee: SRL Co. Ltd

Date of analysis: June 28, 2007

Test No. 200706150036

* Calculated value

13. Recommended Dose

French people enjoy two to three glasses of wine a day in average, which may explain so-called French Paradox. Recommended dosage of 20-40 mg of Resveratrol-P5 or 200-400 mg of Resveratrol-WSP0.5 daily is equivalent to the resveratrol intake accompanied with the wine consumption.

14. Crude Drug Equivalent

Twenty milligram of RESVERATROL-P5 (daily dosage) is equivalent to approximately 200 to 1,000 mL of red wine, 1 kg of fresh grape (with skin), 2 liters of red grape juice, 5 liters of cranberry juice, 200 g of boiled peanuts or 2 kg of peanut butter.

Ref: *Nature Rev. Drug Discov.*, **5**, 493-506, 2006.

15. Commercial Applications

	Applications	Claims	Examples
Food	For Beauty from The Inside	Anti-Ageing, NeuronProtection, Antioxidant, Anti-Inflammation, Metabolic Syndrome Care, Beautifying	Beverages, hard/ soft capsules, tablets, candies, chewing gums, cookies, chocolates, wafers, jerry, <i>etc.</i>
Cosmetic	Topical		Body lotion, body gel, <i>etc.</i>

Solubility in various media (at room temperature)

	water	aqueous ethanol	glycerol	salad oil
-P5	Bad	Good	Good	Bad
-WSP0.5	Good	Good	Bad	Bad

Resveratrol solutions in glycerol or 1,3-butylenglycol (BG) for cosmetic use is available.

16. Packaging

RESVERATROL-P5 (powder, for food),

-WSP0.5 (water-soluble powder, for food)

1 kg Interior packaging: Aluminium Bag
Exterior packaging: Cardboard

RESVERATROL-PC5 (powder, for cosmetic),

-WSPC0.5 (water-soluble powder, for cosmetic)

1 kg Interior packaging: Aluminium Bag
Exterior packaging: Cardboard

17. Storage

Store in cool, dry dark place.

18. Expression

<Food>

RESVERATROL-P5

Grape Extract

-WSP0.5

Cyclodextrin, Grape Extract

<Cosmetics> INCI Name

RESVERATROL-PC5

Vitis Vinifera (Grape) Leaf/Skin/Seed Extract

-WSPC0.5

Maltosyl Cyclodextrin (and) Cyclodextrin (and)
Maltose (and) Vitis Vinifera (Grape) Leaf/Skin/Seed
Extract

PRODUCT STANDARD

PRODUCT NAME : **RESVERATROL-P5** (FOOD)

This product is extracted from grape (*Vitis vinifera*) leaves, seeds and fruit-skin with aqueous ethanol. It guarantees minimum of 25.0 % polyphenols and of 5.0 % trans-resveratrol.

<u>Appearance</u>	Purple to purple-brown powder with slight unique smell.					
<u>Polyphenols</u>	Min. 25.0 %	(Folin-Denis Method)				
<u>trans-Resveratrol</u>	Min. 5.0 %	(HPLC)				
<u>Loss on Drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 °C、 2 hr)				
<u>Purity Test</u>						
(1)Heavy Metals (as Pb)	Max. 10 ppm	(Sodium Sulfide Colorimetric Method)				
(2)Arsenic (as As ₂ O ₃)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)				
<u>Standard Plate Counts</u>	Max. 1×10 ³ cfu/g	(Analysis for Hygienic Chemists)				
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)				
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)				
<u>Composition</u>	<table border="0" style="width: 100%;"> <thead> <tr> <th style="text-align: left;"><u>Ingredient</u></th> <th style="text-align: left;"><u>Content</u></th> </tr> </thead> <tbody> <tr> <td>Grape Extract</td> <td>100 %</td> </tr> </tbody> </table>		<u>Ingredient</u>	<u>Content</u>	Grape Extract	100 %
<u>Ingredient</u>	<u>Content</u>					
Grape Extract	100 %					

PRODUCT STANDARD

PRODUCT NAME : **RESVERATROL-WSP0.5** (FOOD)

This product is extracted from grape (*Vitis vinifera*) leaves, seeds and fruit-skin with aqueous ethanol. It guarantees minimum of 2.5 % polyphenols and of 0.5 % trans-resveratrol. This product is water-soluble.

<u>Appearance</u>	Pale purple powder with slight unique smell.									
<u>Polyphenols</u>	Min. 2.5 %	(Folin-Denis Method)								
<u>trans-Resveratrol</u>	Min. 0.5 %	(HPLC)								
<u>Loss on Drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 °C、 2 hr)								
<u>Purity Test</u>										
(1) Heavy Metals (as Pb)	Max. 10 ppm	(Sodium Sulfide Colorimetric Method)								
(2) Arsenic (as As ₂ O ₃)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)								
<u>Standard Plate Counts</u>	Max. 1×10 ³ cfu/g	(Analysis for Hygienic Chemists)								
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)								
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)								
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Ingredient	Content									
Cyclodextrin	90 %									
Grape Extract	10 %									
Total	100 %									

PRODUCT STANDARD

PRODUCT NAME : **RESVERATROL-PC5** (COSMETIC)

This product is extracted from grape (*Vitis vinifera*) leaves, seeds and fruit-skin with aqueous ethanol. It guarantees minimum of 25.0 % polyphenols and of 5.0 % trans-resveratrol.

<u>Appearance</u>	Purple to purple-brown powder with slight unique smell.					
<u>Polyphenols</u>	Min. 25.0 %	(Folin-Denis Method)				
<u>trans-Resveratrol</u>	Min. 5.0 %	(HPLC)				
<u>Loss on Drying</u>	Max. 10.0 %	(1 g, 105 °C、 2 hr)				
<u>Purity Test</u>						
(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)				
(2)Arsenic (as As₂O₃)	Max. 1 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)				
<u>Standard Plate Counts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)				
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)				
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)				
<u>Composition</u>	<table border="0" style="width: 100%;"> <thead> <tr> <th style="text-align: left;"><u>Ingredient</u></th> <th style="text-align: right;"><u>Content</u></th> </tr> </thead> <tbody> <tr> <td>Vitis Vinifera (Grape) Leaf/Seed/Skin Extract and Resveratrol</td> <td style="text-align: right;">100 %</td> </tr> </tbody> </table>		<u>Ingredient</u>	<u>Content</u>	Vitis Vinifera (Grape) Leaf/Seed/Skin Extract and Resveratrol	100 %
<u>Ingredient</u>	<u>Content</u>					
Vitis Vinifera (Grape) Leaf/Seed/Skin Extract and Resveratrol	100 %					

PRODUCT STANDARD

PRODUCT NAME : **RESVERATROL-WSPC0.5** (COSMETIC)

This product is extracted from grape (*Vitis vinifera*) leaves, seeds and fruit-skin with aqueous ethanol. It guarantees minimum of 2.5 % polyphenols and of 0.5 % trans-resveratrol. This product is water-soluble.

<u>Appearance</u>	Pale purple powder with slight unique smell.	
<u>Polyphenols</u>	Min. 2.5 %	(Folin-Denis Method)
<u>trans-Resveratrol</u>	Min. 0.5 %	(HPLC)
<u>Loss on Drying</u>	Max. 10.0 %	(1 g, 105 °C、 2 hr)
<u>Purity Test</u>		
(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As ₂ O ₃)	Max. 1 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
<u>Standard Plate Counts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>Composition</u>	<u>Ingredient</u>	<u>Content</u>
	Maltosyl Cyclodextrin	} 90 %
	Cyclodextrin	
	Dimaltosyl cyclodextrin	
	Maltose	
	<u>Vitis Vinifera (Grape) Leaf/ Seed/Skin Extract and Resveratrol</u>	<u>10 %</u>
	<u>Total</u>	<u>100 %</u>

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

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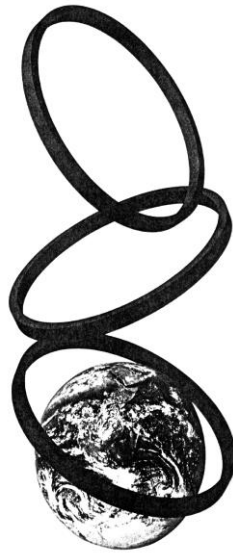
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