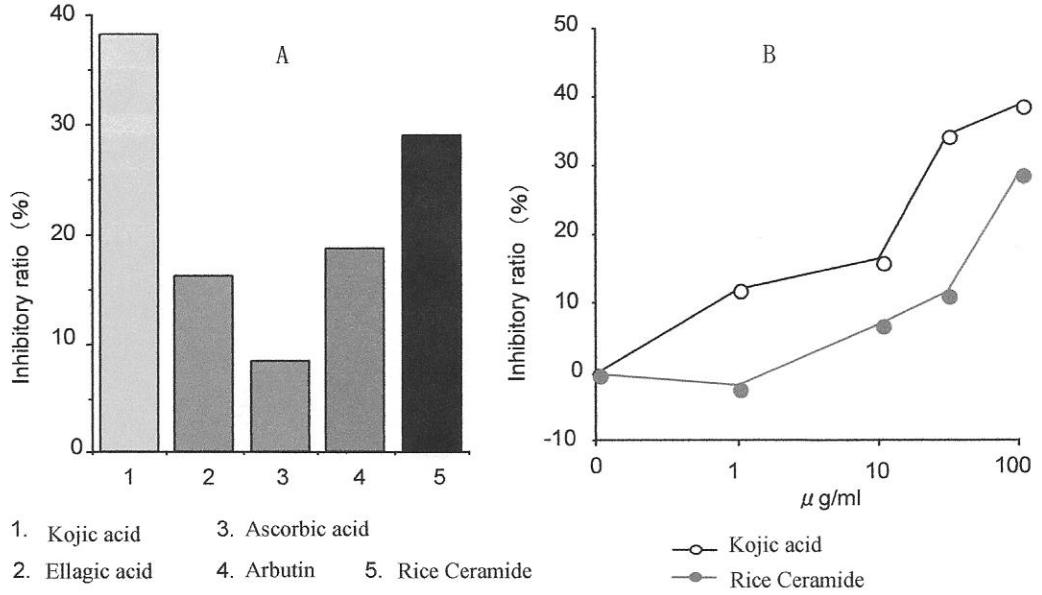
4-2-1. Suppression of Melanin Synthesis in Melanoma

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®.



1. Kojic acid
2. Ellagic acid
3. Ascorbic acid
4. Arbutin
5. Rice 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.

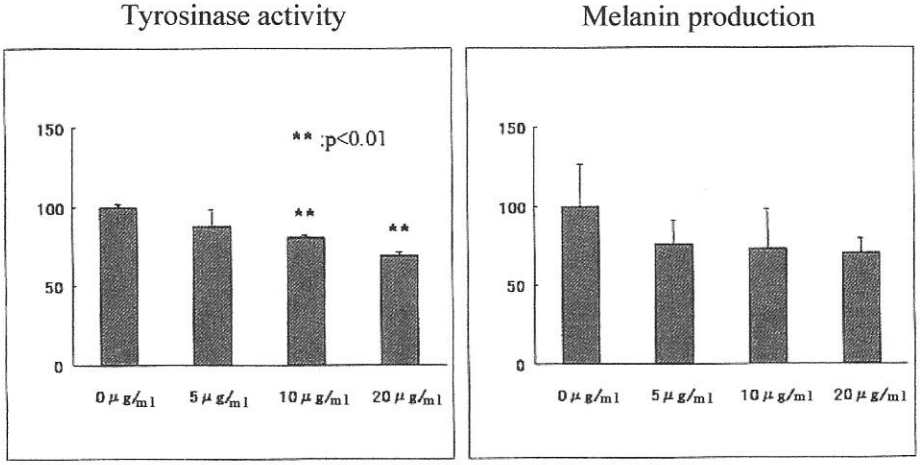


Fig. 21 Effects of rice-derived glycosphingolipid

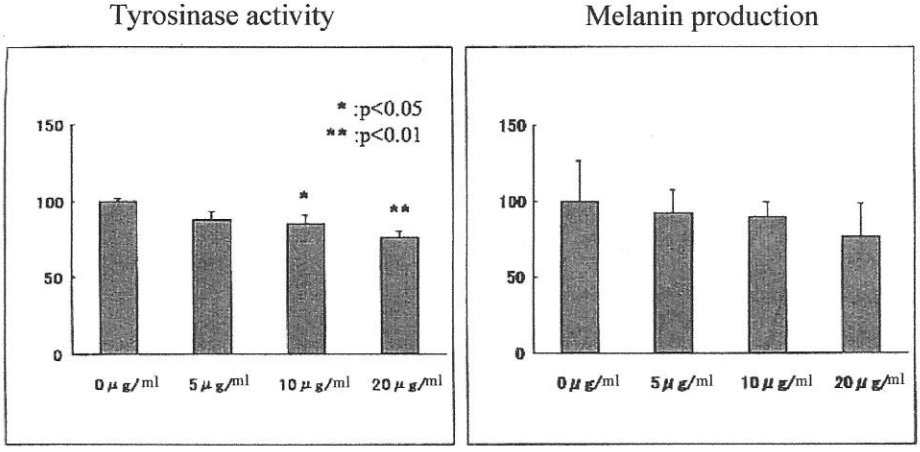


Fig. 22 Effects of rice-derived glycosphingolipid acid-degraded product

[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 grouth 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 μL/well) containing 1% tritonX-100, then mixed for 1 minuite. Ten μL of substrate (10 mM L-DOPA) was added to each well, and incubated for 1 hour at 37 ℃. 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 μL) for 30 minutes at 100 ℃. 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%.

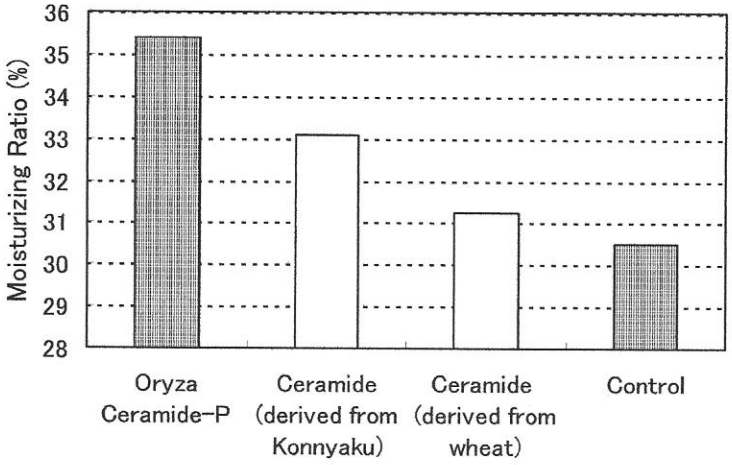


Fig.23 The moisturizing Effects of Ceramide

[Protocol]

Samples

1. ORYZA CERAMIDE®-P (from rice)