



ORYZA OIL & FAT CHEMICAL CO., LTD.

ORYZA CERAMIDETM

Cosmeceutical Food Supplement

- ORYZA CERAMIDETM-PT, PCD
(Powder, Food Grade)
- ORYZA CERAMIDETM-WSP, WSP8
(Water Soluble Powder, Food Grade)
- ORYZA CERAMIDETM-L, L0.8
(Liquid, Food Grade)
- ORYZA CERAMIDETM-PC, PC8
(Powder, Cosmetic Grade)
- ORYZA CERAMIDETM- WSPC, WSPC8
(Water Soluble Powder, Cosmetic Grade)
- ORYZA CERAMIDETM- LC, LC0.8
(Liquid, Cosmetic Grade)



ORYZA OIL & FAT CHEMICAL CO., LTD.
Ver.10.0 YF

ORYZA CERAMIDE™

Cosmeceutical Food Supplement

Rice (*Oryza sativa*) have been widely grown in the Southeast Asia, not only as a chief crop but also as an integral part of traditional culture and lifestyle in some Asian countries.

In recent years, much attention have been focused on rice bran and rice germ, due to its unique bioactive compounds.

In the course of our investigation on rice bran and rice germ for a long time, some products were developed by utilizing its functional compounds, and have been used as medicines, cosmetics, health foods, and food additives.

Recently, glycosphingolipids were extracted from rice bran and rice germ for application in nutritional and cosmeceutical supplement.

1. ORYZA CERAMIDE™

ORYZA CERAMIDE™, brownish in colour, extracted and refined from rice bran or rice germ. It contains a large amount of glycosphingolipid.

The glycosphingolipid of rice bran is similar to the animal glycosphingolipid, in which the backbone is ceramide including sphingoid bases with fatty acid in an amide linkage, and the terminal hydroxyl group is substituted by glucose. There are different species of glycosphingolipids due to its chemical structure of sphingoid bases and different fatty acid components.

Fujino et al. reported more than twenty species of sphingolipids identified in rice bran. ORYZA CERAMIDE™ was found to contain four major constituents. The structures of these constituents were established by analysis of various chromatograph and NMR spectra, as shown in Fig. 1.

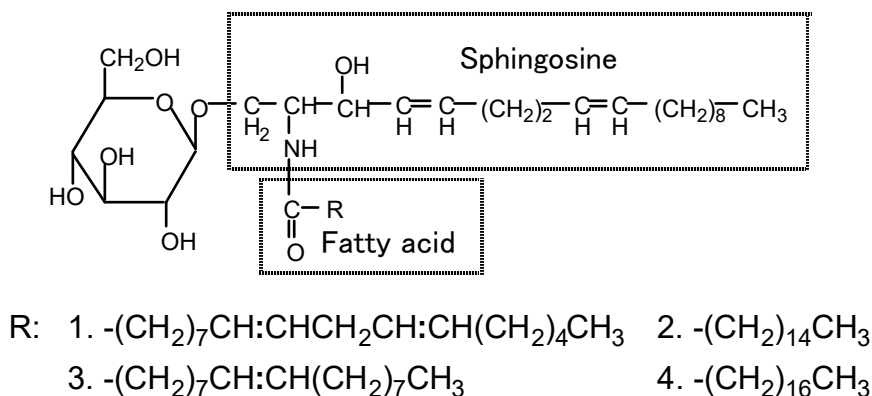


Fig. 1 Structures of ORYZA CERAMIDE™

In a joint research with Professor Igarashi from Hokkaido University Graduate School of Pharmaceutical Sciences, we analyzed the chemical structure of rice-derived glycosphingolipid with high content (No. 7 in Fig. 2) and determined the structure of the main ceramide as shown in Fig. 3.

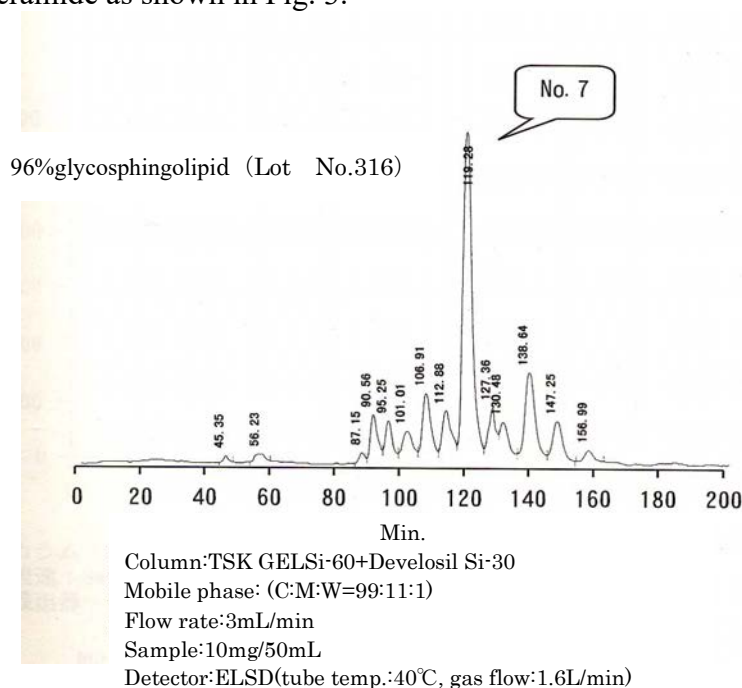


Fig. 2 HPLC chart of rice-derived glycosphingolipid

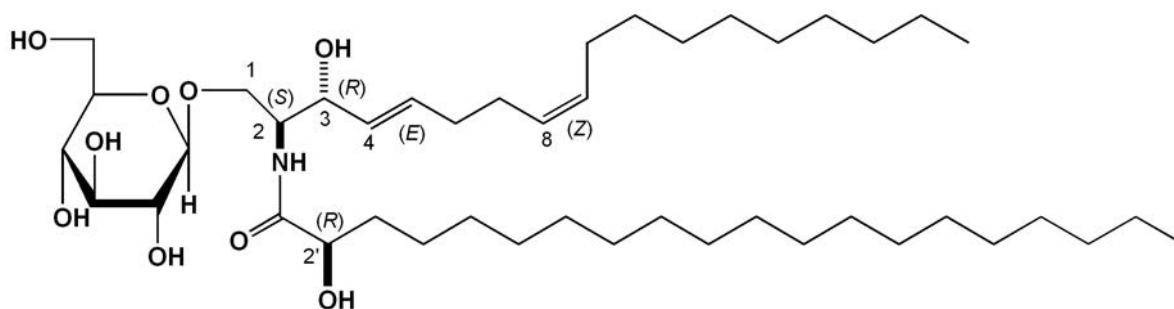


Fig. 3 Structures of rice-derived glycosphingolipid

2. Biological Function of Ceramide in Human

In 1884 the ceramides were found in human brain tissues by Dr. Thudichum. Since then, the presence of several ceramides in skin and biological membrane was observed. Their structures and biological activities have been well elucidated.

The human skin consists of epidermis, corium and tela subcutanea. The epidermis was classified into four layers, namely stratum corneum, stratum granulosum, stratum spinosum and stratum basale as shown in Fig. 4.

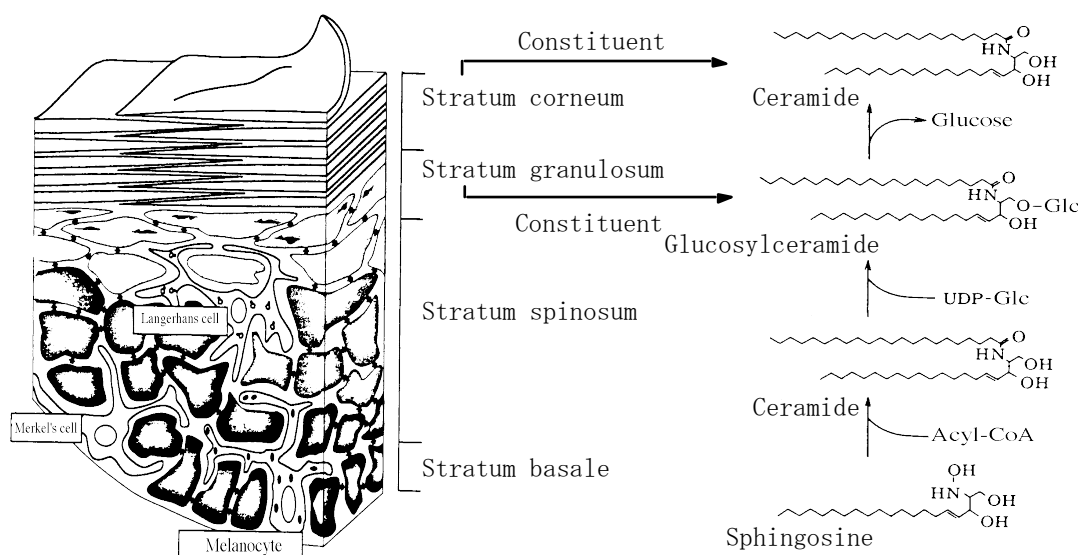


Fig. 4 Structure of Epidermis and Biosynthesis of Ceramides

Nine different species of ceramide were found in skin (as shown in Fig. 5). These ceramides were formed via several biosynthetic processes in epidermis, and were accumulated at stratum corneum as major constituent, about 40~60% of stratum corneum lipids (Fig. 6). In epidermis, these ceramides play important roles for forming lamella phases and maintaining barrier functions.

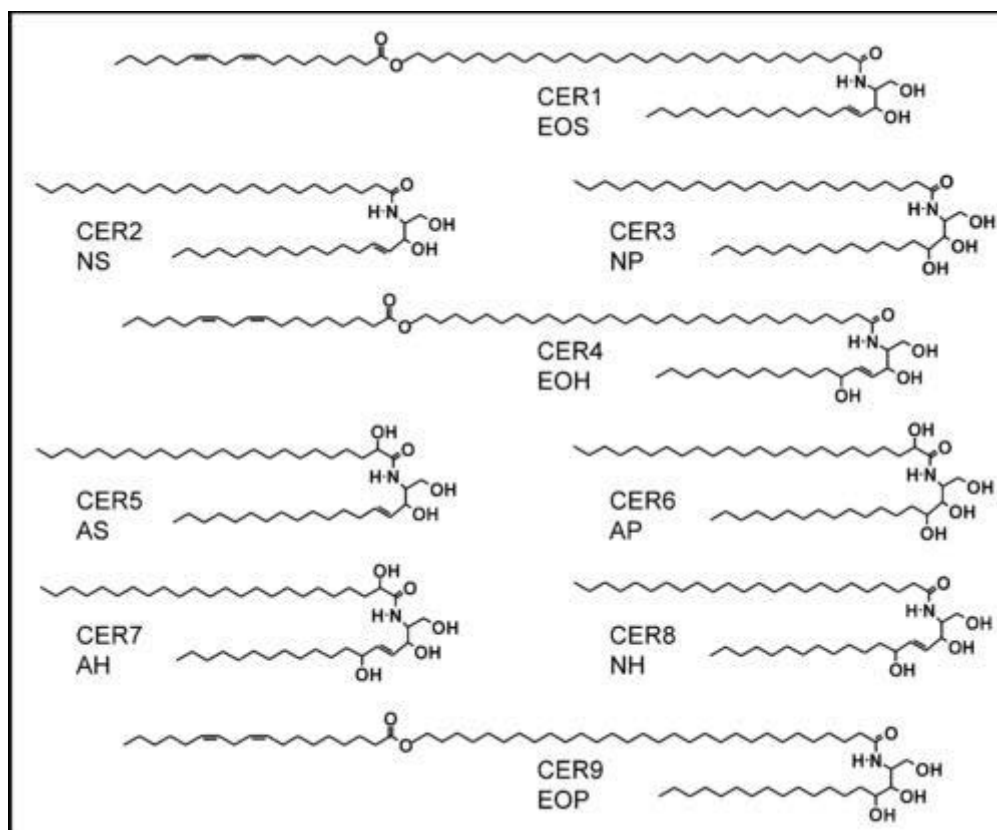


Fig. 5 Species and Structures of Ceramides

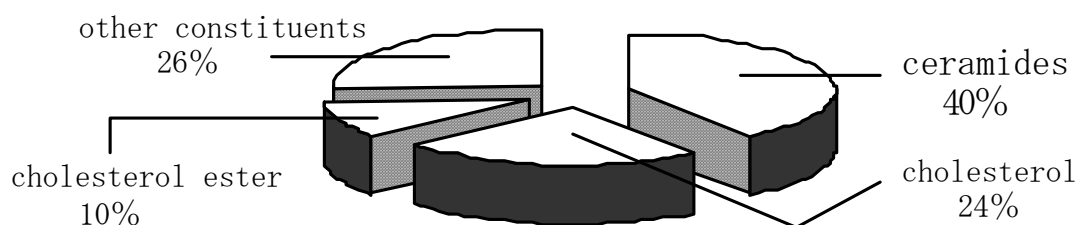
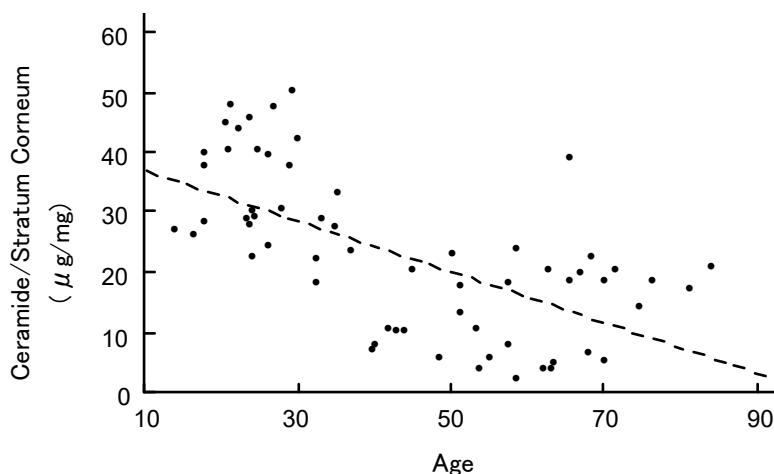


Fig. 6 Composition of Stratum Corneum Lipids

Quantity of ceramides was lower in the stratum corneum of atopic dermatitis, dry skin and aged individual. Study conducted by Imokawa *et. al* demonstrated that the ceramide content declines with increasing age (Fig. 7). As the forearm skin of aged persons (especially those over 70 years old) are usually xerotic, hence that the decrease in ceramide content is associated with dry appearance in xerotic skin. In addition, comparison of total ceramide content of forearm stratum corneum between atopic dermatitis and healthy subjects (Fig. 8) shows that in atopic dermatitis, there is a marked reduction in the amount of total ceramide in both lesional and non-lesional

forearm skin as compared with that of healthy individuals of the same age. This result suggested that ceramide is a key factor for moisture maintenance and barrier function of stratum corneum. Fine lines and wrinkles appear when ceramide content is reduced.



Thus of ceramides is necessary for maintaining healthy youthful looking skin.

Fig. 7 Total Ceramide Content of Stratum Corneum in Healthy Subjects

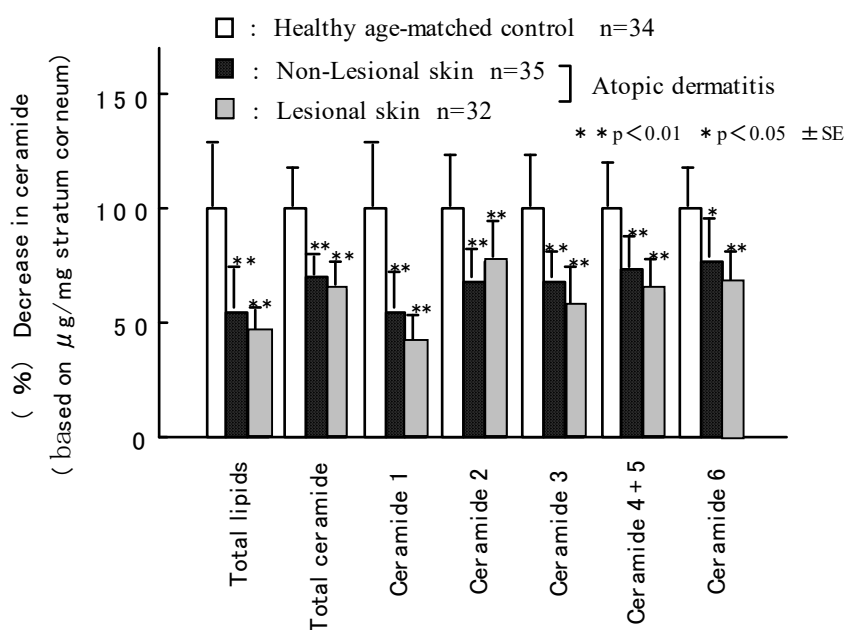


Fig. 8 Comparison of Ceramide Content in Forearm Skin Between Atopic Dermatitis and Healthy Subjects

3. Digestion, Absorption, and Metabolism of Sphingolipids

In order to study digestion, absorption, and metabolism of food-derived sphingolipids, Schmelz and his research group examined how it is metabolized and distributed in the intestinal canal by giving labeled sphingomyelin to model mice in 1994. Sphingomyelin appeared in all parts of the intestinal canal and most of it was broken down to ceramide and its metabolite. Only 1% of sphingomyelin moved from the intestinal canal to the liver in 30 to 60 minutes after administration. This indicates that transport of sphingomyelin and its metabolite from the intestinal canal to other tissues of the body is not very efficient and that absorption and metabolism of sphingolipids vary according to the types. It also indicates that sphingomyelin is hydrolyzed and absorbed in the intestinal canal as a synthetic raw material of bio-complex sphingolipids.

Nyberg and his research group examined the site of digestion and digestive capacity of sphingomyelin in 1997. The group reported that sphingomyelin is digested by sphingomyelinase mainly in the middle and lower areas of the small intestine and that the enzyme plays an important role in the first stage of digestion of sphingomyelin.

4. The Physiological Function and Application of the Plant Glucosylceramide

The synthetic and animal ceramide have been mainly used as materials of cosmetic. Recently, it is discovered that Creutzfeldt-Jakob disease may be contracted from eating or using contaminated animal products, especially that of cattle. Therefore, much attention has been given to the plant glucosylceramide, and some products have been incorporated into cosmetics and food preparations.

ORYZA CERAMIDE™ derived from rice bran suitable to be used as functional food supplements. The whitening and moisturizing effect of this product were determined as follows.

4-1 Moisturizing and Supplementation of Epidermal Ceramide

4-1-1 *In vivo* Examination

We gave rice-derived glucosylceramide (GCFr, purity >99%) or Oryza Ceramide-PT (FGC) to hairless mice for 9 days (Fig. 9A) and measured trans-epidermal water loss

(TEWL). As a result, FGC significantly improved TEWL. Then 10% SDS was treated on the right side of back skin once a day for 3 days and TEWL was measured again (day 12). TEWL of normal and SDS-treated areas was improved (Fig. 9B). Fig. 9C illustrates the difference of TEWL between normal area and SDS-treated area. Both GCFr and FGC significantly improve TEWL.

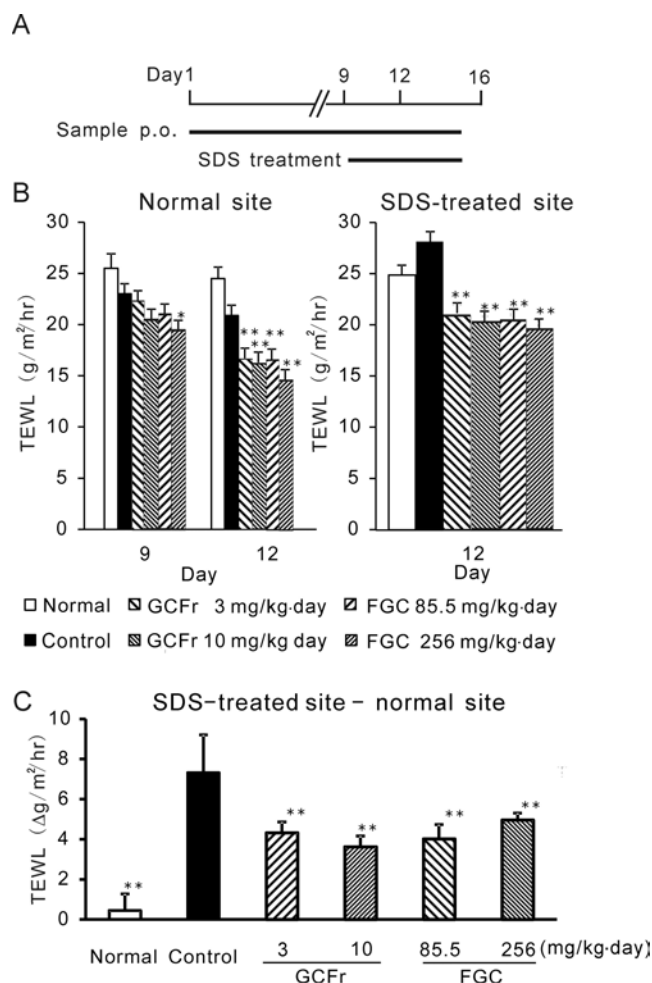


Fig. 9. Improvement of TEWL on mouse skin treated with GCFr and FGC

A) Protocol of treatment with sample and SDS. B) TEWL on normal and SDS-treated skins. C) Difference of TEWL between normal site and SDS-treated site on day 12. GCFr and FGC were given orally to mice for 9 days followed by TEWL measurement on dorsal skin. After the measurement, right half site of dorsal skin was treated with 10% SDS. The treatment was continued from day 9 to 15 and GCFr and FGC were given once a day. TEWL was measured on day 9 and 12. Each column represents mean with the S.E. of 5-6 mice. Significance of differences was examined by Williams method. Asterisks denote significant difference from control at *: $p < 0.05$, **: $p < 0.025$.

On the day 16, SDS-treated skin was removed and lipids were extracted. Lipids were developed by TLC (Fig. 10). As a result, Spots of Ceramide (Cer) a1 and 2, GlcCer EOS and A/B were detected. GCFr increase Cer 1 and centrally GlcCer EOS and A/B were decreased (Table 1). The result shows that conversion of epidermal Cer to GlcCer was enhanced by GCFr to supply Cer damaged by SDS (refer following scheme).

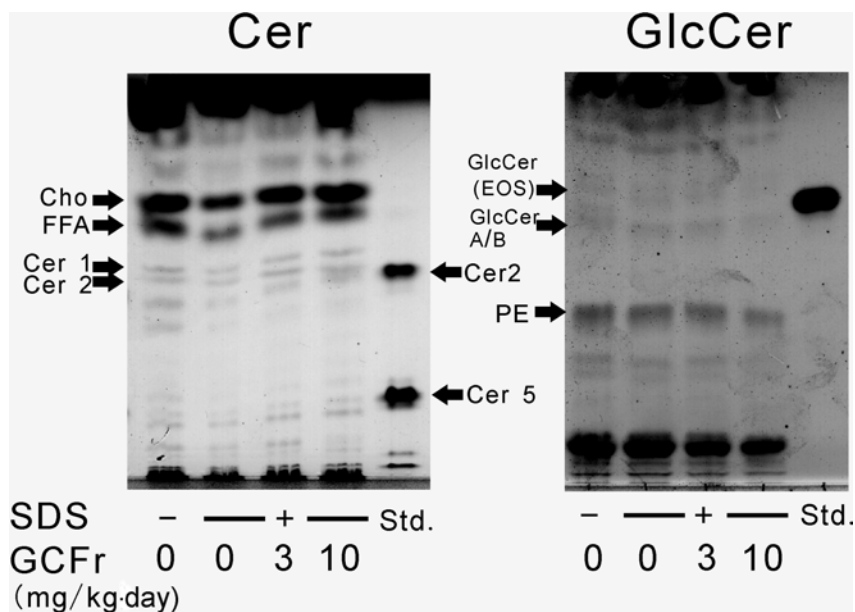
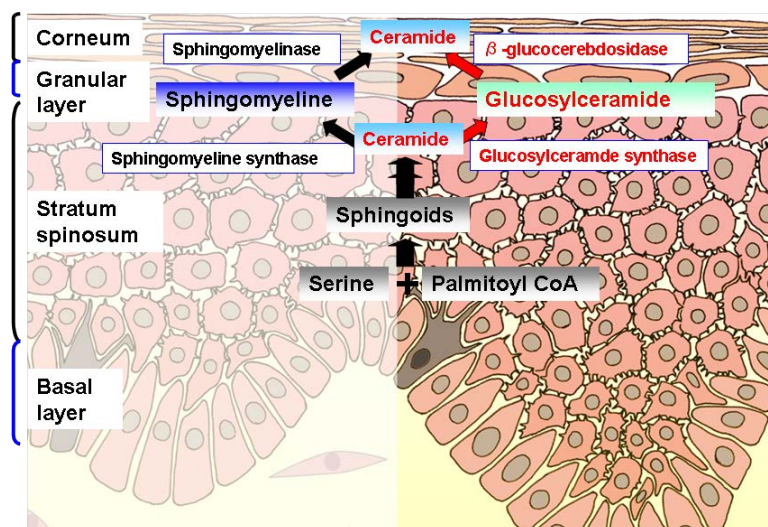


Fig. 10. TLC chromatogram of Cer and GlcCer in mouse skin.

Cho: cholesterol, FFA: free fatty acid, PE: phosphatidylethanolamine. For measurement of Cer and GlcCer, the lipid sample was developed on an HPTLC plate using a mixture of chloroform, methanol and acetic acid (190:9:1) and a mixture of chloroform, methanol and acetic acid (40:12:1), respectively. The spots for Cer were visualized using 10% copper sulfate solution containing 8% phosphoric acid. The spots of GlcCer were visualized using 0.1% orcinol solution containing 10% H₂SO₄.



Imokawa G, *J. Dermatol. Sci.* 55, 1, (2009).

Table 1. Effect of GCFr on the skin lipids in mouse treated with SDS

	Area of spot			
	Cho	FFA	Cer 1	Cer 2
Normal	0.711±0.094	0.469±0.097	0.139±0.028	0.112±0.024
Control	0.669±0.057	0.390±0.054	0.158±0.028	0.126±0.022
GCFr (3 mg/kg·day)	0.732±0.026	0.623±0.096	0.184±0.026	0.137±0.021
GCFr (10 mg/kg·day)	0.840±0.119	0.724±0.088*	0.211±0.036*	0.135±0.020

	Area of spot		
	GlcCer (EOS)	GlcCer A/B	PE
Normal	0.035±0.005	0.044±0.007	0.071±0.020
Control	0.034±0.007	0.042±0.005	0.064±0.008
GCFr (3 mg/kg·day)	0.032±0.009	0.033±0.008	0.103±0.018
GCFr (10 mg/kg·day)	0.016±0.004*	0.017±0.006*	0.069±0.011

Cho: cholesterol, FFA: free fatty acid, PE: phosphatidylethanolamine. The areas of spots were corrected by the area of standard Cer and GlcCer. Each value represents mean with the S.E. of 5-6 mice. Significance of differences was examined by one-way analysis of variance followed by Dunnet's test. An asterisk denotes significant difference from control at *: $p < 0.05$.

Fig. 11 illustrates immunostaining images of Cer and GlcCer in mouse skin. GCFr increase epidermal Cer and decrease GlcCer.

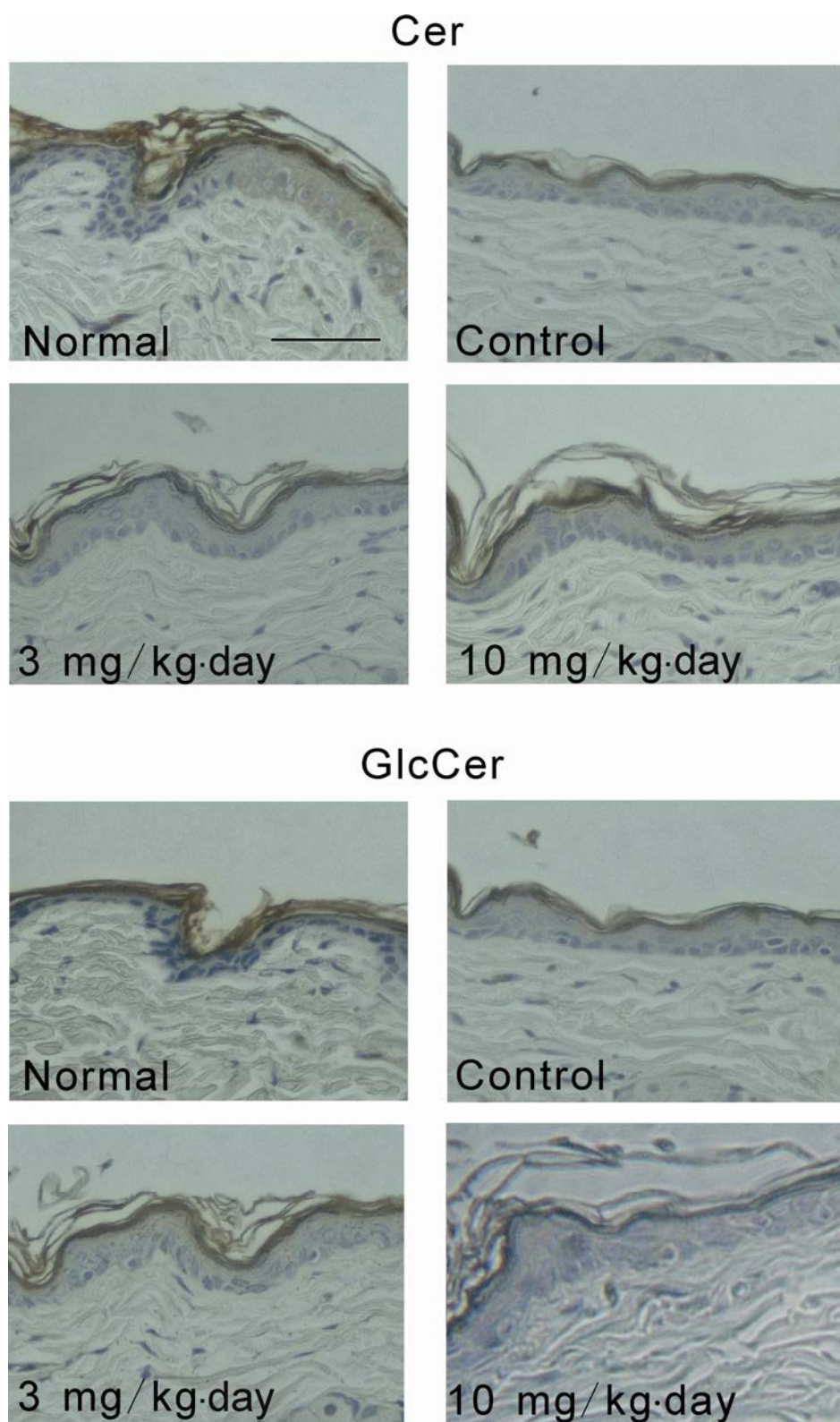


Fig. 11. Immunostaining images of Cer and GlcCer in SDS-treated mice given GCFr. Normal: untreated mouse, Control: SDS treated mouse. GCFr was given orally at 3 and 10 mg/kg·day. The scale bar indicates 50 μ m.

On the other hand, as a result of electron microscopic observation, keratohyalin granules (KG) in s. granulosum were glowed and enlarged (Fig. 12). KG is storage of profilaggrine which is precursor of filaggrine. As filaggrine binds to keratin and strength keratin structure in corneum, GCFr may provide burrier function to corneum.

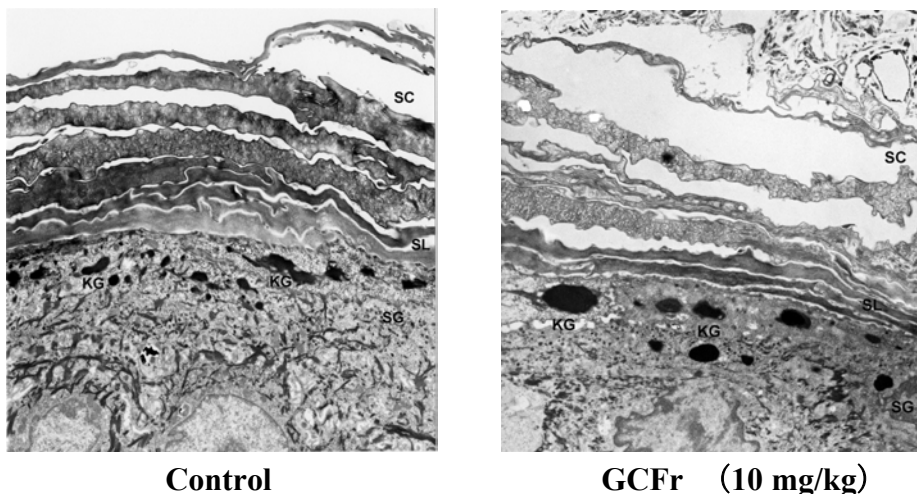


Fig. 12. Cross section images (x 3,000)

SC: corneum, SL: stratum lucidum, SG: stratum granulosum, KG: keratohyalin granule

Fig. 13 illustrates change in lamella structures (lipid bilayers) in corneum. Lamella structure is existing style of ceramide. As lamella structure is cleared by GCFr, some changes including ceramide contents and the existing style were suggested to be occurred.

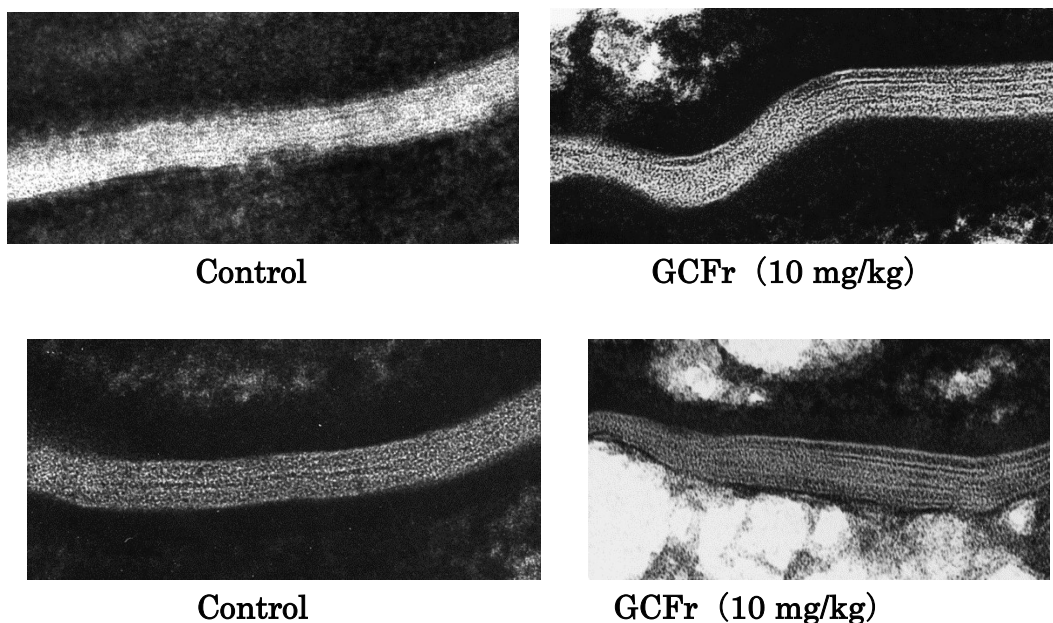


Fig. 13. Lamella structures in corneum (x50,000)

Fig. 14 shows lamella granules existing in s. granulosum. Lamella granule also forms

lipid bilayer same with lamella structure and is storage of GlcCer. As GCFr changes the structure to be clear, supplementation GCFr was suggested to change existing style of GlcCer.

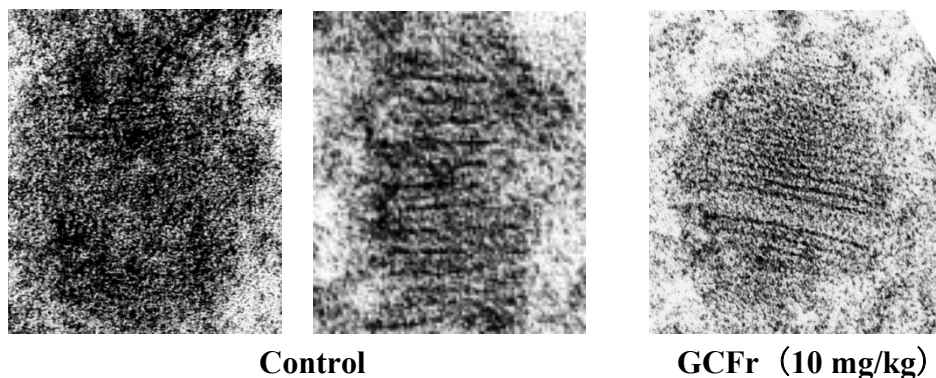


Fig. 14. Lamella granules in stratum granulosum (x300,000)

Fig. 15 is keratin pattern (cytosolic fibrous structure) of a keratinocyte. The cell membrane is cornified envelope (hatched line) and forms strong structure. By the treatment of GCFr, the electron density of conified envelope was increased (highly stained).

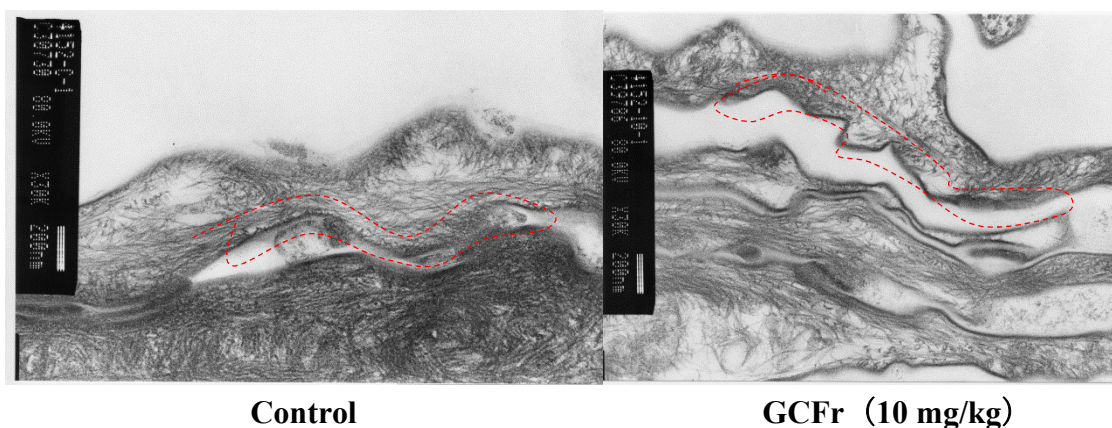


Fig. 15. Keratin pattern and cornified envelope in corneum (x 3,000)
Cornified envelope is consisted of involucrin and loricrin.

Fig. 16 is Western blotting images of enzymes related to synthesis and conversion of GlcCer to epidermal Cer which are called glucocylceramide synthase (GCSase) and β -glucocerebdsosidase (GCCase). As a result, GCFr enhanced expression of GCCase and GCSase. Epidermal GlCCer synthesis and conversion to Cer were suggested to be

enhanced by GCFr. The result reflected the changes of epidermal GlcCer and Cer shown in Table 1 and Fig. 11.

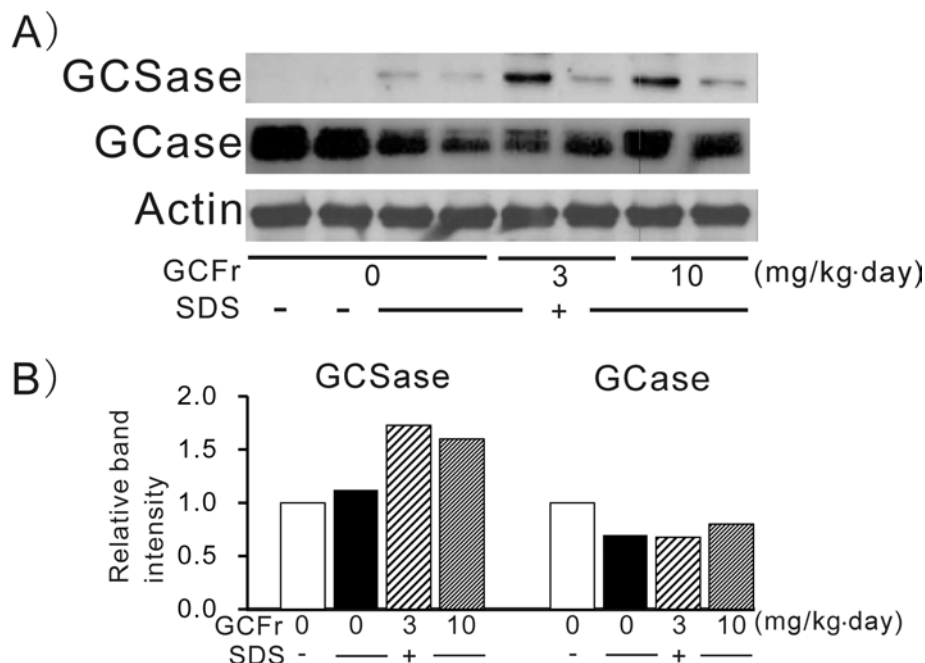


Fig. 16. Effect of GCFr on the expression of GlcCer-metabolizing enzymes in mice. A) The electrophoresis of the tissue protein was performed on 10% SDS-PAGE. B) The intensity of the each band was corrected by that of actin and the mean values (n=2) were indicated as relative values against non-GCFr and SDS-treated group.

These results suggest that oral supplementation of rice-derived glucosylceramide supply humidity and burrier function to corneum by enhancement of epidermal ceramide contents.

4-1-2 *In vitro* Examination

We added major GlcCer in oryza ceramide [GlcCer (d18:2)] into a culture system of human epidermis and cultured for 3 days. As evaluation of Cer and GlcCer contents in the system (Fig. 17), Cer 1, Cer 2, GlcCer EOS and GlcCer A/B were increased by GlcCer (d18:2). (Table 2)

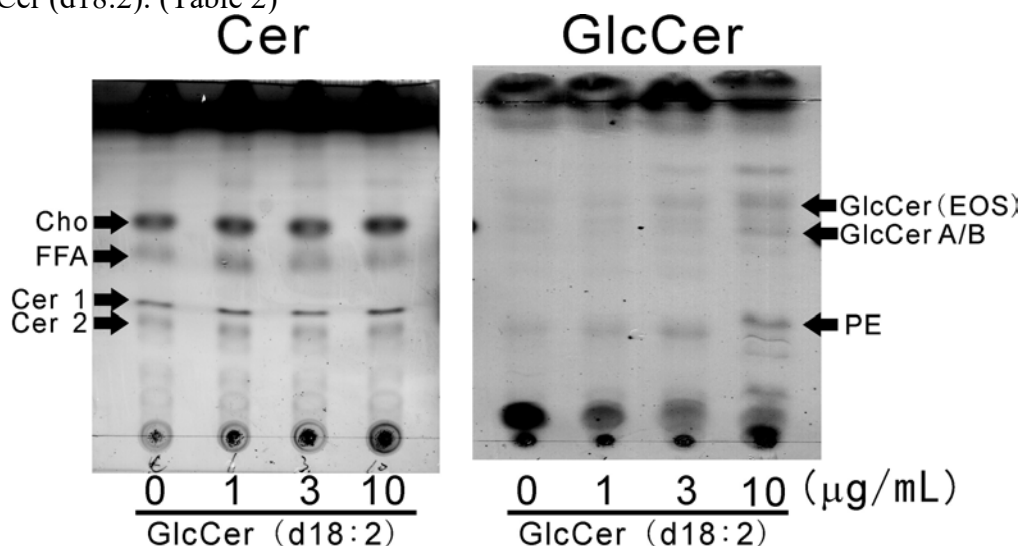


Fig. 17. TLC chromatogram of Cer and GlcCer in epidermal equivalent treated with GlcCer (d18:2).

Cho: cholesterol, FFA: free fatty acid, PE: phosphatidylethanolamine. HPTLC was performed with the same conditions as denoted for Fig. 10.

Table 2. Effect of GlcCer (d18:2) on the lipids in epidermal equivalent.

	Conc. (µg/mL)	Area of spot			
		Cho	FFA	Cer 1	Cer 2
Control	-	0.238±0.036	0.053±0.006	0.048±0.021	0.109±0.011
GlcCer (d18:2)	1	0.247±0.016	0.066±0.010	0.091±0.003*	0.071±0.015
	3	0.263±0.025	0.108±0.018*	0.076±0.001	0.113±0.003
	10	0.308±0.016	0.108±0.018*	0.076±0.006	0.132±0.002*
		Area of spot			
		GlcCer (EOS)	GlcCer A/B	PE	
Control	-	0.034±0.004	0.020±0.004	0.052±0.017	
GlcCer (d18:2)	1	0.049±0.010	0.020±0.009	0.069±0.007	
	3	0.081±0.009*	0.067±0.003**	0.117±0.017*	
	10	0.084±0.005*	0.049±0.010*	0.104±0.010	

Cho: cholesterol, FFA: free fatty acid, PE: phosphatidylethanolamine. The areas of spots were corrected by the area of standard Cer and GlcCer. Each value represents mean with the S.E. of 3 experiments. Significance of differences was examined by one-way analysis of variance followed by Dunnet's test. Asterisks denote significant differences from control at *: $p < 0.05$, **: $p < 0.01$.

Immunostaining images show that GlcCer (d:18:2) enhances Cer and GlcCer contents in epidermis.

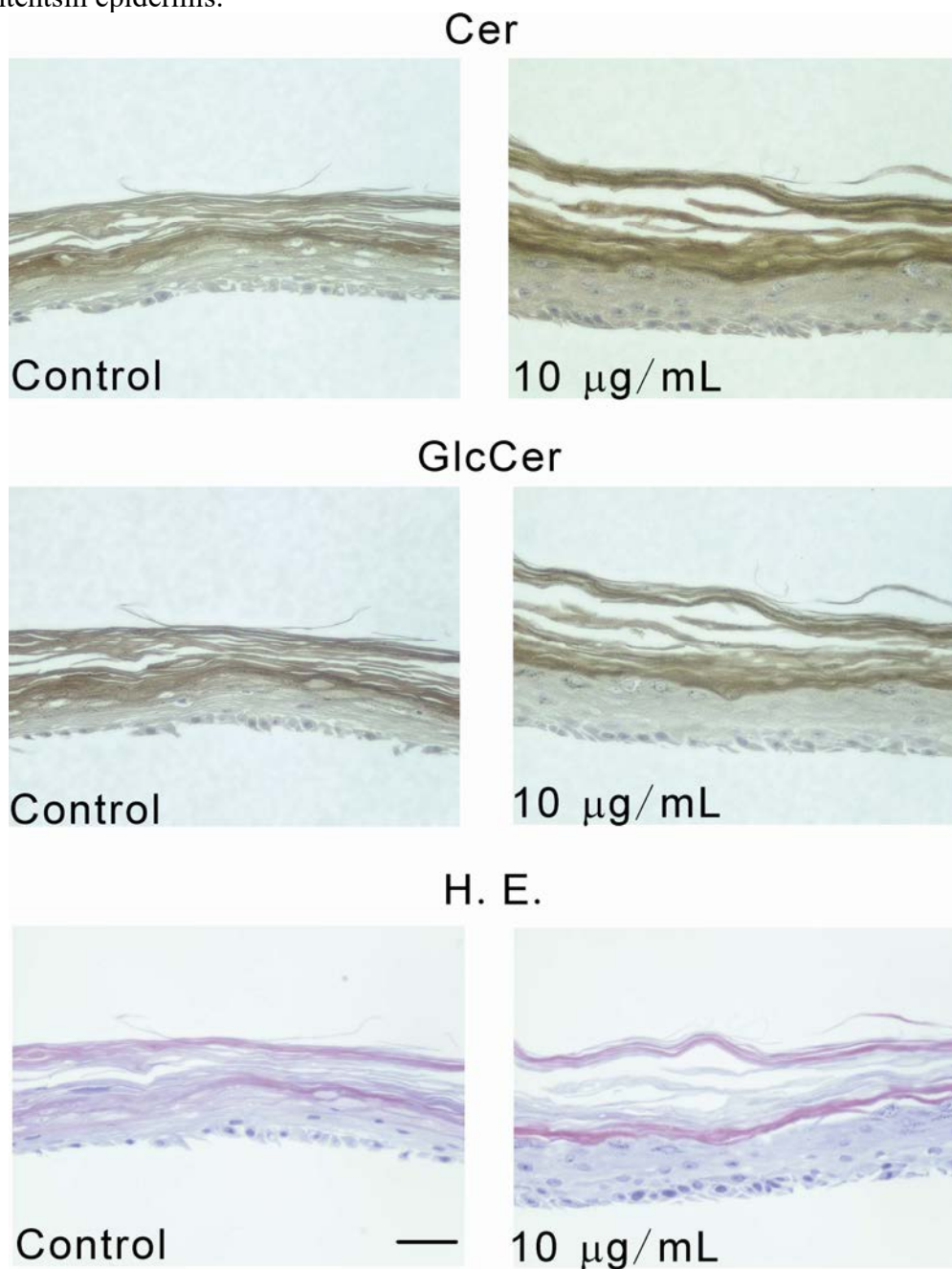


Fig. 18. Immunostaining images of Cer and GlcCer of epidermal equivalent treated with GlcCer (d:18:2).

H.E. hematoxylin-eosin staining. The scale bar indicates 50 µm.

Moreover, GlcCer (d:18:2) did not affect to the expression of Gcase, however it enhanced GCSase and epidermis maturing markers including involucrin and transglutaminase (Fig. 19).

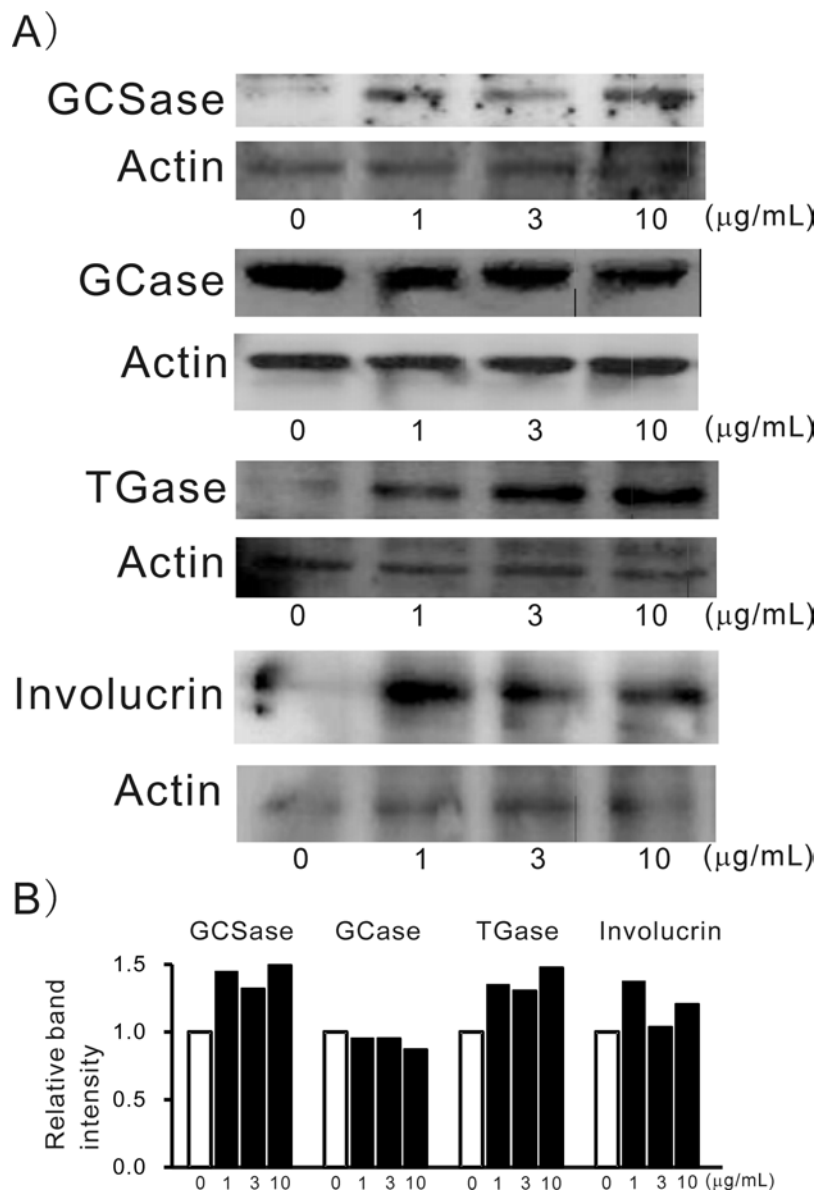


Fig. 19. Effect of GlcCer (d:18:2) on the expression of GlcCer-metabolizing enzymes and differentiation markers of epidermis in epidermal equivalent. The epidermal equivalent was treated with GlcCer (d18:2) for 3 days. A) The electrophoresis of the tissue protein was performed on 10% SDS-PAGE. B) The intensity of each band was corrected by that of actin and the values were indicated as relative values against non-GlcCer (d18:2)-treated group.

4-2. Whitening Effect (*in vitro*)

4-2-1. Suppression of Melanin Synthesis in Melanoma

ORYZA CERAMIDE™ is similar to other ceramides, possess various physiological functions. In this study, the effect of ORYZA CERAMIDE™ on melanogenesis was examined using cultured B16 melanoma cell *in vitro*. The result (as shown in Fig. 20), illustrated that ORYZA CERAMIDE™ is more potent than ascorbic acid, arbutin, and ellagic acid except for kojic acid. Thus it is expected that the whitening effect is achievable by daily consumption of ORYZA CERAMIDE™.

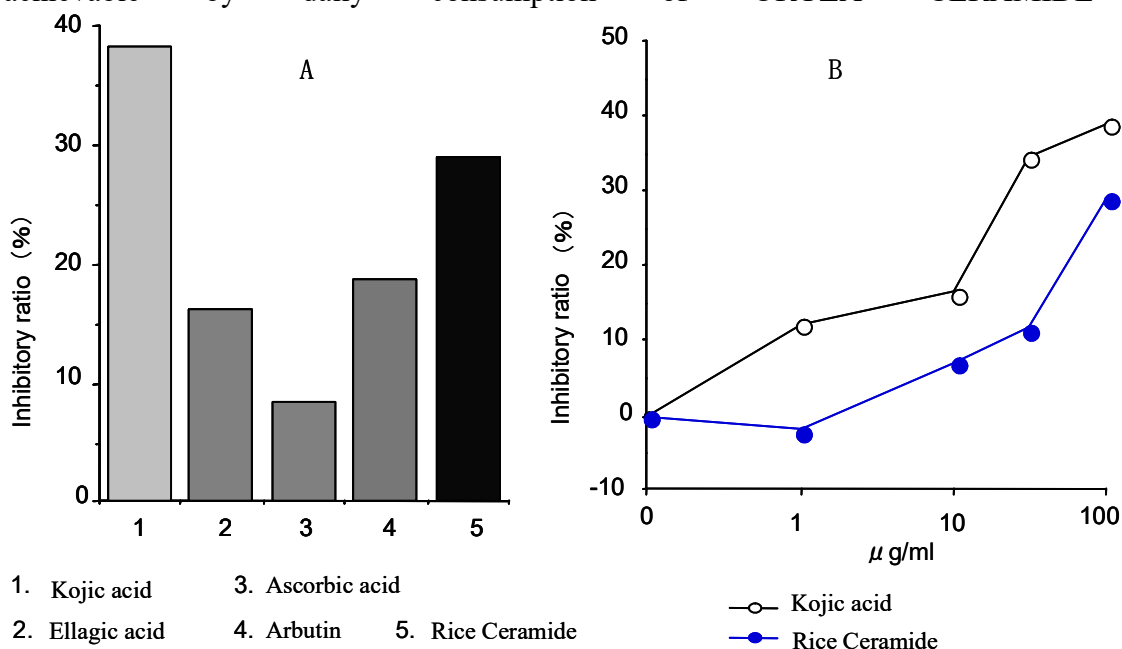


Fig. 20 Inhibitory Effect of ORYZA CERAMIDE™ on Melanin

[Method]

B16 melanoma cells (2×10^3 cells/mL) were placed in dishes (60 mm) and incubated for 24 hours in growth medium (D-MEM containing 10%FCS). The medium was replaced with sample medium [emulsified glycosphingolipids (> 90% of purity)]. After 2-day incubation, the sample-containing medium was replaced with fresh growth medium, followed by another 2-day incubation. The number of cells was counted, and then, cells were lysed with 2 N NaOH and absorbance was measured at 450 nm. The value was normalized by the cell number.

4-2-2. Suppression of Melanin Production in Melanocyte (melan-a)

Professor Igarashi and his assistant Mitsutake of Graduate School of Pharmaceutical Sciences Hokkaido University, examined the effects of rice-origin glycosphingolipid

and its acid-hydrolyzed product* on tyrosinase activity and melanin production using mouse melanocyte. Tyrosinase is an enzyme responsible for melanin formation. As shown in Fig. 21 and 22, both rice-origin glycosphingolipid and its hydrolyzed product showed inhibitory effects on tyrosinase activity and melanin production in a dose-dependent manner. Rice-origin glycosphingolipid is a promising material applicable to skin-lightening foods or cosmetics.

* Rice-origin glycosphingolipid was hydrolyzed in 1 N HCl in methanol. After the hydrolysis, methanol layer was recovered by liquid – liquid distribution, then concentrated and dried.

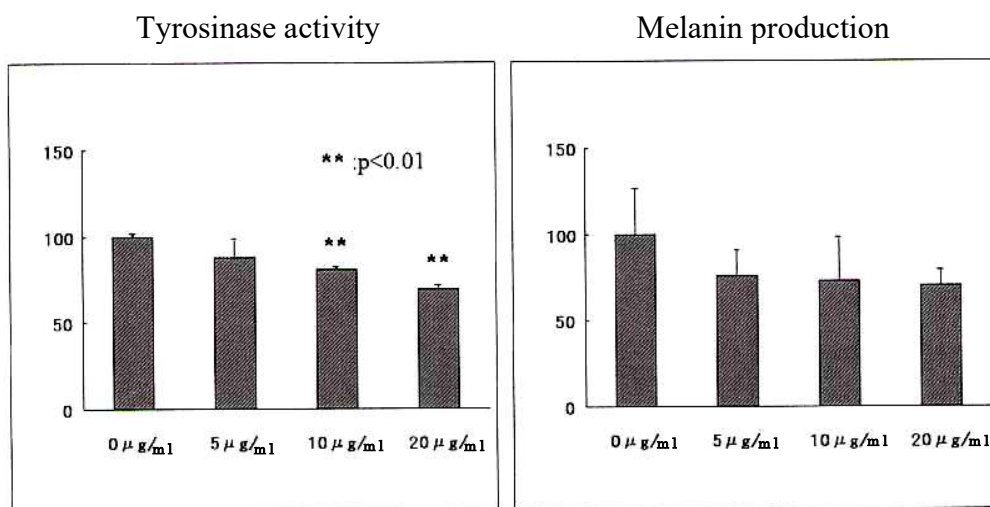


Fig. 21 Effects of rice-derived glycosphingolipid

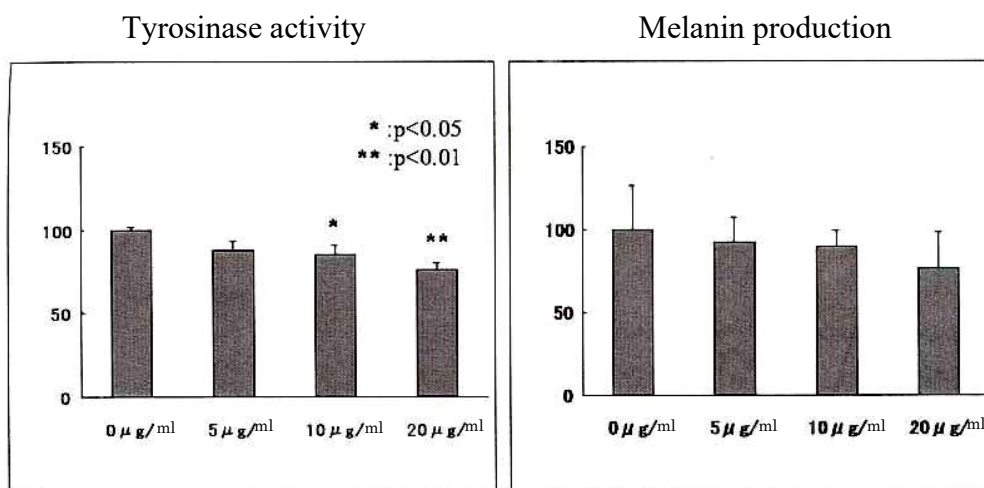


Fig. 22 Effects of rice-derived glycosphingolipid acid-degraded product

[Method]

1) Determination of tyrosinase activity

Mice melanocyte (melan-a cells, 1×10^4 cells/well) were placed in a 96-well plates and incubated for 24 hours in growth medium (RPMI 1640 containing 10% FCS and 200 nM TPA). The medium was replaced with sample-containing medium (glycosphingolipids > 95% of purity). Cells were lysed with PBS (90 μ L/well) containing 1% tritonX-100, then mixed for 1 minute. Ten μ L of substrate (10 mM L-DOPA) was added to each well, and incubated for 1 hour at 37 °C. Absorbance was measured at 475nm. Tyrosinase activity was normalized by the amount of total protein.

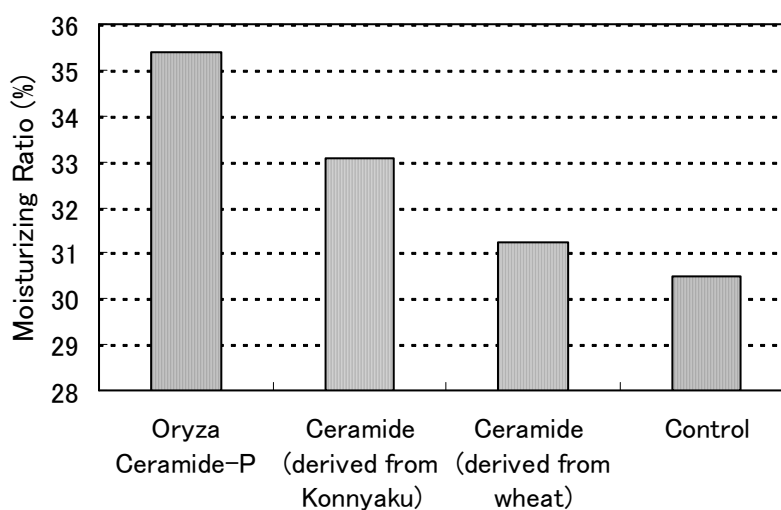
2) Determination of melanin production

Melan-a cells (3×10^5 cells/well) were placed in a 10-cm plates or 6-well plates. The culture condition and sample addition was the same as mentioned in 1). Cells were lysed in 1 N NaOH (500 μ L) for 30 minutes at 100 °C. Absorbance was measured at wavelength of 405 nm. Melanin production was normalized by the amount of total protein.

4-3 The Moisturizing Effects of Oryza Ceramide™ (*in vitro*)

The moisturizing effect of ceramide was established by several clinical studies. In these reports, ceramides were absorbed in the intestine, and circulated into the stratum corneum, and finally work for improving barrier and moistuizing function. Therefore, the moisturizing effect of ORYZA CERAMIDE™ was examined *in vitro*.

The moisturizing effects of ORYZA CERAMIDE™ was compared with other commercially available ceramides. Moisturising effect of various ceramides were compared in Fig. 23. ORYZA CERAMIDE™ – P demonstrated superior moisturizing effect with moisturizing ratio of 35%.



[Protocol]
Samples

Fig.23 The moisturizing Effects of Ceramide

1. ORYZA CERAMIDE™-P (from rice)
2. Ceramide (derived from konnyaku)
3. Ceramide (derived from wheat)

Samples were prepared in 3%(ceramide-base) solution

Condition : Temperature 35 °C, RH 40 %

Preparations

Samples of ceramides were mixed with Basis LP-20H and distilled water as per the following ratio:

Samples : 3 %, Basis LP-20H 5 %, Distilled Water 92 %

[Method]

1 g of test sample was weighed and added to vessel (3cm). Samples were weighed 8 hours later.

Moisturizing ratio was calculated as follows:

Moisturising Ratio (%)=

$\{(\text{Weight at 0 hour} - \text{Weight at 8 hour}) / \text{Original Weight}\} \times 100$

4-4 Activation of Normal Human Dermal Fibroblast Growth by Glycosphingolipids (*in vitro*)

To compare the activating effect of glycosphingolipids of various origin on normal human dermal fibroblast growth.

The effect of various types of ceramide on normal human dermal fibroblast growth is illustrated in Fig 24. The experiment shown that rate of cells growth ORYZA CERAMIDE™ excellent fibroblast growth, that is 163% of cell growth rate at 300 µg/ml performed.

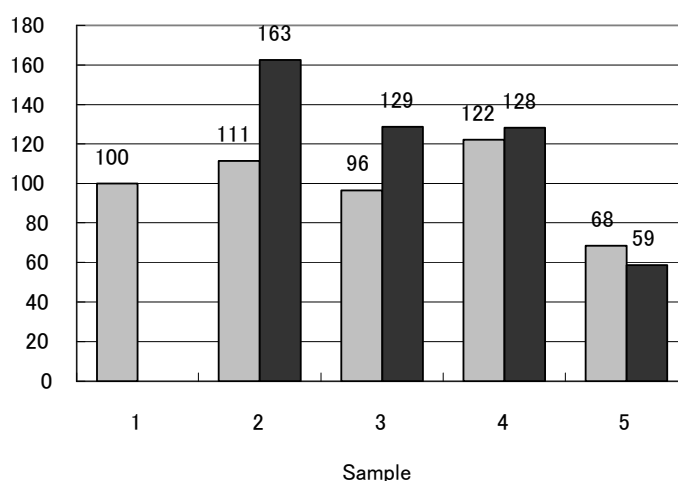


Fig. 24 The activating effect of various glycosphingolipids on normal human dermal fibroblasts cells growth

[Samples]

1. Control

2. ORYZA CERAMIDE™ containing glycosphingolipids > 95 %
3. Konnyaku derived ceramide containing glycosphingolipids > 95 %
4. Corn derived ceramide containing glycosphingolipids > 95 %
5. Wheat derived ceramide containing glycosphingolipids > 95 %

[Method]

Normal human dermal fibroblasts (HS-K) was cultured in RITC80-7 medium containing 10 % plasma FBS, 1000 unit/ml penicillin and 100 µg/ml streptomycin at 5 % CO₂ and 37 °C condition.

The cultured cells were placed in a 96-well microplate with each containing approximately 1x10⁵ cells (100 µl/ml) in RITC80-7 medium (containing 1 % plasma FBS, 1000 unit/ml penicillin and 100 µg/ml streptomycin) for 24 hours. Cultured cells were treated with various samples of ceramides and incubated for 72 hours. Cells growth were determined using Cell Counting Kit-8. The intensity of colour reaction revealed by Cell counting Kit-8 was measured at wavelength 450 nm. The cells growth rate was hence determined.

4-5. Improvement of Barrier Function and Atopic Dry Skin

The ceramides are located in the stratum corneum of skin and play important roles for maintaining barrier function, and protecting the skin against various foreign damages. Study by Imokawa *et al.* confirmed that the symptoms of atopic dry skin was improved by topical application of the ceramides. Meanwhile, Lati *et al.* reported that plant ceramides is beneficial in as antiallergic, antioxidant by inhibiting free radical effect and inhibition of elastase, collagenase and tyrosinase. Hence, ORYZA CERAMIDE™ is suitable to be used as, supplement for prevention against aging and rejuvenate stressed skin.

The effect of rice-derived glycosphingolipid on mouse itch model triggered by compound 48/80 and degranulation from sensitized mast cells were examined. These tests suggest that rice-derived glycosphingolipid reduces histamine release and itch of atopic dermatitis caused by histamine.

4-5-1 Effect on Itch Induced by Compound 48/80 in Mice

It was found that scratching action of mice which glycosphingolipids were fed to, decreased against compound 48/80 injection in a dose dependent manner (Fig. 25).

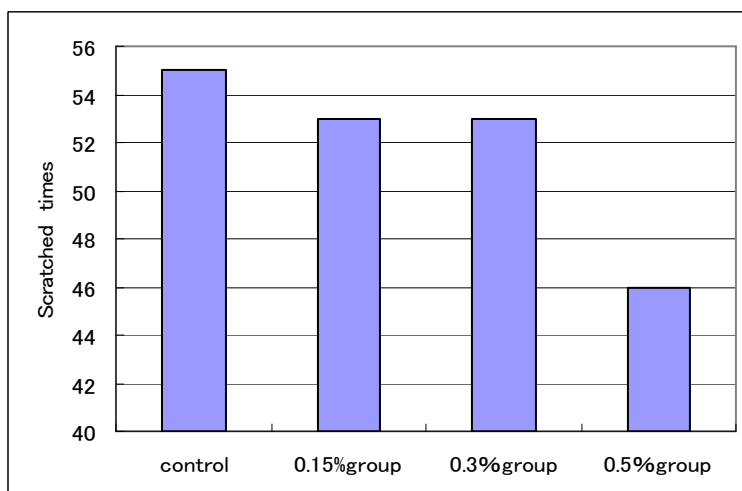


Fig. 25 Effect of rice-derived glycosphingolipid on compound 48/80 induced itch

[Method]

Mice (ddy, male) were fed rice-derived glycosphingolipid (0, 0.15, 0.3, and 0.5%) freely for 3 days. Three % of compound 48/80 solution was injected intradermally on the cervical skin to induce scratching action. The action was monitored for 30 minutes after they started to scratch themselves, and counted the number of scratching.

4-5-2 Effect on Mast Cell Degranulation Induced Itch by Compound 48/80

Effects of glycosphingolipid derived from a variety of plant on the degranulation from mast cells were examined in RBL-2H3. It was found that rice origin glycosphingolipids has the strongest effect in wheat-, devil's tongue-, and corn-origin glycosphingolipid (Table 3) to suppress mast cell degranulation.

Table 3 Inhibitory Effect of Glycosphingolipids on Degranulation from RBL-2H3 Mast Cells

Origin	Inhibition (%)
Rice	87.3±2.2
Wheat	82.2±5.9
Devil's-tongue(konn yaku)	70.8±5.9
Corn	64.2±5.4

Sample concentration: 1 µg/mL, mean ± S.E., n=6

[Method]

RBL-2H3 cells were cultured in Eagle's (MEM) containing 10 % FCS, 1000 unit/mL penicillin, and 10 µg/mL streptomycin. Cells (2.0×10⁵ cells, 400 µL/well) were seeded into 24-well plates. Rat monoclonal antibody DNP (dinitrophenyl)-IgE (Seikagaku Industry) were added to each well (final concentration of 0.45 µg/mL) and cultured for 24 hours for sensitization.

Cells were washed twice with 500 µL of Siraganian buffer (pH 7.2), and then 160 µL of Siraganian buffer containing 5.6 mM glucose, 1 mM CaCl₂, and 0.1% BSA was added. Cells were pre-incubated for 10 minutes at 37 °C, and 20 µL of test sample solution was added. Ten minutes later, antigen [DNP-BSA (dinitrophenyl bovine serum albumin), final concentration of 10 µg/mL] was added and incubated for 30 minutes at 37 °C, to stimulate cells. Stimulation was stopped by cooling for 10 minutes on ice, and then, 50 µL of supernatant were transferred to a 96-well micro plate. Reaction buffer 0.1 M citrate buffer (50 µL) containing 1 mM PNAG (*p*-nitrophenyl-*N*-acetyl -β-glucosaminide) was added, and incubated it for another one hour at 37 °C. Stopping buffer (200 µL, 0.1 M NaHCO₃/Na₂CO₃, pH 10.0) was added to the reaction solution, and the absorbance (wavelength: 410 nm) was measured using a micro plate reader.

The ratio of degranulation was calculated using the following equations.

Rate of β-hexosaminidase release (%) =

$$\frac{\text{Amount of } \beta\text{-hexosaminidase released by stimulation}^{***} - \text{amount of native release}^*}{\text{Total amount of } \beta\text{-hexosaminidase in the cells}^{**} - \text{amount of native release}^*} \times 100$$

Inhibitory ratio of β-hexosaminidase release (%) =

$$\left[1 - \frac{\text{Release rate when the sample is added}}{\text{Release rate when the sample is not added}^{***}} \right] \times 100$$

*Supernatant of culture with no antigen, antibody, or sample

**Supernatant of culture that were sonicated and freezed at -80°C

***Supernatant of culture with antigen and antibody but without the sample

[Test samples]

Glycosphingolipids from rice (> 98 %)

Glycosphingolipids from wheat (> 95 %)

Glycosphingolipids from devil's-tongue (konnyaku) (> 95 %)

Glycosphingolipids from corn (> 95 %)

4-6. Effect of β -sitosterol 3-*O*-glucoside (BSG) Existing in Glucosylceramide Fraction on Enzyme Expressions Involved in Ceramide Production in Human Keratinocytes

Supported by agricultural business subsidies from Ogaki Kyoritsu Bank, we conducted joint study with Professor Imokawa in Chubu University. In the study we found that BSG (Fig. 26) existing with glucosylceramides in Oryza Ceramide® on enhanced ceramide synthesis (Fig. 27). BSG can be found with glucosylceramide by TLC chromatogram (Fig. 28). BSG exists in lipophilic fractions in the other botanical ceramides. However, there are few study about biological function of BSG.

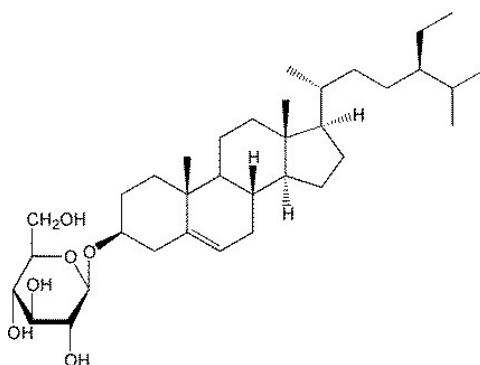


Fig. 26. A chemical Structure of β -sitosterol glucoside (BSG)

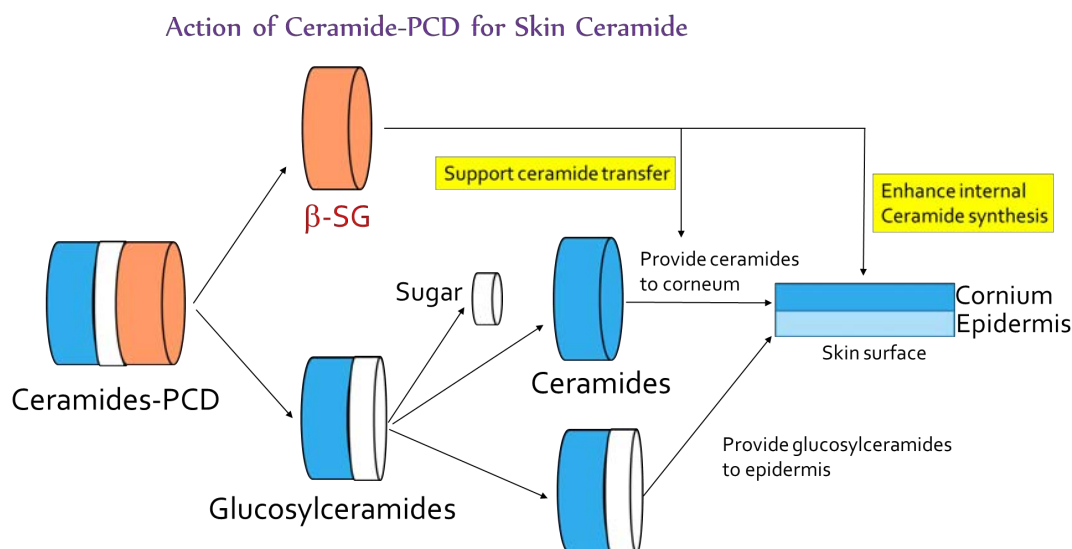


Fig. 27. Composition of Oryza Ceramide® (Ceramide-PCD)

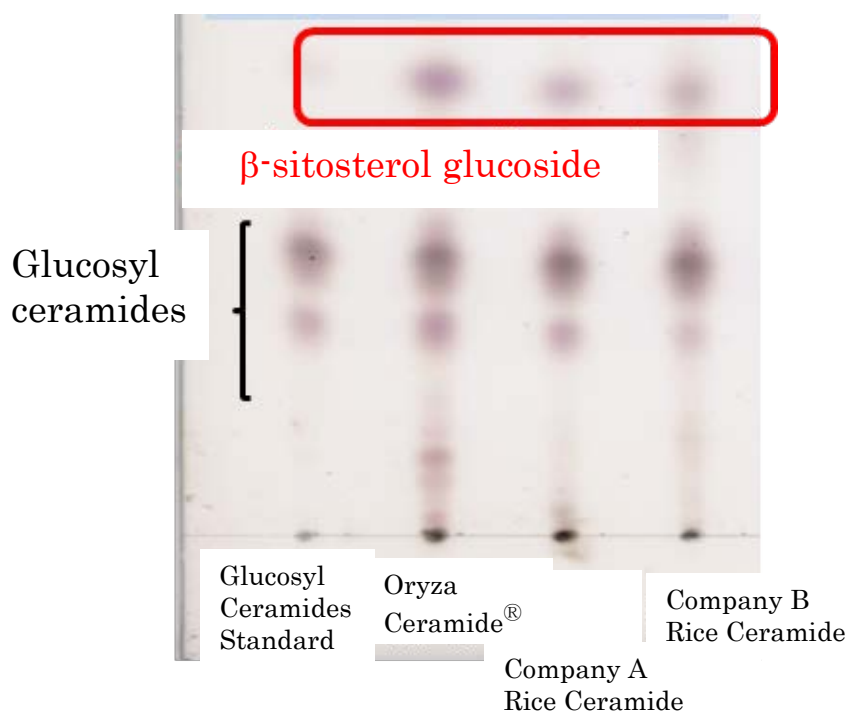


Fig. 28. TLC chromatograms of Rice-derived Ceramide products.

In the study, human keratinocytes (HaCaT) were treated with BSG for 48h and expressions of protein related to de novo ceramide synthesis were evaluated. As a result, BSG (1-10 µg/mL) was found to enhance expressions of ceramide synthase 3 (LASS3) and glucosylceramide synthase (UGCG). (Fig. 29, 30) These enzymes act in the process of dihydroceramide in epidermis converts to ceramide via glucosylceramides. The result supports that BSG can enhance ceramide synthesis in skin (Fig. 31).

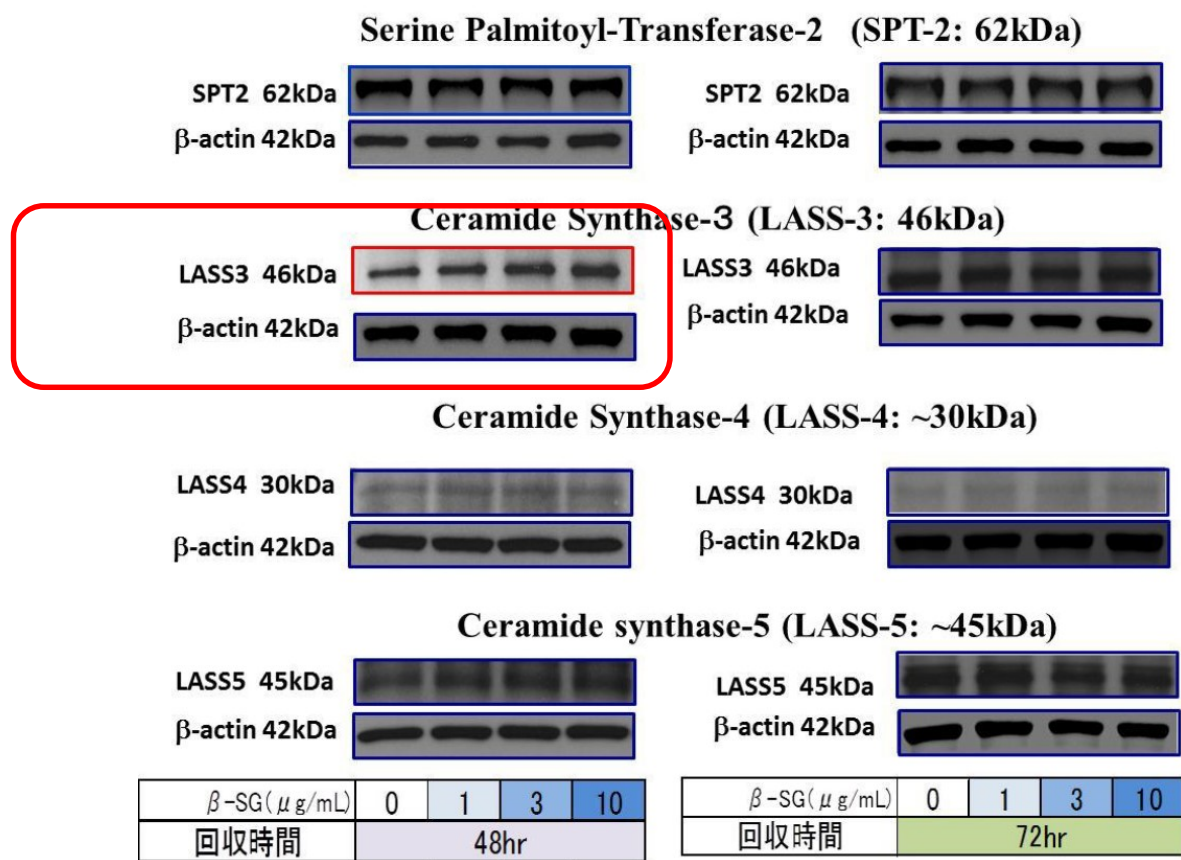
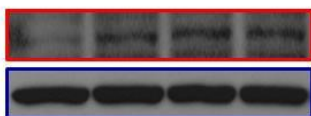


Fig. 29. Effect of BSG (β -SG) on protein expressions which are involved in skin ceramide synthesis.

Glucosylceramide Synthase (UGCG: 38kDa)

UGCG 38kDa
β-actin 42kDa

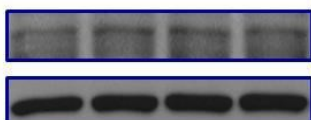


UGCG 38kDa
β-actin 42kDa

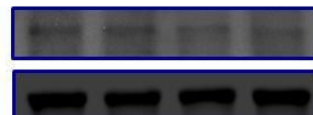


Sphingomyelin Synthase-1 (SMS-1: 49kDa)

SMS1 49kDa
β-actin 42kDa

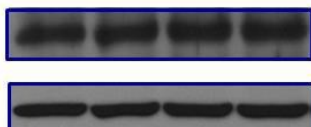


SMS1 49kDa
β-actin 42kDa

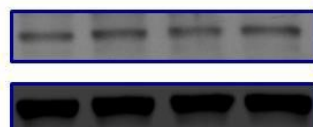


Sphingomyelin Synthase-2 (SMS-2: 42kDa)

SMS2 42kDa
β-actin 42kDa



SMS2 42kDa
β-actin 42kDa



β-SG (μg/mL)	0	1	3	10
回収時間	48hr			

β-SG (μg/mL)	0	1	3	10
回収時間	72hr			

Fig. 30. Effect of BSG (β-SG) on protein expressions which are involved in skin ceramide synthesis (2).

Conclusion

β-SG enhances ceramide production via expression of ceramide synthesizing enzymes.

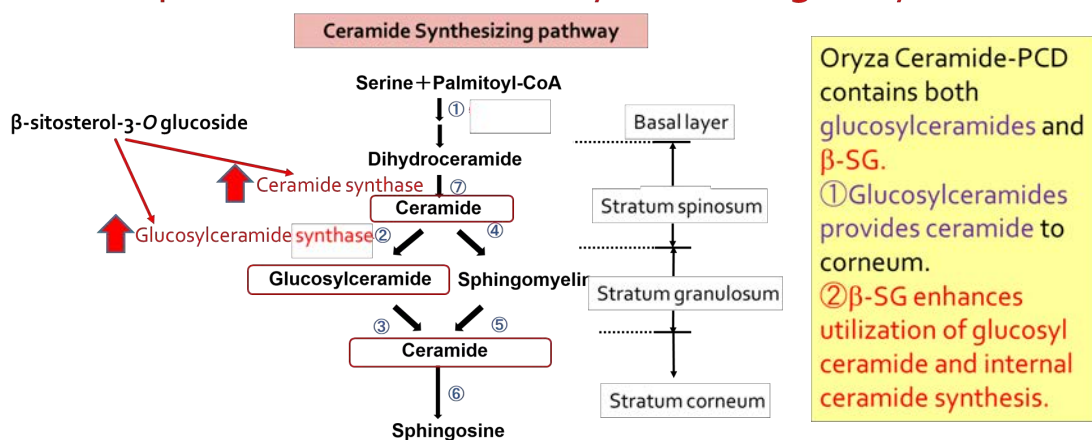


Fig. 31. Summary: Amelioration of skin ceramide synthesis by BSG (β-SG)

4-7. Clinical Test Results of Skin Beautifying Effect (*in vivo*)

This clinical investigation was conducted in OSAKA City University using ORYZA CERAMIDE™. The detail results are described in “CLINICAL INVESTIGATION OF SKIN-BEAUTIFYING EFFECT OF A BEAUTY SUPPLEMENT CONTAINING RICE-DERIVED CERAMIDE”. Please refer to it.

4-8. Clinical Test of ORYZA CERAMIDE™ with Kiwi Seed Extract

ORYZA CERAMIDE™ exhibits moisturizing effect and activation of fibroblasts. On the other hand Kiwi seed extract suppresses activities of 5 α -reductase and lipase involved in acne. We evaluated clinical effect of the oral co-treatment of ORYZA CERAMIDE™ and Kiwi seed extract. As a result, reduction of sebum and improvement of acne and skin condition were observed.

[Method]

Japanese female aged 18 to 34 years old with acne were nominated as subjects. The food sample containing Kiwi seed extract and ORYZA CERAMIDE™ were given for 4 weeks. After 4-week ingestion, the changes of facial factors were evaluated.

[Result]

1. Gross diagnosis

Acne is classified into comed, papule, pustule, abscess, and nodule. The acne score was set as comed: 1, papule: 2, pustule: 3, abscess: 4, and nodule: 5 and acne symptoms were evaluated. After 4- week ingestion of the sample, significant improvement of the score was observed. (Fig. 32).

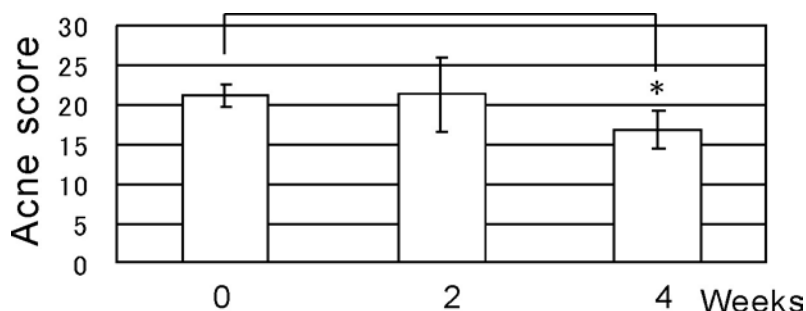


Fig. 32. Change in Acne Score (*: $p < 0.05$)

2. Porphyrin

Prophyrin is typical metabolite form *P. acne* and reveal the existence of *P. acne*. As shown in Fig. 33, The number of porphyrin sites in the face were decreased toward ingestion term. Hence, the number of *P. acne* was found to be decreased by the ingestion of the sample.

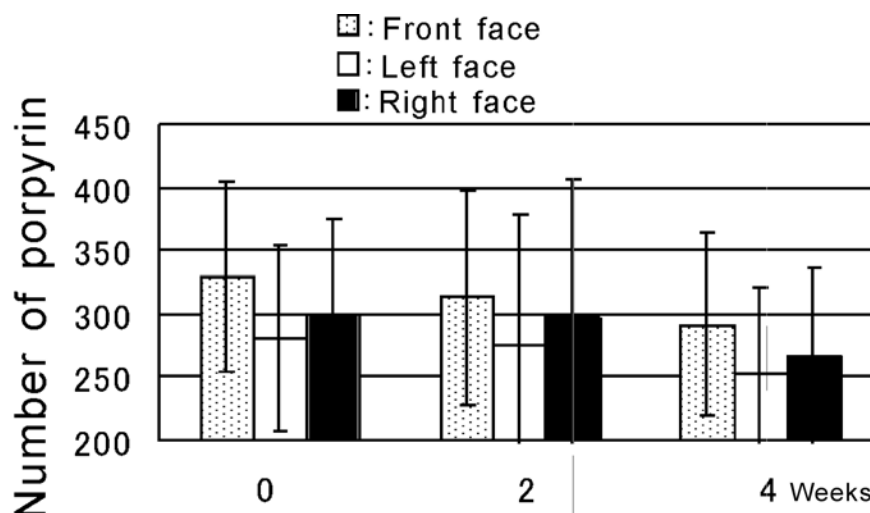


Fig. 33. Change in porphyrin sites

3. Sebum

The amount of sebum was decreased in both points of central forehead and the top of left cheek. Significant difference was observed on the top of left cheek at 2 weeks (Fig. 34).

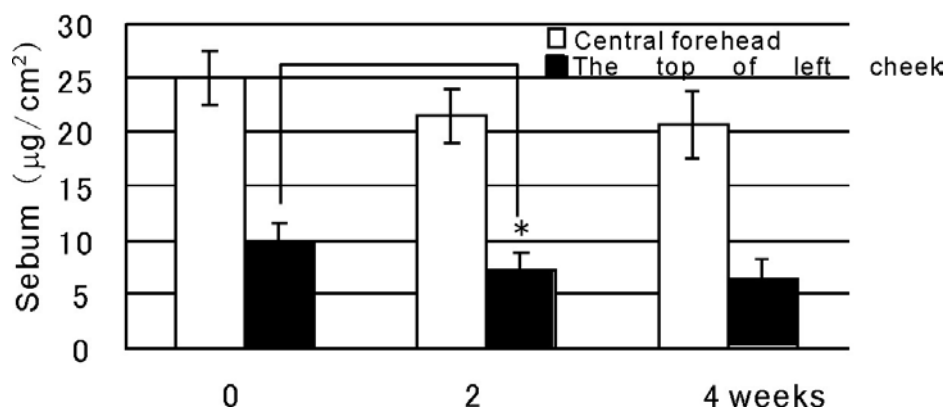


Fig.34. Change in sebum

4. Smoothness

Smoothness at left and right faces was slightly increased (Fig. 35).

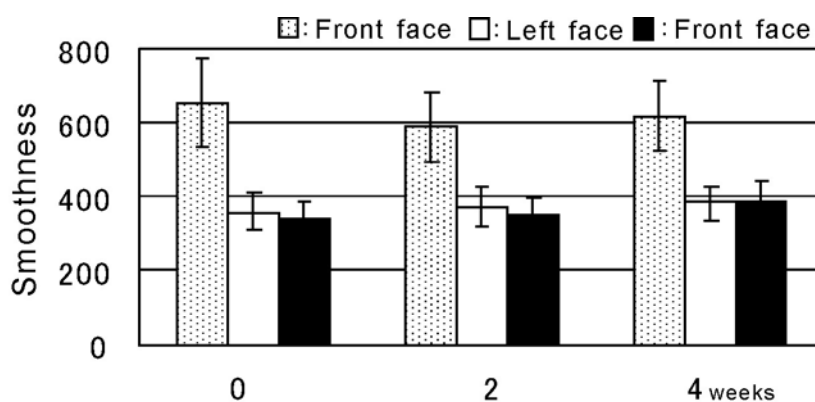


Fig. 35. Change in smoothness

5. Change in skin disease specific QOL by Skindex-16

Skindex-16 is a scale of QOL in skin and consists of 16 question belongs to the fields of symptom, emotion and function. Each field is evaluated 0 to 100 points and maximum total score is 300. Increase in the score reveals low QOL. As a result, Emotion and total score were decreased. Significant decrease was observed at 2 weeks in total score (Fig. 36). This result suggests that the sample improved QOL of the subject with acne.

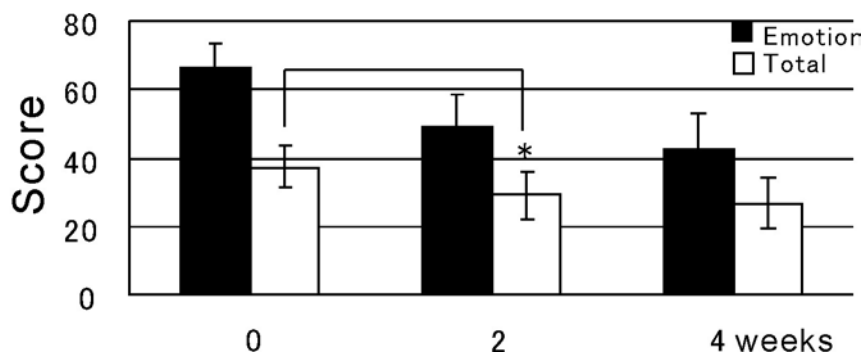


Fig. 36. Change in QOL (*: $p < 0.05$)

4-9. Effect to Prevent Colon Cancer

In Japan, stomach cancer is the highest cause of death, followed by colon cancer among digestive cancer. Incidence of colon cancer is increasing as diet becomes more and more westernized. In a joint research with Professor Yoshimi and coworkers of Ryukyu

University, preventive effect of rice-origin glycosphingolipid to colon cancer. The result indicated that rice-origin glycosphingolipid is potentially a safe, natural food to prevent colon cancer.

A carcinogen, azoxymethane (AOM) was hypodermically injected to F344 rats (5-week old, male) once a week for the first two weeks to induce aberrant crypt foci (ACF) and mucin depleted foci (MDF) both of which are precancerous lesions of colon cancer. In groups 2 and 3 (groups that received G₁CM administration at the initiation stage), the number of ACF and MDF cases was significantly fewer, compared to group 1 (the positive control group) in the fourth week of the test. In groups 4 and 5 (groups that received G₁CM administration at post-initiation stage), the number of ACF and MDF cases of each group in the eighth week was also significantly fewer, compared to group 1.

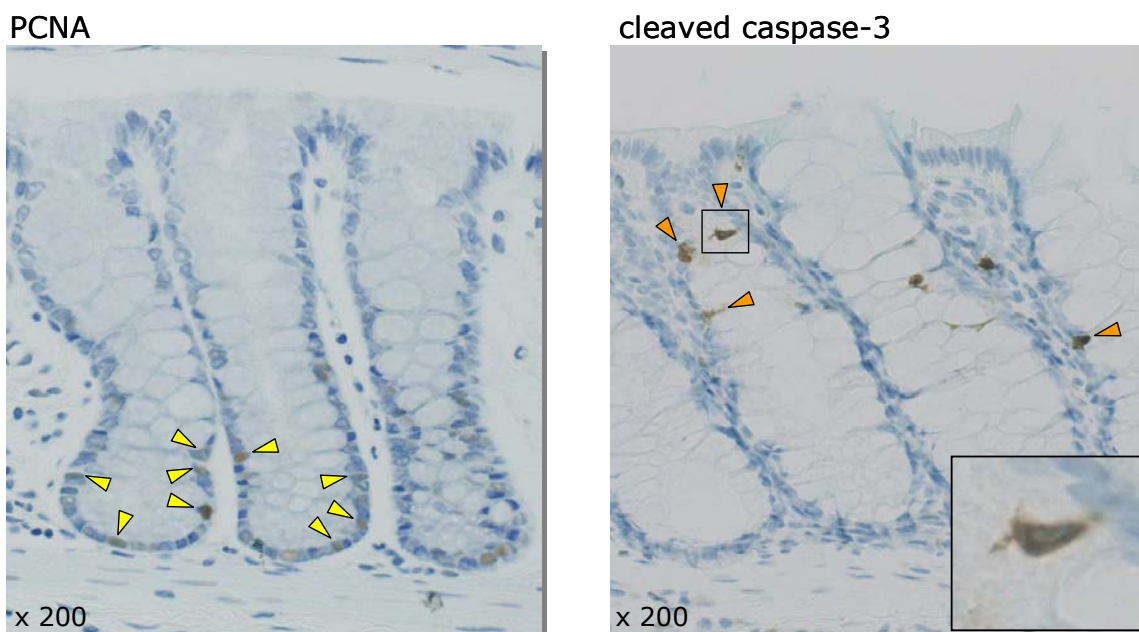
These data indicate that rice-origin glycosphingolipid prevents precancerous lesions from getting worse when it is administered in the promotion stage of tumorigenesis, even without simultaneous administration. Actually, no large intestinal mucosa was found in groups 2, 3, 4 and 5, which G₁CM was fed to.

Immuno histochemical staining suggests that rice-origin glycosphingolipid induces apoptosis of cancer cells.

Table 4. Effect of rice-derived glycosphingolipid on colon cancer induced by AOM in rats

Group	Conc. (ppm)	N	Number of ACF		Number of MDF	
			4 w	8w	4w	8w
1	0	12	75 ± 17	111 ± 33	9.2 ± 5.2	17.1 ± 6.6
2	100	12	46 ± 9 <i>p</i> <0.01	90 ± 28	0.8 ± 0.8 <i>p</i> <0.05	10.8 ± 5.9 <i>p</i> <0.05
3	250	12	45 ± 14 <i>p</i> <0.01	75 ± 20 <i>p</i> <0.05	0.8 ± 0.4 <i>p</i> <0.05	6.8 ± 2.7 <i>p</i> <0.05
4	100	12	-	89 ± 28	-	10.0 ± 5.1
5	250	12	-	78 ± 15 <i>p</i> <0.05	-	9.6 ± 3.1 <i>p</i> <0.01
6	250	3, 6	0	0	0	0
7	0	3, 6	0	0	0	0

(mean ± standard deviation)



- PCNA immunoreactivity is positive in nucleus (▼)
- Cleaved caspase-3 positive cells are mainly localized in upper half of the crypt (▼), and its immunoreactivity shows cytoplasmic pattern in high power magnification.

Fig. 37. PCNA and caspase-3

[Method]

F344 rats (5 week-old, male) were divided into seven groups and hypodermically injected azoxymethane (AOM, 20 mg/kg) once week for the first two weeks to induce ACF and MDF. In the forth and eighth weeks, their were picked up large intestines, fixed with formalin, stained with Alcian Blue (pH 2.5), then counted the number of ACF and MDF cases under an optical microscope. In addition, immunohistochemical staining with antibodies of proliferating nuclear antigen (PCNA) and cleaved caspase-3 was performed to confirm tumorigenesis (PCNA) and apoptosis (caspase-3).

Group 1: Basic feed before the AOM administration (the first week) to the end of the test (the eighth week).

Groups 2 and 3: Fed with pure glycosphingolipid(>90% purity) (G₁CM 100 or 250 ppm) before the AOM administration(the first week) to the fourth week of the test, and then basic feed to the end of the test (the eighth week).

Groups 4 and 5: Basic feed before the AOM administration (the first week) and then, feed with 100 or 250 ppm of G₁CM from the third week of the test to the end (the eighth week).

Groups 6 and 7: AOM was not administrated. Fed with 250 ppm of G₁CM or basic feed throughout the test period.

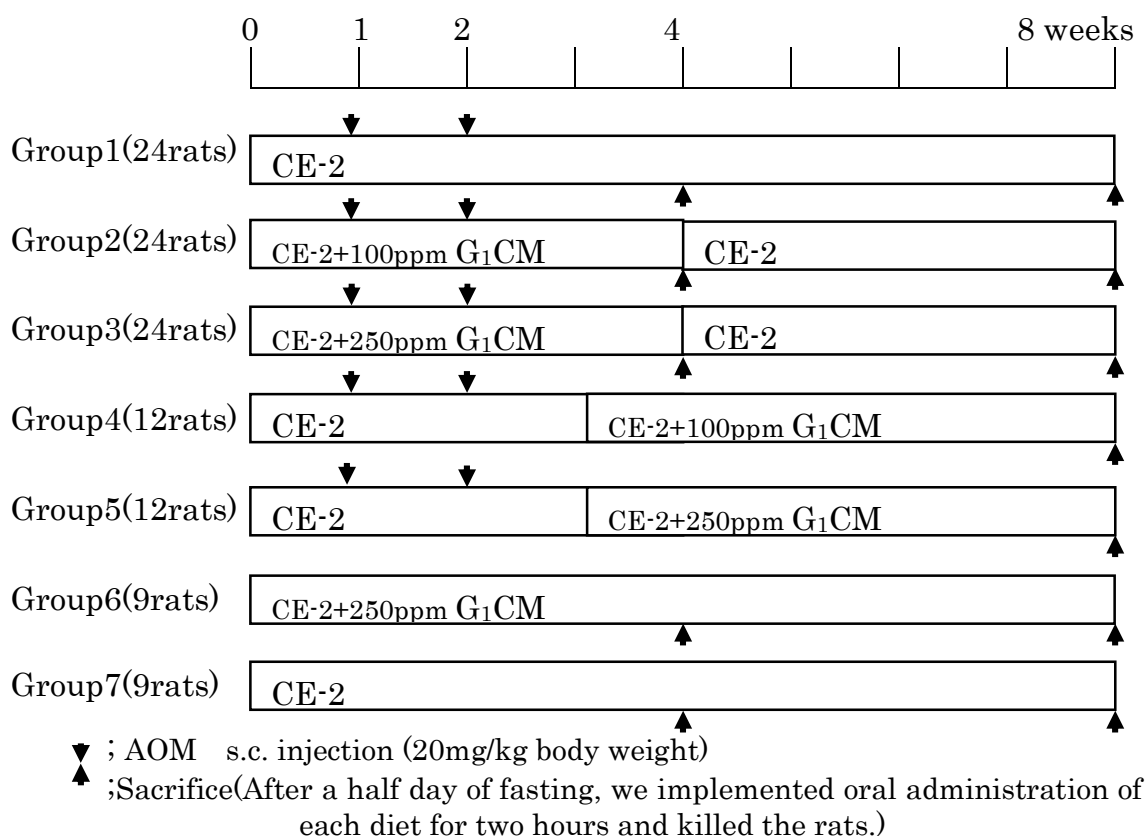


Fig. 38 Protocol of AOM induce rats colon cancer model

References

- 1) Eva-Maria Schmelz et al., *J. of Nutrition*, **124**(5), 702(1994)
- 2) Merrill A. H., Jr., et al., *FASEB Journal*, **3A**, 469(1989)
- 3) Futerman A. H., *CHAPTER 4, Current Topics in Membranes*, Vol.**40**, 93(1994)
- 4) Elian Lati., *Fragrance Journal*, **23**(1), 81(1995)
- 5) Genji Imokawa, *Yukagaku*, **44**(10), 751(1995)
- 6) Lena N. et al., *J.Nutr.Biochem.*, **8**(3),122(1997)

4-10. Effect on Squamous Cell Carcinoma

As a result of joint study with professor Okazaki and Hujiwara in Tottori University, ORYZA CERAMIDE™ was found to suppress growth of squamous cell carcinoma and enhance life span in mice.

The carcinoma was transplanted to cervix and dorsal skin of NOD/SCID mice. After growth of the carcinoma up to approx. 5 mm diameter, ORYZA CERAMIDE™ (300 mg/kg, 24 mg/kg as sphingo glycolipid) was given orally. As a result significant decrease in the volume of carcinoma (Fig. 39), enhance of apoptosis (Fig. 40) and life time extension (Fig. 41) were observed.

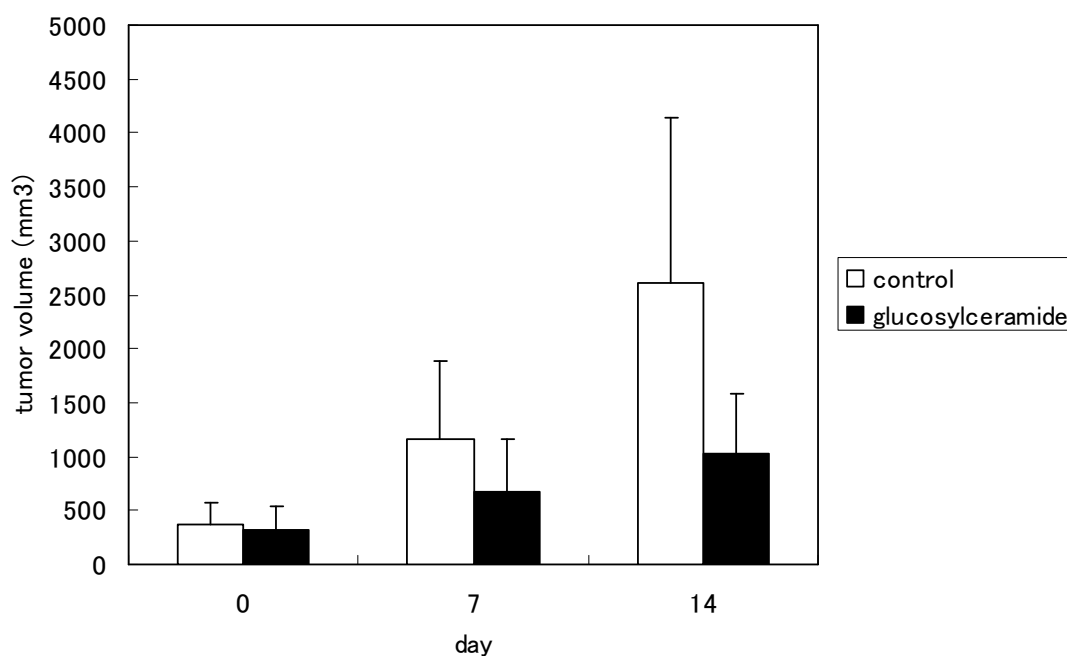


Fig. 39. The volume of carcinoma

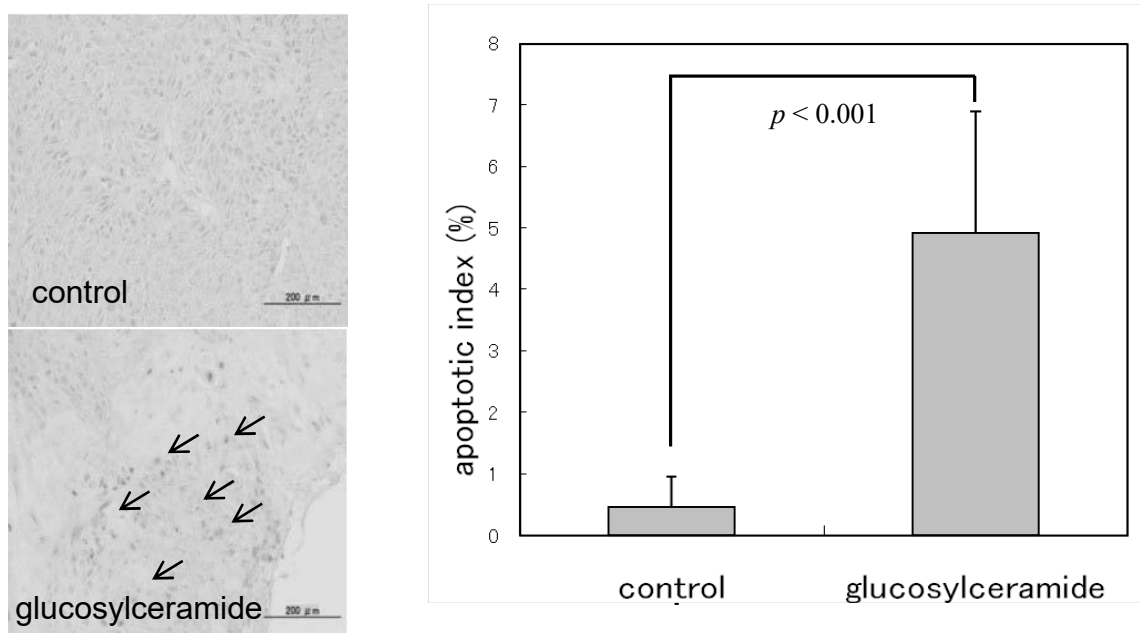


Fig. 40. Enhance of apoptosis

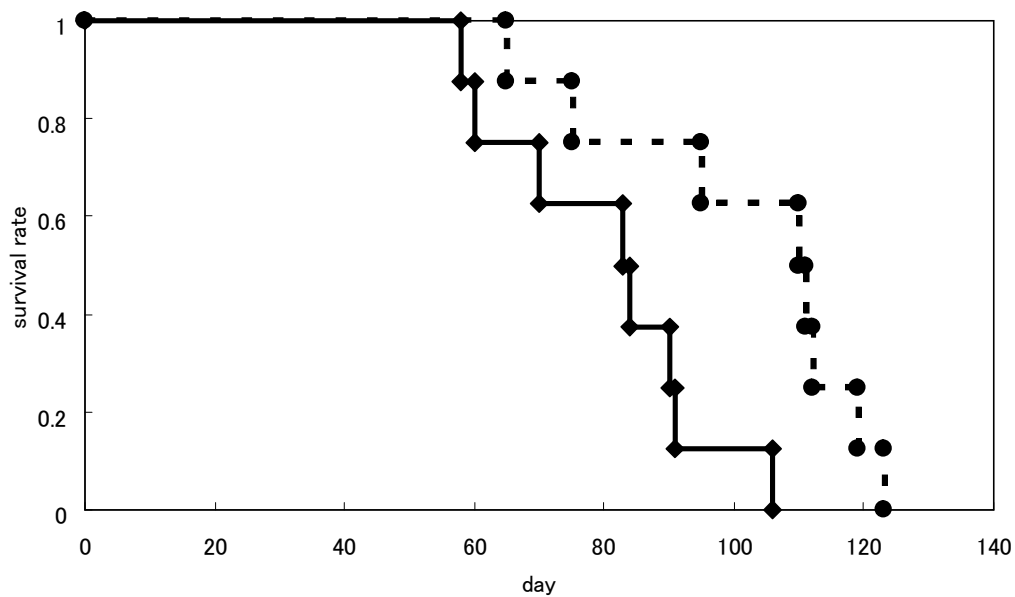
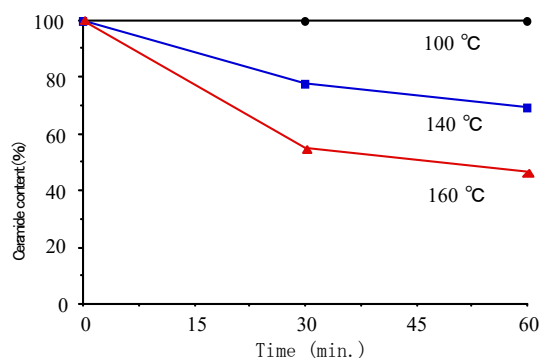


Fig. 41. Mortality

5. Stability

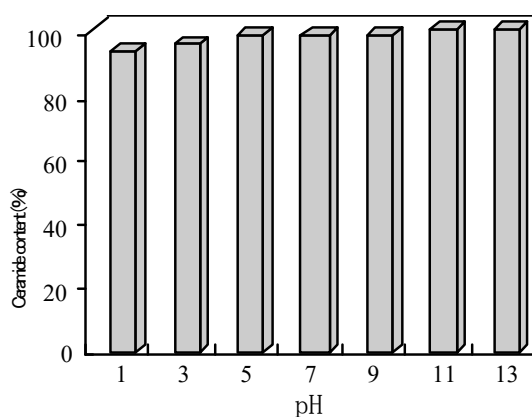
(1) Thermo stability

The pyrolysis of ORYZA CERAMIDE™ does not occur at a normal food processing temperature for 60 min.



(2) pH stability

ORYZA CERAMIDE™ remained stable at a all pH range field.



* The ceramide concentration in 90% ethanol solution (pH 6.8, unregulated) was set 100%.

6. Nutrition Information

Items (/100 g)	ORYZA CERAMIDE™			
	PT	PCD	WSP WSP8	L L0.8
Water (g)	1.2	1.8	1.8	40.2
Protein (g)	5.3	3.8	3.8	0.4
Fat (g)	19.6	33.8	33.8	5.9
Ash (g)	66.3	2.5	2.5	0.2
Available carbohydrate (g)	5.6	58.1	58.3	53.3
Dietary fiber (g)	2.0	0.0	0.0	0.0
Sodium (mg)	337	524	524	52
Energy (kcal)	224	552	552	268

7. Safety Profile

(1) Residual Agricultural Chemicals

The rice bran and rice germ-derived extract containing glycosphingolipids (without binder) is conformed to regulation stipulated for 447 residual agricultural chemical compounds. No residual agricultural chemicals detected as confirm by test trustee.

Test trustee : Masis Co. Ltd. Date : September 5, 2006

(2) Acute Toxicity (LD₅₀)

ORYZA CERAMIDE™ (5000 mg/kg) were given orally to mice (5 weeks old) and fed with laboratory chow for 2 weeks. No toxic effect observed, at 5000 mg/kg.

LD₅₀ (in mouse) is deduced to be > 5000 mg/kg.

(3) Four-weeks repeated dose toxicity test

Toxicity on 28-day repeated dose was conducted on Slc:ddY male and female mice (4 weeks old). 60 mg/kg (corresponding to 3.6 g/human when a human weight would be 60 kg) of rice-derived sphingolipids was given to mice any restrictions on test condition during the 28-day period. No abnormal changes observed in organs, weight and blood profile of rats at end of test.

(4) Other Information about Safety

Glycosphingolipid in ORYZA CERAMIDE™ (mainly sphingosine derivative extracted from rice bran) is registered as an emulsifier of food additive. This safe material is the only ceramide approved by the Ministry of Health, Labour and Welfare among various types of ceramides.

8. Recommended Daily Dosage

Product	Recommended Dosage (mg/day)	
	Whitening effect	Moisturizing effect
ORYZA CERAMIDE™-PT, PCD (powder)	30~50	20~40
ORYZA CERAMIDE™-P8T (powder)	11~19	7.5~15
ORYZA CERAMIDE™-WSP (water soluble powder)	30~50	20~40
ORYZA CERAMIDE™-WSP8 (water-soluble powder)	11~19	7.5~15
ORYZA CERAMIDE™-L (milky liquid)	300~500	200~400
ORYZA CERAMIDE™-L0.8 (milky liquid)	113~188	75~150

9. Applications

Applications	Examples
Health foods	Soft-capsule, Tablet, Hard-capsule, etc.
Foods	Candy, Gum, Cake, Cookie, Wafer, Drink, Nutritional oil, etc.
Cosmeceuticals	Face care (lotion, milk, cream, etc.) Body care (body lotion, body cream, etc.) Cleansing cosmetics (soap, etc.) Makeup cosmetics (lipstick, foundation, etc.) etc.

10. Packaging

Powder		Water soluble powder		Liquid	
Food	Cosmetic	Food	Cosmetic	Food	Cosmetic
ORYZA CERAMIDE™-					
PT	PC	WSP	WSPC	L	LC
PCD	PC8	WSP8	WSPC8	L0.8	LC0.8
P8T					
1kg Exterior packaging : Cardboard box Interior packaging : Polyvinylidene coating bag and Can				5kg Exterior packaging : Cardboard box Interior packaging : A double layered plastic bag	

11. Storage

Store in cool and dark place. Avoid humidity.

12. Expression

<Food>

Product name	Expression
ORYZA CERAMIDE™ -PT, PCD -P8T -WSP, WSP8 -L, L0.8	Rice Ceramide Rice Extract (including rice ceramide) Rice Extract (including rice glycosphingolipid)

<Cosmetic>

Product name	INCI name
ORYZA CERAMIDE™ -PC, PC8	Cyclodextrin Oryza Sativa (Rice) Bran Oil Glycosphingolipids
ORYZA CERAMIDE™ -WSPC, WSPC8	Maltosyl Cyclodextrin Cyclodextrin Maltose Oryza Sativa (Rice) Bran Oil Glyco sphingolipids
ORYZA CERAMIDE™-LC	Glycerin Water Polyglyceryl-10 Oleate Lecithin Oryza Sativa (Rice) Bran Oil Glycosphingolipids
ORYZA CERAMIDE™-LC0.8	Glycerin Water Polyglyceryl-10 Oleate Oryza Sativa (Rice) Bran Oil Glycosphingolipids

PRODUCT STANDARD

PRODUCT NAME

ORYZA CERAMIDE™-PT

(FOOD)

This product is extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Gramineae*). It contains a minimum of 3.0 % glycosphingolipid.

<u>Appearance</u>	Light yellowish powder with slight unique aroma	
<u>Glycosphingolipid</u>	Min. 3.0 %	(Densitometry Method) (HPLC Light Scattering Method)
<u>Loss on Drying</u>	Max. 5.0 %	(Analysis for Hygienic Chemists, 1 g, 105°C, 2 h)
<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>	<u>Ingredient</u>	<u>Content</u>
	Rice extract	25.00 %
	Dextrin	1.13 %
	Calcium carbonate	65.60 %
	Sodium caseinate (milk)	5.00 %
	Pullulan	2.00 %
	Enzymatic lysolecithin (soybean)	0.57 %
	Glycerin ester of fatty acid	0.50 %
	Xanthan gum	0.20 %
	Total	100.00 %

**PRODUCT STANDARD**

PRODUCT NAME

ORYZA CERAMIDE™-PCD

(FOOD)

This product is extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Gramineae*). It contains a minimum of 3.0 % glycosphingolipid.

<u>Appearance</u>	Slightly yellow powder with slightly unique aroma									
<u>Glycosphingolipid</u>	Min. 3.0 %	(Densitometry Method) (HPLC Light Scattering Method)								
<u>Loss on Drying</u>	Max. 8.0 %	(Analysis for Hygienic Chemists, 1 g, 105°C, 2 h)								
<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 Metho d, 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><tr><th>Ingredients</th><th>Contents</th></tr><tr><td>Rice extract</td><td>40 %</td></tr><tr><td>Cyclodextrin</td><td>60 %</td></tr><tr><td>Total</td><td>100 %</td></tr></table>		Ingredients	Contents	Rice extract	40 %	Cyclodextrin	60 %	Total	100 %
Ingredients	Contents									
Rice extract	40 %									
Cyclodextrin	60 %									
Total	100 %									

**PRODUCT STANDARD**

PRODUCT NAME

ORYZA CERAMIDE™-WSP

(FOOD)

This product is extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Gramineae*). It contains a minimum of 3.0 % glycosphingolipid. This product is water-soluble.

<u>Appearance</u>	Slightly yellow powder with slightly unique aroma	
<u>Glycosphingolipid</u>	Min. 3.0 %	(Densitometry Method) (HPLC Light Scattering Method)
<u>Loss on Drying</u>	Max. 5.0 %	(Analysis for Hygienic Chemists, 1 g, 105°C, 2 h)
<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>	<u>Ingredients</u>	<u>Contents</u>
	Rice extract	40 %
	Cyclodextrin	60 %
	Total	100 %

PRODUCT STANDARD

PRODUCT NAME

ORYZA CERAMIDE™-WSP8

(FOOD)

This product is extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Gramineae*). It contains a minimum of 8.0 % glycosphingolipid. This product is water-soluble.

<u>Appearance</u>	Slightly yellow powder with slightly unique aroma									
<u>Glycosphingolipid</u>	Min. 8.0 %	(Densitometry Method) (HPLC Light Scattering Method)								
<u>Loss on Drying</u>	Max. 5.0 %	(Analysis for Hygienic Chemists, 1 g, 105°C, 2 h)								
<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><tr><td>Ingredients</td><td>Contents</td></tr><tr><td>Rice extract</td><td>40 %</td></tr><tr><td>Cyclodextrin</td><td>60 %</td></tr><tr><td>Total</td><td>100 %</td></tr></table>		Ingredients	Contents	Rice extract	40 %	Cyclodextrin	60 %	Total	100 %
Ingredients	Contents									
Rice extract	40 %									
Cyclodextrin	60 %									
Total	100 %									

PRODUCT STANDARD

PRODUCT NAME

ORYZA CERAMIDE™-L

(FOOD)

This product is emulsifying liquid including rice bran glycosphingolipid extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Gramineae*). It contains a minimum of 0.30 % glycosphingolipid.

<u>Appearance</u>	Light yellowish liquid with slight unique aroma	
<u>Glycosphingolipid</u>	Min. 0.30 %	(Densitometry Method) (HPLC Light Scattering Method)
<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>	<u>Ingredients</u>	<u>Content</u>
	Glycerin	46 %
	Purified water	40 %
	Glycerin ester of fatty acid	10 %
	Rice extract	4 %
	Total	100 %

PRODUCT STANDARD

PRODUCT NAME

ORYZA CERAMIDE™-L0.8

(FOOD)

This product is water-soluble emulsion containing glycosphingolipids extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Gramineae*). It contains a minimum of 0.80 % glycosphingolipid.

<u>Appearance</u>	Light yellowish liquid with slight unique aroma	
<u>Glycosphingolipid</u>	Min. 0.80 %	(Densitometry Method) (HPLC Light Scattering Method)
<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>	<u>Ingredients</u>	<u>Content</u>
	Glycerin	46 %
	Purified water	40 %
	Glycerin ester of fatty acid	10 %
	Rice extract	4 %
	Total	100 %

**PRODUCT STANDARD**

PRODUCT NAME

ORYZA CERAMIDE™-PC

(COSMETIC)

This product is extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linn e (*Gramineae*). It contains a minimum of 3.0 % glycosphingolipid.

<u>Appearance</u>	Yellowish powder with slightly unique smell.											
<u>Certification Test</u> Cyclodextrin	Add 2 ml of iodine reagent to 0.2 g of this product and boil it in water bath. Yellow brown precipitate is formed at the room temperature.											
<u>Glycosphingolipid</u>	Min. 3.0 %	(Densitometry Method) (HPLC Light Scattering Method)										
<u>Loss on Drying</u>	Max. 8.0 %	(1g, 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>	<table><tr><td><u>Ingredient</u></td><td><u>Content</u></td></tr><tr><td>Cyclodextrin</td><td>60 %</td></tr><tr><td>Oryza Sativa (Rice) Bran Oil</td><td>37 %</td></tr><tr><td><u>Glycosphingolipids</u></td><td>3 %</td></tr><tr><td>Total</td><td>100 %</td></tr></table>		<u>Ingredient</u>	<u>Content</u>	Cyclodextrin	60 %	Oryza Sativa (Rice) Bran Oil	37 %	<u>Glycosphingolipids</u>	3 %	Total	100 %
<u>Ingredient</u>	<u>Content</u>											
Cyclodextrin	60 %											
Oryza Sativa (Rice) Bran Oil	37 %											
<u>Glycosphingolipids</u>	3 %											
Total	100 %											

**PRODUCT STANDARD**

PRODUCT NAME

ORYZA CERAMIDE™-PC8

(COSMETIC)

This product is extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Gramineae*). It contains a minimum of 8.0 % glycosphingolipid.

<u>Appearance</u>	Yellowish powder with slight unique aroma.											
<u>Certification Test</u> Cyclodextrin	Add 2 ml of iodine reagent to 0.2 g of this product and boil it in water bath. Yellow brown precipitate is formed at the room temperature.											
<u>Glycosphingolipid</u>	Min. 8.0 %	(Densitometry Method) (HPLC Light Scattering Method)										
<u>Loss on Drying</u>	Max. 5.0 %	(1g, 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>	<table><tr><th>Ingredients</th><th>Contents</th></tr><tr><td>Cyclodextrin</td><td>60 %</td></tr><tr><td>Oryza Sativa (Rice) Bran Oil</td><td>32 %</td></tr><tr><td>Glycosphingolipids</td><td>8 %</td></tr><tr><td>Total</td><td>100 %</td></tr></table>		Ingredients	Contents	Cyclodextrin	60 %	Oryza Sativa (Rice) Bran Oil	32 %	Glycosphingolipids	8 %	Total	100 %
Ingredients	Contents											
Cyclodextrin	60 %											
Oryza Sativa (Rice) Bran Oil	32 %											
Glycosphingolipids	8 %											
Total	100 %											

PRODUCT STANDARD

PRODUCT NAME

ORYZA CERAMIDE™-WSPC

(COSMETIC)

This product is extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Gramineae*). It contains a minimum of 3.0 % glycosphingolipid. This product is water-soluble.

<u>Appearance</u>	Slightly yellow powder with slightly unique smell.	
<u>Certification Test</u> Cyclodextrin	Add 2 ml of iodine reagent to 0.2 g of this product and boil it in water bath. Yellow brown precipitate is formed at the room temperature.	
<u>Glycosphingolipid</u>	Min. 3.0 %	(Densitometry Method) (HPLC Light Scattering Method)
<u>Loss on Drying</u>	Max. 5.0 %	(1 g, 105 °C, 2 h)
<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>Ingredients</u>	<u>Contents</u>
	Maltosyl Cyclodextrin	60 %
	Cyclodextrin	
	Maltose	
	Dimaltosyl Cyclodextrin	37 %
	Oryza Sativa (Rice) Bran Oil	
	Glucosyl Ceramid	3 %
	Total	100 %

**PRODUCT STANDARD**

PRODUCT NAME

ORYZA CERAMIDE™-WSPC8

(COSMETIC)

This product is extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Gramineae*). It contains a minimum of 8.0 % glycosphingolipid. This product is water-soluble.

<u>Appearance</u>	Yellowish powder with slightly unique smell.	
<u>Certification Test</u> Cyclodextrin	Add 2 ml of iodine reagent to 0.2 g of this product and boil it in water bath. Yellow brown precipitate is formed at the room temperature.	
<u>Glycosphingolipid</u>	Min. 8.0 %	(Densitometry Method) (HPLC Light Scattering Method)
<u>Loss on Drying</u>	Max. 5.0 %	(1g, 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)

Composition

Ingredients	Contents
Maltosyl Cyclodextrin	60 %
Cyclodextrin	
Dimaltosyl Cyclodextrin	
Maltose	32 %
Oryza Sativa (Rice) Bran Oil	
Glucosyl Ceramide	8 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

ORYZA CERAMIDE™-LC

(COSMETIC)

This product is water-soluble emulsion containing glycosphingolipids extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Graminea*). It contains a minimum of 0.30 % glycosphingolipid.

Appearance

Slightly brown liquid with slightly unique smell.

Certification Test

(1) Glycerin

10 g of this product is dissolved in 50 ml of ethanol in separating funnel. Ethanol layer is evaporated. When 0.5 g of potassium hydrogensulfate is added in residue and heated, it occurs irritating smell.

(2) Lecithin

1 g of this product, 5 g of potassium sulfate, 0.5 g copper sulfate and 20 ml of sulfuric acid are heated in kjeldahl frask. After solution changes transparent blue, heat for 2 hours. After cool, 20 ml of water is added. 10 ml of ammonium molybdate solution is added in 5 ml of this solution, then it occurs yellow precipitate.

Glycosphingolipid

Min. 0.30 % (Densitometry Method)
(HPLC Light Scattering Method)

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 ×10² cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts

Max. 1 ×10² cfu/g (Analysis for Hygienic Chemists)

Coliforms

Negative (Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Glycerin	40.0 %
Water	38.0 %
Polyglyceryl-10 oleate	13.0 %
Lecithin	5.0 %
Oryza Sativa (Rice) Bran Oil	3.7 %
Glycosphingolipids	0.3 %
Total	100.0 %

PRODUCT STANDARD

PRODUCT NAME

ORYZA CERAMIDE™-LC0.8

(COSMETIC)

This product is water-soluble emulsion containing glycosphingolipids extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linne (*Graminea*). It contains a minimum of 0.80 % glycosphingolipid.

<u>Appearance</u>	Slight yellowish liquid with slight unique aroma.															
<u>Certification Test</u>																
Glycerin	10 g of this product is dissolved in 50 ml of ethanol in separating funnel. Ethanol layer is evaporated. When 0.5 g of potassium hydrogensulfate is added in residue and heated, it occurs irritating smell.															
<u>Glycosphingolipid</u>	Min. 0.80 %	(Densitometry Method) (HPLC Light Scattering Method)														
<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>	<table><tr><th>Ingredients</th><th>Contents</th></tr><tr><td>Glycerin</td><td>46.0 %</td></tr><tr><td>Water</td><td>40.0 %</td></tr><tr><td>Polyglyceryl-10 oleate</td><td>10.0 %</td></tr><tr><td>Oryza Sativa (Rice) Bran Oil</td><td>3.2 %</td></tr><tr><td>Glycosphingolipids</td><td>0.8 %</td></tr><tr><td>Total</td><td>100.0 %</td></tr></table>		Ingredients	Contents	Glycerin	46.0 %	Water	40.0 %	Polyglyceryl-10 oleate	10.0 %	Oryza Sativa (Rice) Bran Oil	3.2 %	Glycosphingolipids	0.8 %	Total	100.0 %
Ingredients	Contents															
Glycerin	46.0 %															
Water	40.0 %															
Polyglyceryl-10 oleate	10.0 %															
Oryza Sativa (Rice) Bran Oil	3.2 %															
Glycosphingolipids	0.8 %															
Total	100.0 %															



ORYZA OIL & FAT CHEMICAL CO., LTD.

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 :

ORYZA OIL & FAT CHEMICAL CO., LTD.

No.1, Numata Kitagata-cho, Ichinomiya-city, Aichi-pref.,

493-8001 JAPAN

TEL : +81 (0) 586 86 5141

FAX : +81 (0) 586 86 6191

URL/http : //www.oryza.co.jp/

E-mail : info@oryza.co.jp



Tokyo sales office:

5F Diamant-building 1-5 Kanda-suda-cho

Chiyoda-ku, Tokyo, 101-0041 JAPAN

TEL :+81-3-5209-9150 FAX :+81-3-5209-9151

E-mail: tokyo@oryza.co.jp

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Newly added data: 4-6. Effect of β -sitosterol 3-O-glucoside (BSG) Existing in Glucosylcermaide Fraction on Enzyme Expressions Involved in Ceramide Production in Human Keratinocytes, page 27.

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ORYZA OIL & FAT CHEMICAL CO., LTD.