



ORYZA OIL & FAT CHEMICAL CO., LTD.

# ***RED RICE EXTRACT***

**For Metabolic Syndrome  
(Anti-hypercholesterolemia and  
Anti-hypertriglyceridemia) and Antioxidative**

- **RED RICE EXTRACT-P**  
(Powder, Food Grade)
- **RED RICE EXTRACT-WSP**  
(Water-soluble Powder, Food Grade)
- **RED RICE EXTRACT-PC**  
(Powder, Cosmetic Grade)
- **RED RICE EXTRACT-WSPC**  
(Water-soluble Powder, Cosmetic Grade)
- **RED RICE EXTRACT-LC**  
(Water-soluble Liquid, Cosmetic Grade)



**ORYZA OIL & FAT CHEMICAL Co. LTD**

ver. 1.3 YF

**RED RICE EXTRACT**  
**For Metabolic syndrome**  
**(Anti-hypercholesterolemia and**  
**Anti-hypertriglyceridemia) and**  
**Antioxidative**

## 1. Introduction

Metabolic syndrome, characterized by abdominal obesity, hyperglycemia, dyslipidemia and hypertension leading to arteriosclerosis, is a modern chronic condition increasing in incidence. For prevention and remedy of metabolic syndrome, the Japanese congress has approved an additional 57.1 billion Yen (approximately 500 Million US Dollars) to the fiscal 2008 budget, demonstrating the high social concern about this life-style-associated syndrome. Demand for functional food preventing and beneficial metabolic syndrome will thus increase. In addition, safe and reliable food as well as raw material with traceable origin and certified process is gaining in popularity.

As purple rice and green rice, red rice is a pigmented rice with a long tradition as one of the major crops in Southeast Asia. The ancient rice cultured back in the Yayoi period (from about 300 BC to 250 BC) in Japan is believed to be red rice. “Red pilaf”, a typical Japanese dish served at various celebratory occasions, allegedly also originates from red rice. To date, only a few studies have addressed the issue of benefits of red rice in the diet. Nevertheless, anti-oxidative, arteriosclerosis-preventive and anti-cancer functionalities have been reported.<sup>1-3)</sup> In 2007, a project “application of pigmental compounds from ancient rice (red rice and purple rice) in prevention and remedy of metabolic syndrome” was launched at Oryza Oil & Fat Chemical Co., Ltd, as a part of the Japanese government granted program to support innovation and challenge in medium- and small-sized enterprises. During this project, we found an anti-dyslipidemic effect in the pigmental components from red rice such as polyphenol and procyanidine. “Red rice extract” can thus be considered as a beneficial food additive for metabolic syndrome and as suitable for various health products targeting dyslipidemia and hyperglycemia.



Fig. 1. Red Rice

References:

- 1) Finocchiaro F., Ferrari B., Gianinetti A., Dall'asta C., Galaverna G, Scazzina F, Pellegrini N. Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. *Mol. Nutr. Food Res.* **51** (8), 1006-19 (2007).
- 2) Ling W. H., Cheng Q. X., Ma J., Wang T. Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. *J. Nutr.* 131 (5), 1421-6 (2001).
- 3) Koide T., Kamei H., Hashimoto Y., Kojima T., Hasegawa M. Antitumor effect of hydrolyzed anthocyanin from grape rinds and red rice. *Cancer Biother. Radiopharm.* 11(4), 273-7 (1996).

## 2. Polyphenolic Constituents

Polyphenolic constituents in red rice are red pigmental proanthocyanidine. In collaboration with the group of Prof. Murakami at the Department of Pharmacology of Osaka University, we have recently elucidated the chemical structure of these compounds (Fig. 2).

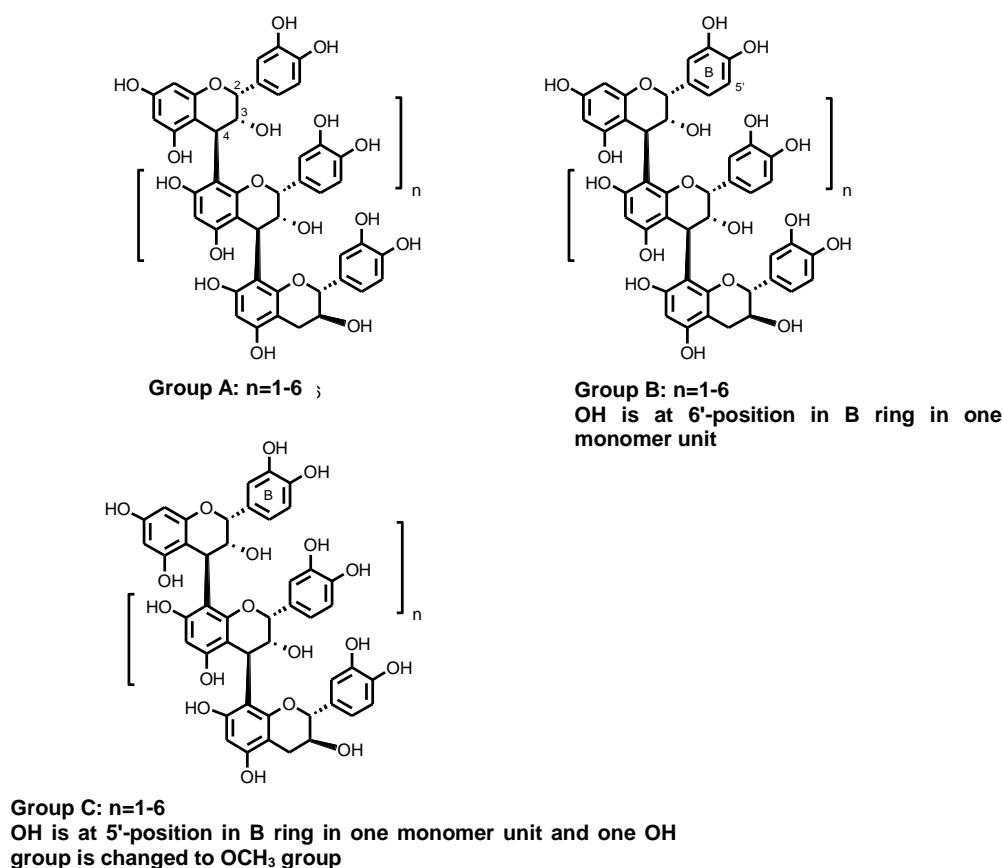


Fig. 2. Chemical Structure of Proanthocyanidine in Red Rice

### 3. Anti-oxidative Effect

As shown in Fig. 3, a dose-dependent SOD-like activity and DPPH radical scavenging activity was found in Red Rice Extract-P, with IC<sub>50</sub> values of 170µg/ml and 64µg/ml, respectively.

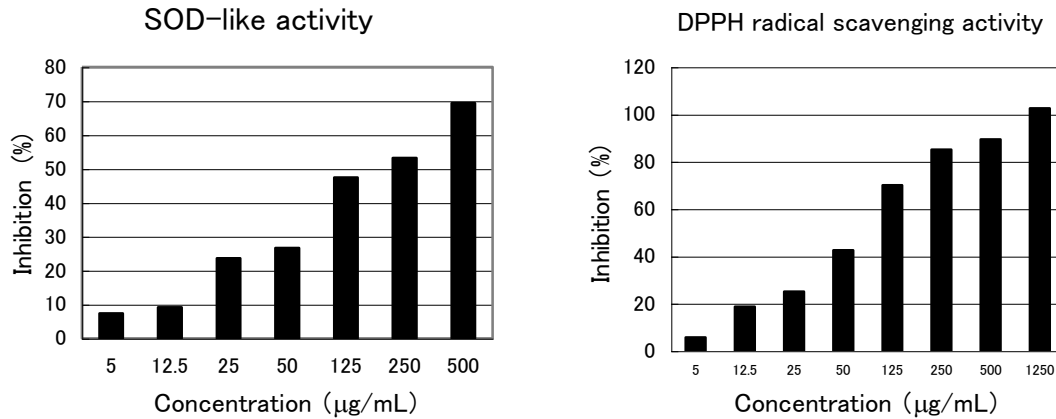


Fig. 3. SOD-like activity and DPPH radical scavenging activity

### 4. Improve Metabolic Syndrome

Metabolic syndrome are defined when two of the following criteria are met: abdominal fat accumulation (waist size of >85 cm for male and >90cm for female), hypertension (systolic blood pressure: >130 mmHg, diastolic blood pressure: 85 mmHg), hyperglycemia (fasting blood glucose: >110 mg/dL) and hypertriglyceridemia (triglyceride: >150 mg/dL or HDL-cholesterol: <40 mg/dL). As will be described below, we found that red rice extract can improve these parameters.

#### (1) Improve hypertriglyceridemia (*in vivo*)

Mice were given free access to a high fat diet for 12 days. Red Rice Extract-P (200mg/kg) was administrated once a day orally to the test group. The mice were fasted for 20 hours before organ and blood were sampled for analysis. As summarized in table 1, body weight gain was suppressed and liver-weight was reduced in the test group. A significant reduction of triglyceride was found in both liver and blood, demonstrating the effect of Red Rice Extract in preventing diet-induced hyperglyceridemia.

Table 1. Effect of Red Rice Extract on lipid parameters in high-fat -diet-fed mice

	Control	Red Rice Extract 200 (mg/kg)
Body weight		
Initial (g)	37.1±0.4	37.4±0.4
12 Days after (g)	45.1±1.0	40.9±1.0
Increase (g)	6.9±0.5	6.1±0.5
Organ weights		
Liver (g)	1.45±0.04	1.31±0.05
Epididymal fat (mg)	1559±84	1863±178
Retrorenal (mg)	622±33	635±64
Liver lipids		
<b>Triglyceride (mg/g)</b>	<b>31.3±3.6</b>	<b>22.9±1.5*</b>
Cholesterol (mg/g)	8.3±0.5	7.9±0.5
Blood		
<b>Triglyceride (mg/dL)</b>	<b>176±19</b>	<b>105±11*</b>
Cholesterol (mg/dL)	228±12	230±24
Glucose (mg/dL)	195±13	178±13

N=6, Mean±SE, \*:  $p < 0.05$ , \*\*:  $p < 0.01$

## (2) Improve Hypercholesterolemia

Mice were given free access to a cholesterol-diet (CE-2: 53.5% sucrose, 30% mild casein, 10% butter, 1% cholesterol and 0.5% sodium cholic acid) for 6 days. Red Rice Extract-P (200 mg/kg/day) was administered once a day orally to the test group. The mice were fasted for one day before cholesterol was measured in liver and blood. As shown in table 2, blood cholesterol concentration was significantly lower in the test group (194±26 versus 365±40), suggesting the effect of Red Rice Extract in improving diet-induced hypercholesterolemia.

Table 2. Effect of Red Rice Extract on cholesterol in high cholesterol diet-fed mice.

	High cholesterol diet		
	Normal	Control	Red Rice Extract (200 mg/kg)
<b>Serum cholesterol (mg/dL)</b>	<b>141±7**</b>	<b>365±40</b>	<b>194±26**</b>
Liver cholesterol (mg/dL)	4.7±1.1*	8.1±0.8	7.9±0.7

N=7, Mean±S.E., \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

## (3) Enhancing lipid metabolism in hepatocyte (*in vitro*)

Since red rice extract was found to improve hyperlipidemia, we next examined its influence on expression of genes involved in lipid metabolism using HepG2 hepatocytes. As shown in Fig. 4, a low dose of Red Rice Extract (0.1µg/ml) readily increased expression of AMPK (AMP-activated protein kinase, promotes  $\beta$ -oxidation of fatty acid) and PPAR $\alpha$  (peroxisome proliferator-activated receptor).  $\beta$ -oxidation of fatty acid occurs in both mitochondria and peroxisome. Red Rice Extract significantly increased expression of ACOX1 coding for acyl

CoA oxidase, the rate-limiting enzyme in  $\beta$ -oxidation in peroxisome, while not influencing expression of CPT1A coding for carnitine palmitoyl transferase, the rate-limiting enzyme in  $\beta$ -oxidation in mitochondria. Thus, Red Rice Extract promotes likely mainly  $\beta$ -oxidation in peroxisome in hepatocytes.

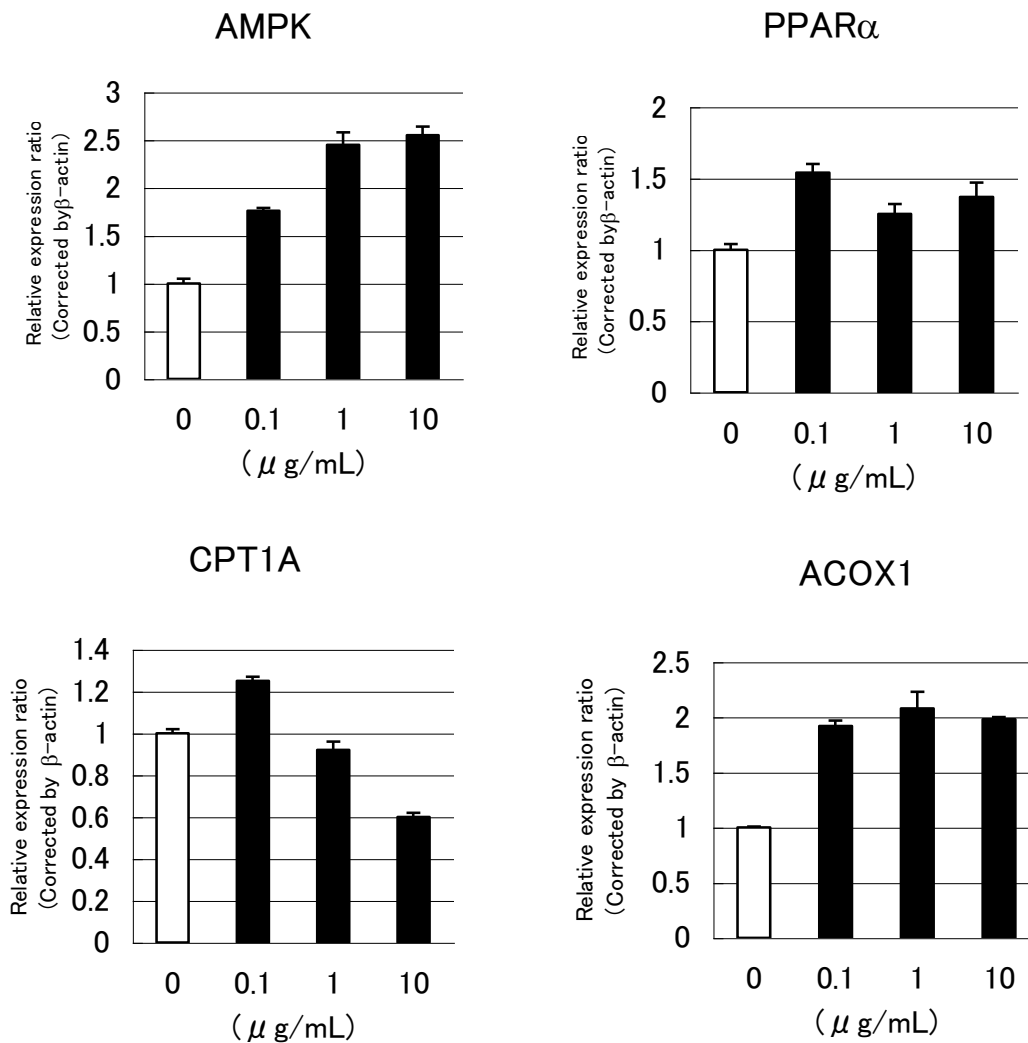
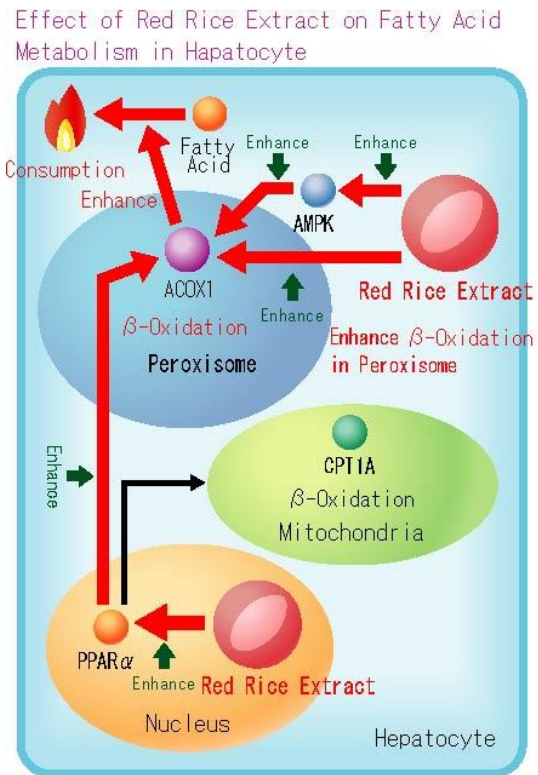


Fig. 4. Effect of Red Rice Extract on expression of genes coding for enzymes involved in fatty acid metabolism in hepatocytes (N=7)

Further experiments revealed that Red Rice Extract does not influence fat absorption in the intestinal tract and fat accumulation in liver. This data suggests that the beneficial effect of Red Rice Extract for hyperlipidemia is via stimulation of lipid metabolism in the liver.

**[Method]** 500  $\mu$ L HepG2 cell suspension in D-MEM medium supplemented with 10% FCS was placed to each well of a 24-well plate at  $8 \times 10^4$  cells/mL. After culturing for 24 hours, 50  $\mu$ L solution containing various amount of Red Rice Extract-P was added and the cultivation was continued for another 20 hours. Total RNA was subsequently prepared from the harvested cells and used for reverse-transcription for production of c-DNA. Expression of genes was investigated by means of real-time PCR using the c-DNA as the template.

Fig. 5. Mechanism of enhancing effect of Red Rice Extract on fatty acid metabolism in hepatocytes



#### (4) Proanthocyanidine enhance ACOX1 expression

To further explore the mechanism of the enhancing effect of Red Rice Extract on lipid metabolism, we examined influence of the proanthocyanidine components of Red Rice Extract on ACOX1 expression. As shown in Fig. 6, 10  $\mu$ g/mL of proanthocyanidine mixture led to increased ACOX1 expression, suggesting that the enhancing effect of Red Rice Extract on lipid metabolism is via the functionality of the Proanthocyanidine components.

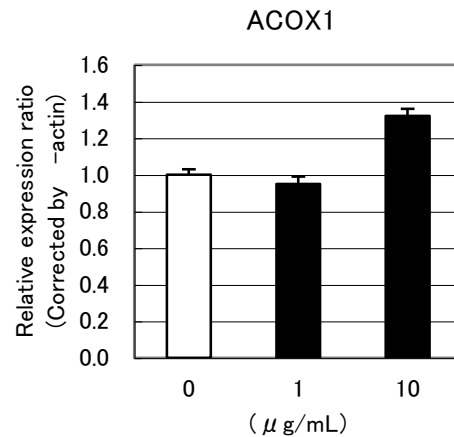


Fig. 6. Proanthocyanidine enhances ACOX1 expression

## 5. Effect of Red Rice Extract-P on human subjects

### Continuing intake in healthy male subjects

Red Rice Extract-P was administrated to 7 healthy male volunteers (employees at our company) aged from 27 to 62 years for 3 weeks, 50 mg daily.

Body weight, BMI and obesity index were slightly decreased after the intake period. Blood triglyceride was also reduced after the intake. Noteworthily, in two hypertriglyceridemic subjects, a drastic reduction of blood triglyceride from >500 mg/dL to < 200mg/dL was achieved after the 3 weeks of intake of the Red Rice Extract, suggesting that Red Rice Extract is especially effective for improving hypertriglyceridemia while not influencing triglyceride index in normal range. Similar effect was observed for total cholesterol. In all 3 subjects with cholesterol of > 200 mg/dL, Red Rice Extract intake led to reduction of this index. In two of them, the reduction was statistically significant. These results suggest that the remedial effect of Red Rice Extract for metabolic syndrome is via reduction of triglyceride and cholesterol.

**Table 3. Effect of 3-week intake of Red Rice Extract**

	before	after
<b>Body weight (kg)</b>	<b>66.6±15.4</b>	<b>66.3±14.9</b>
Body fatty percentage (%)	20.9±5.8	22.9±6.4 p<0.05
<b>BMI (kg/m<sup>2</sup>)</b>	<b>23.6±4.5</b>	<b>23.5±4.3</b>
Impedance (Ω)	479±82	504±73 p<0.05
Adipose mass (kg)	14.6±6.1	16.0±7.1 p<0.05
<b>Obesity (%)</b>	<b>7.1±20.5</b>	<b>6.9±19.5</b>
Blood glucose (mg/dL)	92±3	94±7
Free fatty acid	0.7±0.3	0.6±0.2
Tryglyceride (mg/dL)	<b>253±210</b>	<b>174±136</b>
Phospholipid (mg/dL)	243±46	229±20
Total cholesterol (mg/dL)	211±28	200±18
LDL-cholesterol (mg/dL)	117±28	118±25
HDL-cholesterol (mg/dL)	57±14	57±18

Values are given in average of the 7 subjects ± standard deviation



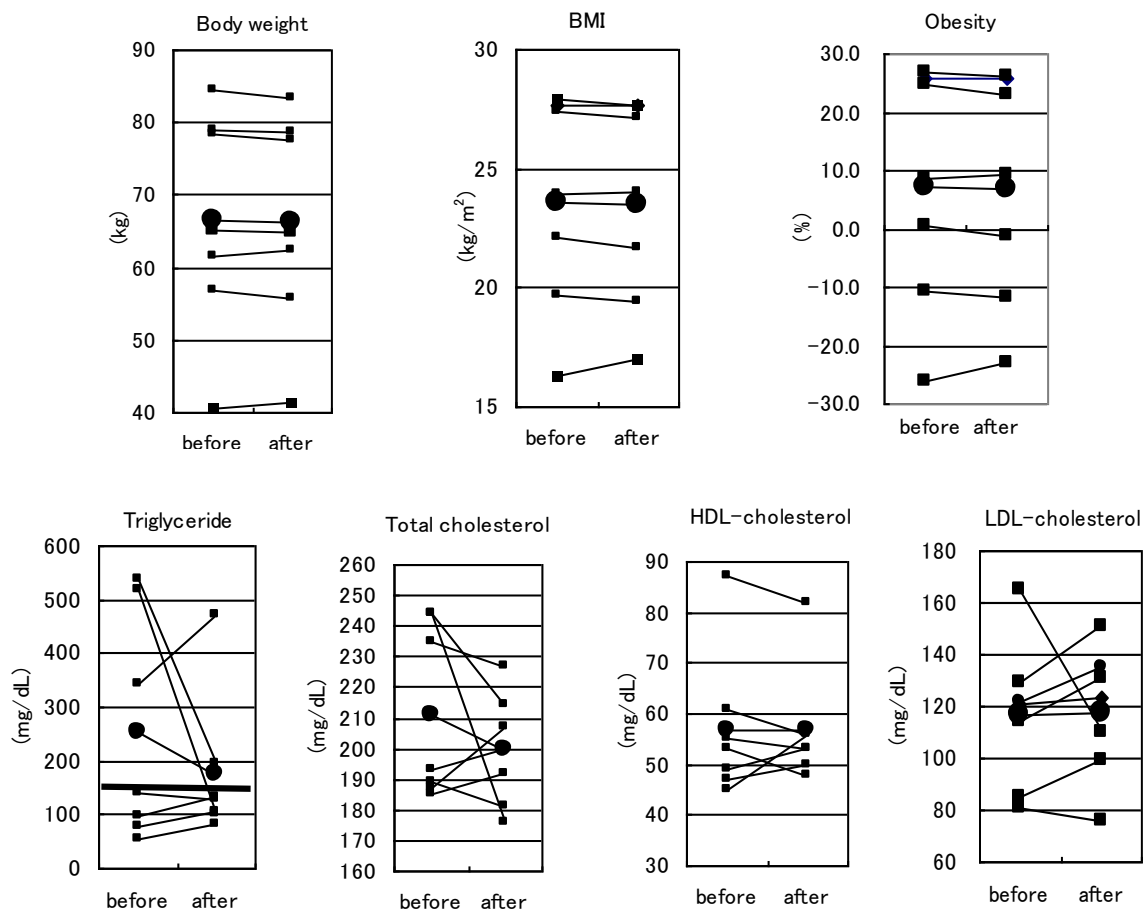
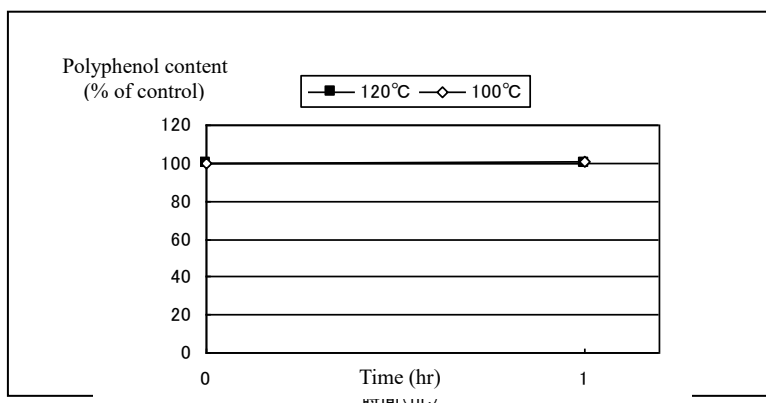


Fig. 7. Parameters before and after 3-weeks intake of Red Rice Extract (● average)

## 6. Stability of Red Rice Extract

### (1) Thermostability

Red Rice Extract-P was heated at 100°C and 120°C for 1 hour. No alteration was observed neither in the property of the extract nor in the polyphenol content (Fig. 8). Red Rice Extract-P is thus stable at temperature up to 120°C.



## (2) pH Stability

Red Rice Extract-WSP was dissolved in solutions of various pH and kept at room temperature in dark for one week. Subsequent analysis revealed that the polyphenol components remained stable over a pH range of 3 - 8 (Fig. 9).

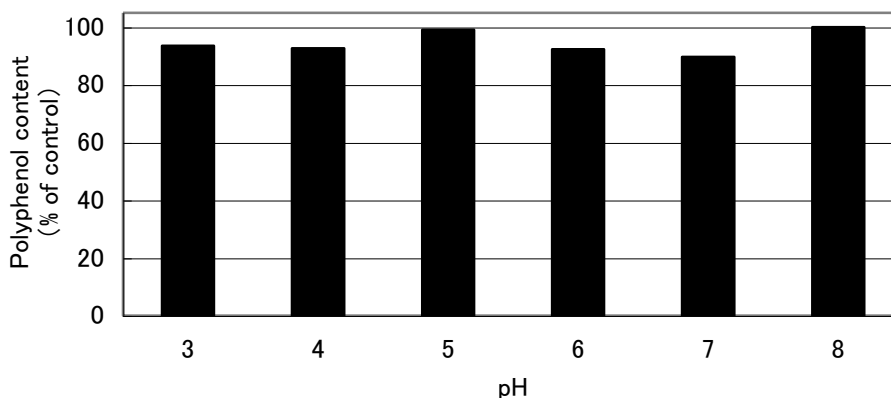


Fig.9. pH stability of Red Rice Extract-WSP

## 7. Nutrition facts of Red Rice Extract-P

Component		Note	Method
Water	1.6 g/100g		Vaccum-heat trying
Protein	3.4 g/100g	1	Combustion
Lipid	4.9 g/100g		Acid fat dissolution
Ash	3.4 g/100g		Direct ashing
Carbonhydrate	86.7 g/100g	2	
Energy	405 kcal/100g	3	Modified out-watering
Dietary fiber	< 2.7 g/100		Enzymatic-weight method
Sodium	6 mg/100g		Atomic absorption spectrometry

Note 1) N=6.25

Note 2) 100 - (moisture + protein + fat + ash + dietary fiber)

Note 3) Factors for calculating the energy value : protein 4; lipid 9; sugar 4; dietary fiber 2

Test trustee: SRL Co. Ltd.

Date of issue of report: October 15, 2007

Report issue No.: 200710010036

## 8. Safety profile of Red Rice Extract

### (1) Residual agricultural chemicals

Red Rice Extract-P was examined for residues of 498 agricultural chemicals according to the food hygiene regulation and pesticide legislation. All items were below the detection limits.

Test trustee: Masis Co., LTD  
Date of issue of the report: October 15, 2007  
Contract No.: 15161

## (2) Lovastatin

Lovastatin is an anti-hypercholesterolemic drug unapproved in Japan. „Red yeast rice”, a fermented rice, contains trace of Lovastatin. Recently, high concentration of Lovastatin was detected in a food supplement consisting of red yeast rice in the USA which turned out to be due to an intentional drug-blending. Consequently, FDA requested a recall of the product. Though “red rice” and “red yeast rice” are not related to each other, both of them contains the words “red” and “rice” which may cause confusion. We thus examined Red Rice Extract-P for the present of Lovastatin. No Lovastatin was detected.

Test trustee: Japan Food Analytic Center  
Date of issue of the report: November 9, 2007  
Contract No.: 307100639-02

## (3) Acute Toxicity (LD<sub>50</sub>)

Following the Guideline for Single-Dose Toxicity Tests of Pharmaceutical Products, Red Rice Extract (no diluent, polyphenol content: 30%) was administrated orally to fasted ddY mice of 5-weeks of age at 1000 mg/kg. The mice were observed for 2 weeks. No difference was found between the test group and control group regarding death and body weight change. No abnormalities were found at autopsy at the end of the test period. The oral administration LD<sub>50</sub> value for Red Rice Extract was thus above 1000mg/kg for both male and female mice.

## (4) Ames Test

Ames test was conducted for Red Rice Extract-P using *S. typhimurium* TA100 and TA98. At concentration of 19.5~5000 µg/plate, no increase in the frequency of revertant colonies were found with or without metabolic activation. Red Rice Extract-P was thus non-mutagenic under the test condition.

## (5) Human Intake Test

As described above, 50 mg Red Rice Extract-P was administrated to 7 male volunteers daily for 3 weeks. No abnormality was observed in any of the following blood parameters: total bilirubin, total protein, albumin, AST, ALT, γ-GTP, LDL-cholesterol, total cholesterol, triglyceride, phospholipid, free fatty acid, HDL-cholesterol, sodium, calcium, serum iron, TIBC, UIBC, urea nitrogen, urea acid, glycemia.

## 9. Recommended Dosage

Recommended daily dose of Red Rice Extract-P is 50 mg.

## 10. Application

	Application	Dosage form
Food	Metabolic syndrome, hyperlipidemia, anti-oxidation	Beverage, hard and soft capsule, tablet, candy, chewing gum, gum, cookies, chocolate, wafer, jelly, etc.
Cosmetics	Whitening, moisturizing, anti-aging	Lotion, pack, bodygel

## 11. Packaging

Red Rice Extract-P (powder, food grade), -WSP (soluble powder, food grade)

5kg Interio packaging: aluminium-coated plastic bag  
Exterio packaging: cardboard box

Red Rice Extract-PC (powder, cosmetics grade),  
-WSPC (water-soluble powder, cosmetics grade)

5kg Interio packaging: aluminium-coated plastic bag  
Exterio packaging: cardboard box

Red Rice Extract –LC (soluble liquid, cosmetics grade)

5kg Interio packaging: cubic polyethylene container  
Exterio packaging: cardboard box

## 12. Storage

Store in cool, dry and dark place.

## 13. Expression

<Food>

**RED RICE EXTRACT-P**

Example: Red Rice Extract, dextrin

**RED RICE EXTRACT-WSP**

Example: Red Rice Extract, cyclodextrin

\* please refer to your local standard and regulation.

<Cosmetics>

**RED RICE EXTRACT-PC**

INCI Name: Dextrin (and) Oryza Sativa (Rice) Bran Extract

**RED RICE EXTRACT-WSPC**

INCI Name: Cycloextrin (and) Oryza Sativa (Rice) Bran Extract

**RED RICE EXTRACT-LC**

INCI Name: Water (and) Butylene Glycol (and) Oryza Sativa (Rice) Bran Extract

PRODUCT STANDARD  
PRODUCT NAME

**RED RICE EXTRACT-P**

(FOOD)

This product is extracted from red rice (*Oryza sativa* L.) with aqueous ethanol. It guarantees minimum of 5 % polyphenols.

<u>Appearance</u>	Pale blown to red brown powder with slight unique smell.	
<u>Polyphenols</u>	Min. 5 %	(Folin-Denis Method)
<u>Proanthocyanidin</u>	Red spot is detected	(TLC)
<u>Loss on Drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105 °C, 2 h)

Purity Test

(1) Heavy Metals (as Pb <sub>2</sub> )	Max. 30 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As <sub>2</sub> O <sub>3</sub> )	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
<u>Standard Plate Counts</u>	Max. 3 × 10 <sup>3</sup> cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. 1 × 10 <sup>3</sup> cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Maltodextrin	83%
Red Rice Extract	17%
Total	100%

PRODUCT STANDARD  
PRODUCT NAME

**RED RICE EXTRACT – WSP**

(FOOD)

This water-soluble product is extracted from red rice (*Oryza sativa* L.) with aqueous ethanol. It guarantees minimum of 1 % polyphenols.

<u>Appearance</u>	Pale blown to red brown powder with slight unique smell.									
<u>Polyphenols</u>	Min. 1 %	(Folin-Denis Method)								
<u>Proanthocyanidin</u>	Red spot is detected	(TLC)								
<u>Loss on Drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105 °C, 2 h)								
<u>Purity Test</u>										
(1) Heavy Metals (as Pb <sub>2</sub> )	Max. 30 ppm	(Sodium Sulfide Colorimetric Method)								
(2) Arsenic (as As <sub>2</sub> O <sub>3</sub> )	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)								
<u>Standard Plate Counts</u>	Max. 3 × 10 <sup>3</sup> cfu/g	(Analysis for Hygienic Chemists)								
<u>Moulds and Yeasts</u>	Max. 1 × 10 <sup>3</sup> cfu/g	(Analysis for Hygienic Chemists)								
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)								
<u>Composition</u>	<table border="0"> <thead> <tr> <th><u>Ingredient</u></th> <th><u>Content</u></th> </tr> </thead> <tbody> <tr> <td>Maltodextrin</td> <td>95 %</td> </tr> <tr> <td>Red Rice Extract</td> <td>5 %</td> </tr> <tr> <td>Total</td> <td>100 %</td> </tr> </tbody> </table>		<u>Ingredient</u>	<u>Content</u>	Maltodextrin	95 %	Red Rice Extract	5 %	Total	100 %
<u>Ingredient</u>	<u>Content</u>									
Maltodextrin	95 %									
Red Rice Extract	5 %									
Total	100 %									

PRODUCT STANDARD  
PRODUCT NAME

**RED RICE EXTRACT –PC**

(COSMETIC)

This product is extracted from red rice (*Oryza sativa* L.) with aqueous ethanol. It guarantees minimum of 5 % polyphenols.

<u>Appearance</u>	Pale blown to red brown powder with slight unique smell.									
<u>Polyphenols</u>	Min. 5 %	(Folin-Denis Method)								
<u>Proanthocyanidin</u>	Red spot is detected	(TLC)								
<u>Loss on Drying</u>	Max. 10.0 %	(1 g, 105 °C, 2 h)								
<u>Purity Test</u>										
(1) Heavy Metals (as Pb <sub>2</sub> )	Max. 30 ppm	(The Second method of The Japanese Standards of Quasi-Drug Ingredient)								
(2) Arsenic (as As <sub>2</sub> O <sub>3</sub> )	Max. 1 ppm	(The Third method of The Japanese Standards of Quasi-Drug Ingredient)								
<u>Standard Plate Counts</u>	Max. 1 × 10 <sup>2</sup> cfu/g	(Analysis for Hygienic Chemists)								
<u>Moulds and Yeasts</u>	Max. 1 × 10 <sup>2</sup> cfu/g	(Analysis for Hygienic Chemists)								
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)								
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<u>Ingredient</u>	<u>Content</u>									
Maltodextrin	87 %									
<u>Oryza Sativa (Rice) Extract</u>	<u>17 %</u>									
Total	100 %									

PRODUCT STANDARD  
PRODUCT NAME

**RED RICE EXTRACT – WSPC**

(COSMETIC)

This water-soluble product is extracted from red rice (*Oryza sativa* L.) with aqueous ethanol. It guarantees minimum of 1 % polyphenols.

<u>Appearance</u>	Pale blown to red brown powder with slight unique smell.									
<u>Polyphenols</u>	Min. 1 %	(Folin-Denis Method)								
<u>Proanthocyanidin</u>	Red spot is detected	(TLC)								
<u>Loss on Drying</u>	Max. 10.0 %	(1 g, 105 °C, 2 h)								
<u>Purity Test</u>										
(1) Heavy Metals (as Pb <sub>2</sub> )	Max. 30 ppm	(The Second method of The Japanese Standards of Quasi-Drug Ingredient)								
(2) Arsenic (as As <sub>2</sub> O <sub>3</sub> )	Max. 1 ppm	(The Third method of The Japanese Standards of Quasi-Drug Ingredient)								
<u>Standard Plate Counts</u>	Max. 1 × 10 <sup>2</sup> cfu/g	(Analysis for Hygienic Chemists)								
<u>Moulds and Yeasts</u>	Max. 1 × 10 <sup>2</sup> cfu/g	(Analysis for Hygienic Chemists)								
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)								
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Maltodextrin	95 %									
<u>Oryza Sativa (Rice) Extract</u>	<u>5 %</u>									
Total	100 %									





ORYZA OIL & FAT CHEMICAL CO., LTD., striving for development of new functional additives and material for your health and beauty.

**From product planning to OEM – For any additional information and assistance, please contact:**

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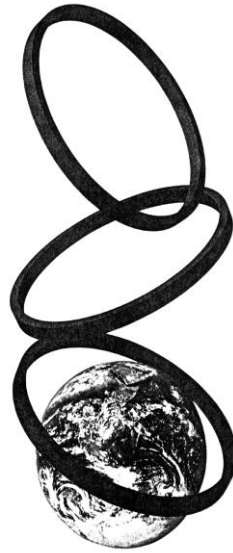
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