ORYZA TRITERPENOID

Ingredient with anti-hyperlipidemia & Skin rejuvenating

■ ORYZA TRITERPENOID–C
(Powder, Cosmetic Grade)

ORYZA OIL & FAT CHEMICAL CO., LTD.

Ver.1.0
1. Introduction

Rice remains as the major crop planted in Japan for generations. The bioactive components of rice and rice bran has been reviewed and examined. Rice Oil is rich in sterol with renowned blood cholesterol lowering effect. However, the most commonly used resource to derive sterol is soybean oil. Rice oil has sterol content that is 5x higher than soybean oil and β-sitosterol remain as the main functional component. Nevertheless, it is believed that rice contain specific sterol which are absent in other oils due to its unique physiological effects.

Oryza Oil & Fat Chemical Co., Ltd. with its very own patent, has developed and commissioned the production of ORYZA TRITERPENOID. ORYZA TRITERPENOID is 100% rice derived (hydrolyzed and refined from rice bran & rice germ) with distinctive sterol compositions.

2. Triterpenoid

Steroid is a compound with cyclopentanone hydrophenanthrene ring \((C_{17}H_{28})\). Sterol, however, has a structure with hydroxyl group located at 3- position ranging from \(C_{27}\) to \(C_{30}\). Sterols are widely exists as free form, esters of fatty acid and glycoside with wide distribution among animals and plants. The main sterol found in animals are represented by sterol \(C_{27}\) while β-sitosterol, stigmasterol and campesterol are commonly found in plants. Plant sterols are renowned for its cholesterol lowering effects with preventive effects against colon cancer, prostatic hypertrophy and platelet
aggregation. ORYZA TRITERPENOID is extracted and purified from rice oil containing cycloartenol, 24-methylene cycloartanol, campesterol, cycloartanol and cyclobranol of triterpene alcohol as illustrated in Fig. 1. Triterpene alcohol belongs to sterol of C\textsubscript{30}. Researches conducted at the R&D of ORYZA OIL & FAT CHEMICAL CO., LTD. revealed that ORYZA TRITERPENOID exert unique health promoting effects that differs from other plant sterols.

Fig. 1. Components of ORYZA TRITERPENOID
Gas chromatograph (GC) was conducted to analyze the content of ORYZA TRITERPENOID. Analysis by GC confirmed that ORYZA TRITERPENOID contains campesterol, β-sitosterol, cycloartenol, and 24-methylenecycloartanol (Fig. 2).

![Gas chromatogram of ORYZA TRITERPENOID](image)

**Fig. 2.** Gas chromatogram of ORYZA TRITERPENOID

_[Method]_

ORYZA TRITERPENOID is dissolved in carbon disulfide for GCMS as follows:

- **Column**: Rtx-1 [I.D. 0.25mm×15m]
- **Solution Injection Method**: Split method
- **Sampling time**: 1.5min
- **Injector temperature**: 300°C
- **Oven temperature**: 150°C (1.5min)→(15°C /min)→250°C →(5°C /min)→320°C (3min)
- **Carrier gas**: Helium
3. The Mechanism of ORYZA TRITERPENOID on Fat Absorption Inhibition

Like other plant sterols, ORYZA TRITERPENOID lower elevated blood cholesterol level as illustrated in Fig. 3. Bile acids secreted by gall bladder is responsible for the metabolism of cholesterol in the human body. Cholesterol is normally dissolved by bile and transported to the blood. Sterol with similar chemical structure competes with cholesterol for bile absorption. Hence, absorption of cholesterol is inhibited resulting in lowering of blood cholesterol level.

In addition, ORYZA TRITERPENOID is inhibitory against pancreatic lipase in the metabolism pathway. Pancreatic lipase is responsible for the emulsification of lipids prior to intestinal absorption. Inhibition of pancreatic lipase will thus inhibit fat absorption and lower or reduce elevated blood triglyceride levels.

Fig. 3. Mechanism of Action of ORYZA TRITERPENOID on fat absorption
4. ORYZA TRITERPENOID – Physiological Effects

Plant sterols exist in the form of lipid. Studies shown that plant sterols are indeed beneficial to health, e.g. lowering of elevated blood cholesterol level, promote healthy urinary function and preventive against inflammation and risk of cancers.

(1) Pancreatic Lipase Inhibitory Activities

The effect of ORYZA TRITERPENOID on pancreatic lipase was examined in vitro. As shown in Fig. 4, ORYZA TRITERPENOID demonstrated a dose dependent inhibitory effect against pancreatic lipase.

![Inhibitory effects of ORYZA TRITERPENOID on pancreatic lipase](image)

Fig. 4. Inhibitory effects of ORYZA TRITERPENOID on pancreatic lipase

[Method]

Porcine pancreatic lipase (Sigma) was used. Inhibitory effect was measured by Lipase Kit-S (Dainippon Pharmaceutical).

(2) Inhibition of Micelle Formation (Simulation in vivo condition)

Orally administered lipids / fats are emulsified prior intestinal absorption. Lipids usually form micelles with bile acids and phosphatides for emulsification. Inhibition of micelle formation will inevitably prevent the absorption of fat. In vivo condition was simulated in test tubes to examine the effects of ORYZA TRITERPENOID on micelle formation. As shown in Fig.5, ORYZA TRITERPENOID demonstrated a dose-dependent inhibition against micelle formation where emulsification is inhibited. Hence, ORYZA TRITERPENOID prevents fat absorption.
Enhancement of Fibroblast growth

The effect of ORYZA TRITERPENOID on fibroblast growth was evaluated in vitro. As illustrated in Fig.6, ORYZA TRITERPENOID demonstrated concentration-dependent acceleration of collagen synthesis in vitro.

[Method]

Fibroblasts were seeded in 96-welled micro plate containing Dulbecco’s modified MEM (DMEM) with 5% fetal bovine serum (FBS) for breeding. Medium was replaced 24 hours later in DMEM containing 5% FBS and different concentration of ORYZA TRITERPENOID. Ascorbate Magnesium Phosphate (VC-PMG, Nikko Chemicals) was used as positive control. Fibroblasts were further cultured for 48 hours followed by ELISA test. Cells were dissolved in 0.1% Triton X-100 solution, quantity of protein was
determined as cellular toxicity index. Culture media and collagen were coated at 4°C for one day on ELISA plate. Culture was then treated with 1% bovine serum albumin (BSA) and blocked at 37°C for 1 hour. Anti-Human Collagen Type I antibody (Rabbit) was diluted with 0.3% BSA solution for primary antibody response. Reaction was conducted at 37°C for 1 hour. Meanwhile, Histofine PO (Rabbit) was diluted with 0.3% BSA solution for secondary antibody response. Similarly, reaction was conducted for 1 hour.

Phosphoric acid-citric acid buffer 0.3mg/ml (0.1M, pH 4.0) was added to solution of 2,2 Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) for 20 minutes reaction prior to absorbance measurement at wavelength 405nm using micro plate reader. 

Reference: COSMOS TECHNICAL CENTRE CO., LTD.

5. ORYZA TRITERPENOID – Typical Composition

Composition of the active components of ORYZA TRITERPENOID as tabulated below:

<table>
<thead>
<tr>
<th>Description</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campesterol</td>
<td>14</td>
</tr>
<tr>
<td>Cycloartenol</td>
<td>39</td>
</tr>
<tr>
<td>24-methylenecycloartanol</td>
<td>42</td>
</tr>
<tr>
<td>Cyclobranol</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

6. ORYZA TRITERPENOID – Thermal Stability

As shown below, ORYZA TRITERPENOID is highly stable at 100°C up to 60 minutes.
7. ORYZA TRITERPENOID – Nutritional Information

<table>
<thead>
<tr>
<th>Description</th>
<th>Results</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>3.0 g/100g</td>
<td>Heat drying method under ordinal pressure</td>
</tr>
<tr>
<td>Protein*1</td>
<td>0.0g/100g</td>
<td>Kieldahl method</td>
</tr>
<tr>
<td>Fat</td>
<td>94.9 g/100g</td>
<td>Acid fat dissolution method</td>
</tr>
<tr>
<td>Ash</td>
<td>1.3 g/100g</td>
<td>Direct ashing method</td>
</tr>
<tr>
<td>Carbohydrate*2</td>
<td>0.8g/100g</td>
<td></td>
</tr>
<tr>
<td>Energy*3</td>
<td>857 kcal/100g</td>
<td></td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td>0.0 g/100g+</td>
<td>Prosky method</td>
</tr>
<tr>
<td>Sodium</td>
<td>270 mg/100g</td>
<td>Atomic absorption spectrophotometory</td>
</tr>
</tbody>
</table>

*1) N=6.25  
*2) 100 – (moisture + protein + fat + ash)  
*3) Factors for calculating the energy value: protein, 4; fat, 9; carbohydrate, 4; dietary fiber, 2  

Test trustee: SRL, Inc.  
Date of issue of the test result report: April 13, 2005  
Research result issue number: No. 20050331022

8. ORYZA TRITERPENOID – Safety

(1) Residual Agricultural Chemicals

<table>
<thead>
<tr>
<th>Description</th>
<th>Result</th>
<th>Detection Limit</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHC</td>
<td>Not Detected</td>
<td>1ppm</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>DDT</td>
<td>Not Detected</td>
<td>1ppm</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>Aldrin</td>
<td>Not Detected</td>
<td>1ppm</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>Not Detected</td>
<td>1ppm</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>Endrin</td>
<td>Not Detected</td>
<td>1ppm</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>Parathion</td>
<td>Not Detected</td>
<td>1ppm</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>Phenitorothion</td>
<td>Not Detected</td>
<td>1ppm</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>Malathon</td>
<td>Not Detected</td>
<td>1ppm</td>
<td>Gas Chromatography</td>
</tr>
</tbody>
</table>

Test trustee: Kyusai analysis institute Co., LTD.  
Date of issue of the test result report: April 14, 2005  
Research result issue number: No. 20050329-2
(2) Ames Test

Salmonella typhimurium and Escherichia coli were treated with suspension containing ORYZA TRITERPENOID using Ames plate incorporation method at five dose levels, in triplicate, both and without the addition of a rat liver homogenate metabolising system. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material (ORYZA TRITERPENOID), either with or without metabolic activation. ORYZA TRITERPENOID was considered to be non-mutagenic under the conditions of this test.

Test trustee: SafePharm Laboratories
Date of issue of the test result report: March 21, 2005
Research result issue number: 1600/006

9. ORYZA TRITERPENOID – Commercial Applications

<table>
<thead>
<tr>
<th>Applications</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cosmetics</td>
<td>Base cosmetics (lotion, milk, cream, etc.)</td>
</tr>
<tr>
<td></td>
<td>Body cosmetics (body lotion, body cream, etc.)</td>
</tr>
<tr>
<td></td>
<td>Cleansing cosmetics (soap, etc.)</td>
</tr>
<tr>
<td></td>
<td>Makeup cosmetics (lipstick, foundation, etc.)</td>
</tr>
</tbody>
</table>

10. Packaging

ORYZA TRITERPENOID -C [Cosmetics grade, powder]
5kg  Interior packaging: aluminum-coated plastic bag
     Exterior packaging: 18L tin and cardboard box

11. Storage

Store in cool, dry dark place. Avoid humidity.
**PRODUCT STANDARD**

**PRODUCT NAME**

**ORYZA TRITERPENOID-C**
(COSMETIC)

This product is hydrolysed and refined from rice bran and rice germ of *Oryza sativa* Linne (*Gramineae*). It guarantees a minimum 90% total triterpenoids.

**Appearance**

White coloured powder. Neutral smell.

**Certification Method**

Dissolve 100 mg of sample in 25 ml of chloroform in a volumetric flask. Prepare the standard solution by dissolving 0.05 g of standard stigmasterol in 25 ml of chloroform to achieve concentration of 2 μg/ml. Proceed for GC analysis. GC analysis is performed according to the following conditions for 2 μl of test solution and standard solution. The peak of triterpenoid is found in the GC chromatogram of test solution.

<GC condition>

<table>
<thead>
<tr>
<th>Column</th>
<th>SE30 60~80 mesh (4 mm φ × 2 m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column temperature</td>
<td>250°C</td>
</tr>
<tr>
<td>Detector temperature</td>
<td>300°C (FID)</td>
</tr>
<tr>
<td>Injector temperature</td>
<td>300°C</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>He</td>
</tr>
<tr>
<td>Flow rate</td>
<td>Adjust to about 17 min of retention time of stigmasterol in carrier gas flow rate</td>
</tr>
</tbody>
</table>

**Triterpenoid**

Min. 90.0 % (GC)

**Melting point**

90~115°C

**Loss on Drying**

Max. 1.0 % (Analysis for Hygienic Chemists, 1g, 105°C, 1h)

**Residue on Ignition**

Max. 0.5 % (The Second Method of The Japanese Standards of Quasi-Drug Ingredients, 1g)
Purity Test

(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)

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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice Triterpenoid</td>
<td>100 %</td>
</tr>
</tbody>
</table>
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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