**Nutritional Cosmetics**
*Beauty from Within*

Nutritional cosmetics is an emerging area of intense research and marketing and encompasses the concept that orally consumed dietary products can support healthier and more beautiful skin. There are numerous dietary ingredients now being marketed for their potential skin health and beauty benefits and many of these are supported by growing scientific evidence. The purpose of this book is to compile the scientific evidence showing the potential benefits of some of the more extensively researched ingredients. As far as possible, information about the benefits of ingredients consumed orally for skin health is presented.

The information contained in this book will help provide insights into an emerging research area and provide scientific background for the potential clinical effectiveness for some of the better researched nutricosmetic ingredients.

- Reviews the most-popular and most-researched nutricosmetic ingredients
- Presents information specifically about the benefits of ingredients consumed orally for skin health
- Considers the benefits of whey protein, rosemary, soy – and green tea and milk thistle, specifically, for protection against sun damage and photocarcinogenesis
- Provides information on antioxidants, incl: potential benefits of botanical antioxidants; carotenoids; coenzyme Q10; healthy fruits; olive fruit; and natural enzymes.

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# 16

# Natural Products Supporting the Extracellular Matrix: Rice Ceramide and Other Plant Extracts for Skin Health

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## Summary

Natural cosmeceutical products are attracting increasing attention, especially from the ecological and safety point of view. Collagen, hyaluronic acid,

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elastin, and ceramides are components of the skin's extracellular matrix. Ceramides and glucosylceramides are contained in the stratum corneum of the epidermis and play roles in the barrier function and moisturization of the skin. Some of these components can be extracted and purified from plants. Rice ceramide extracted from rice germ and bran consists of at least six types of glucosylsphingolipids. This extract supports skin moisturizing and barrier function, suppresses melanin synthesis, promotes cell proliferation, and acts anti-inflammatorily and antiallergically. Therefore, application of rice ceramide in cosmeceutical products and as dietary supplements should be beneficial for maintaining a healthy skin extracellular matrix. Besides rice ceramide, we found that litchi seed extract, purple rice extract, and grape extract inhibit skin-tissue-degrading enzymes, for example collagenase, hyaluronidase, and elastase. Selective and combined application of these extracts is thus expected to help maintain skin health.

## 16.1 Introduction

In development of new skin care products, raw materials with novel physiological functions and proven safety play a key role. Choice of synthetic or natural materials for cosmetic composition depends on their cost, safety, and effectiveness. With a safe, novel, and ecological image, more and more consumers are favoring natural cosmetic components recently. In addition, consciousness of environmental aspects has led to the increased demand for organic cultivation, non-solvent extraction, and non-animal- and non-fish-derived materials. We have developed a number of extracts from various plants for application in foods and cosmetics. Ceramide, a skin component with moisturizing activity, can either be synthesized or extracted from plants such as rice, wheat, and corn. The demand for plant-derived ceramides, which can be used for both cosmetics and foods, is increasing. In this chapter, we introduce skin-healthy effects of rice-derived ceramide. We also describe beauty effects of litchi seed extract, purple rice extract, and grape extract.

## 16.2 Rice Ceramides

### 16.2.1 Ceramides in Skin

In 1884, ceramides were found in human brain tissues, and later in skin and mucosal tissue. Human skin consists of epidermis, corium, and tela



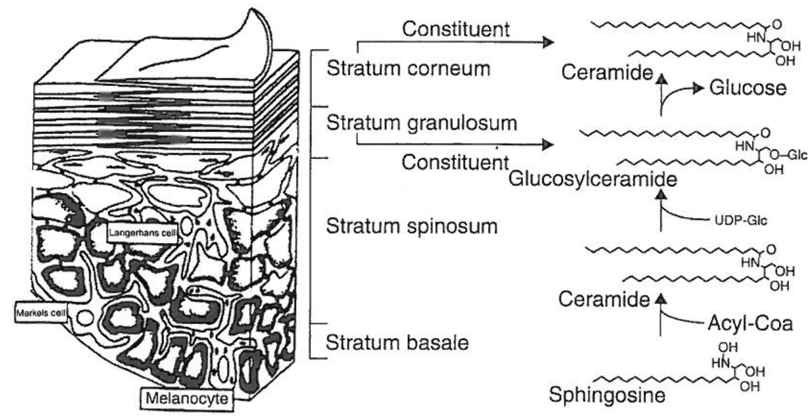


**Figure 16.1** Sphingolipids and their biosynthesis scheme in human skin.

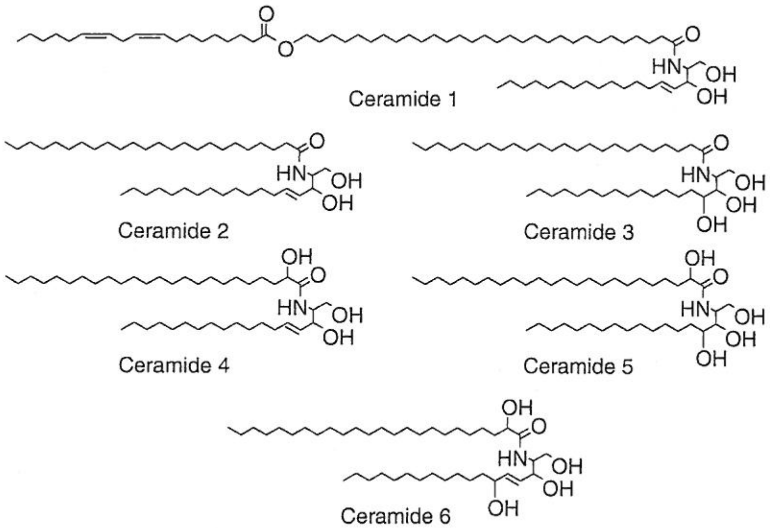


**Figure 16.2** Ceramides in stratum corneum.

subcutanea. The epidermis is classified into four layers, namely the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale (Fig. 16.1). More than six types of ceramides have been identified in human skin (Fig. 16.2) [1,2]. These ceramides are produced via several biosynthetic pathways in the epidermis and accumulated in the stratum corneum as the major constituent lipids (40–60% of the total lipids). In the epidermis, ceramides play an important role in forming lamella phases and in maintaining barrier function [3].

Imokawa et al. [4] demonstrated that the content of skin ceramides declines with age (Fig. 16.3). Forearm skin of elderly persons (especially those older than 70 years) is often xerotic, suggesting an association between ceramide decrease and skin drying. Marked reduction of ceramides was found in both lesional and nonlesional forearm stratum corneum of patients with atopic dermatitis (Fig. 16.4). These findings suggest that ceramides are a key factor for moisture maintenance and barrier function of the stratum corneum. A decrease in ceramide content is also thought to be associated with wrinkle formation. Thus, a sufficient amount of ceramides is considered to be essential for maintaining healthy skin.

### 16.2.2 Rice Ceramide

Rice (*Oryza sativa* L.) has been widely grown in Southeast Asia, not only as a major crop but also as an integral part of traditional culture and lifestyle in many Asian countries. In recent years, attention has been focused on rice bran and rice germ for its unique bioactive compounds and non-GMO profile. We have developed a number of products extracted from rice bran and rice germ as functional ingredients in medicines and cosmetics, as dietary supplements, and as food additives. One such functional compound is rice ceramide, which supports barrier function and moisture of skin. Rice ceramide contains a large amount of glucosphingolipids, which have

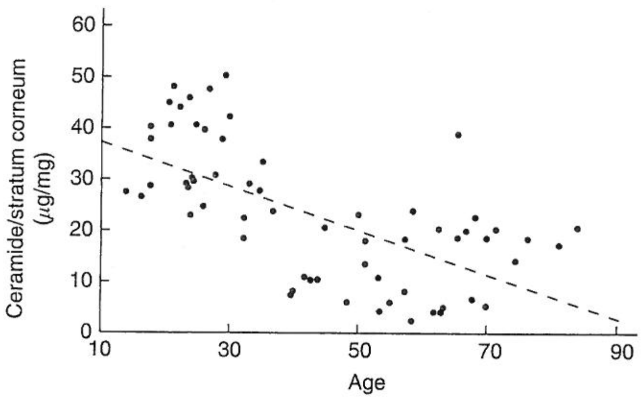


**Figure 16.3** Ceramide content of the stratum corneum in healthy subjects of various ages [4].



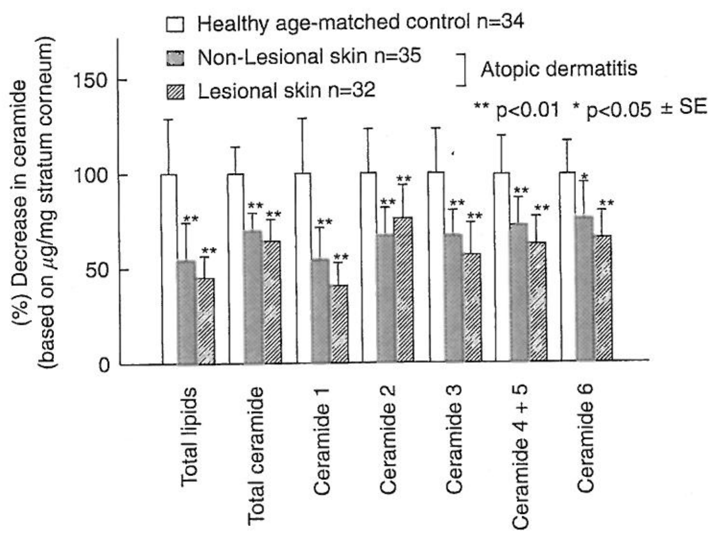


**Figure 16.4** Ceramide contents in forearm skin of healthy subjects and atopic dermatitis patients [4].

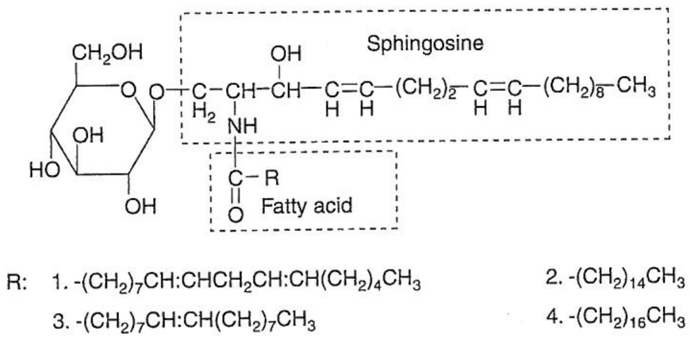


**Figure 16.5** Major glucosphingolipids in rice bran.

similar chemical structures to those from animals. Rice-derived glucosphingolipids consist of sphingoid bases conjugated with fatty acids by amide linkages. The terminal hydroxyl group is substituted to glucose. Glucosphingolipids are classified into different species depending on the structure of their sphingoid bases and fatty acids. Koga et al. identified more than 20 types of sphingolipids in rice bran [5]. We have identified four additional types of ceramides that are the major glucosphingolipids in rice bran (Fig. 16.5).

### 16.2.3 Digestion, Absorption, and Transport of Ceramides

Digestion, absorption, and metabolism of plant-derived sphingolipids have been studied by Schmelz et al. [6]. They examined the distribution and metabolism of sphingolipids in the intestine by tracing radio-labeled sphingomyelin in mice. Sphingomyelin appeared in all parts of the intestine, and most of it was catabolized to ceramides and their metabolites. Only 1% of undigested sphingomyelin was transferred from intestine to liver 30–60 minutes after administration. Transport of sphingomyelin and its metabolites from the intestinal canal to other tissues is thus not efficient. The absorption and metabolism of sphingolipids vary depending on the types of ceramides. Duan et al. [7] reported that sphingomyelin is digested by alkaline sphingomyelinase mainly in the middle and lower areas of the small intestine and that alkaline sphingomyelinase plays an important role in the first stage of digestion. Other researchers reported the effectiveness of oral application of ceramides for skin diseases. Kimata [8] gave ceramide (1.8 mg/day) to children with moderate atopic dermatitis for 2 weeks and confirmed that the treatment improved skin symptoms and reduced allergic responses. Asai and Miyachi [9] reported that topical application of rice ceramides for 3 weeks enhanced water contents in the stratum corneum. Thus, it is likely that at least a part of digested ceramides can be absorbed and can reach damaged skin, where they improve skin condition by retaining moisture.

### 16.2.4 Cosmeceutical Function of Rice Ceramide

In the past, mainly synthetic and animal-derived ceramides have been used for cosmetics. In recent years, risk of bovine spongiform encephalopathy has raised a safety concern for using cattle-derived ceramides. Consequently, more attention has focused on plant ceramides such as rice ceramide, which is considered suitable for both food and cosmetics. In this section, we review several cosmeceutical functions of rice ceramide. Most importantly, rice ceramide possesses an excellent moisturizing effect for skin, superior to that of ceramides from other plants, such as elephant foot and wheat, as demonstrated in our *in vitro* experiments (Fig. 16.6). Also, topical application of ceramide has been reported to improve skin moisturization [9,10]. Moreover, ceramide is effective in improving atopic dermatitis in animals and humans [11–13]. These observations suggest that ceramide is effective for supplementation of moisture for dry skin.

In addition, ceramide possesses an antipigmentation activity. We found that rice ceramide (100 μg/ml) inhibited melanin production in B16 melanoma



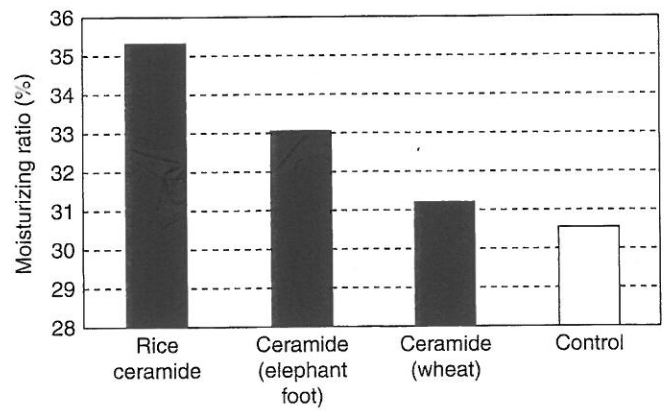


**Figure 16.6** Moisturizing effect of plant-derived ceramides. Each ceramide was suspended in water at 3% solution in test tubes, and kept for 8 hours at 35°C and 4% humidity.

cells *in vitro* (Fig. 16.7[a]). At the same concentration, activity of rice ceramide was higher than that of ascorbic acid, arbutin, and ellagic acid. Similar to kojic acid, rice ceramide exhibited a similar concentration-dependent activity in inhibiting melanogenesis (Fig. 16.7[b]).

In collaboration with Professor Igarashi at Hokkaido University, we found that suppression of melanin formation by ceramides involves inhibition of tyrosinase, which is a key enzyme for melanin synthesis. As shown in Fig. 16.8, rice glucosphingolipids exhibited an inhibitory effect on tyrosinase and on melanin production in a concentration-dependent manner. In addition to the tyrosinase inhibitory activity, ceramide has been reported to affect other pathways leading to melanin production. Kim et al. [14] found that C(2)-ceramide suppressed proliferation of mouse melanocytes *in vitro* via inhibition of the Akt/protein kinase B (PKB) activation that produces phosphorylated Akt/PKB. Moreover, using human melanocytes, they found that C(2)-ceramide decreased the protein expression of microphthalmia-associated transcription factor, which is required for tyrosinase expression. They further reported that C(2)-ceramide induced delayed activation of extracellular signal–regulated protein kinase (ERK) and Akt/PKB, which may lead to suppression of cell growth and melanogenesis [15]. These findings suggest that ceramide is effective in preventing skin pigmentation.

Ceramides also enhance proliferation of dermal fibroblasts. As illustrated in Fig. 16.9, except wheat-derived ceramide, all ceramides enhanced cell

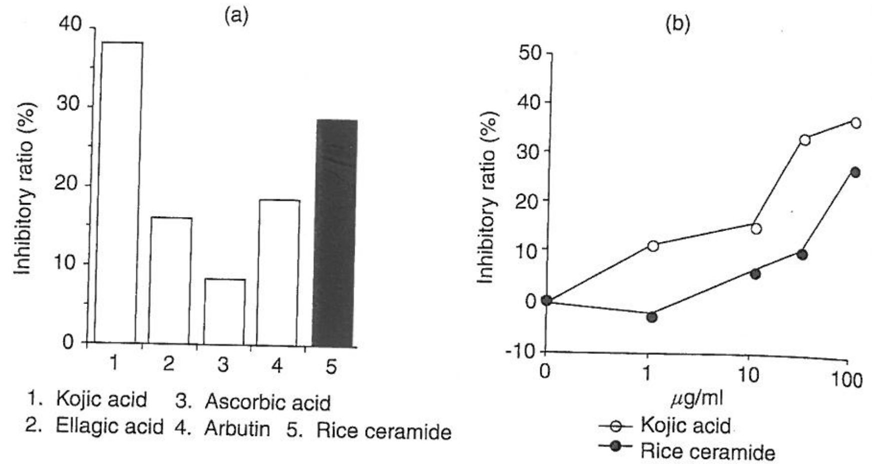


**Figure 16.7** Inhibitory effect of rice ceramide on melanin production in melanoma. (a) Comparison of rice ceramide with positive control at 100 μg/ml. (b) Dose response of kojic acid and rice ceramide. B16 melanoma cells ($2\times10^3$ cells/ml) were pre-incubated for 24 hours and the medium was replaced with new media containing 100 μg/ml emulsified glucosphingolipids (>90% of purity) or kojic acid. After 2-day incubation, the medium was replaced with fresh media, followed by another 2-day incubation. Cells were lysed in 2N NaOH, and absorbance was measured at 450 nm. The value was normalized by the cell number.

proliferation at 300 μg/ml whereas rice ceramides exhibited the most potent proliferative effect. The proliferative effect of ceramides seems to be mainly via the mitogenic activity of sphingosine-1-phosphate [16,17]. In contrast, apoptotic activity on murine keratinocytes has been reported for ceramide [18]. Marchell et al. [19] also reported that ceramide inhibits mitosis and induces terminal differentiation and apoptosis in keratinocytes. However, they also found mitogenic activity in glucosylceramide. Rice ceramides contain large amount of glucosylceramides (Fig. 16.5) and are thus expected to have an overall proliferative effect for keratinocytes.

We described in a previous section that topical and oral application of ceramides is effective against atopic dermatitis. Besides their moisturizing effect and barrier function, ceramides exhibit antiallergic and anti-inflammatory activities. We evaluated the effect of rice ceramide against itch in mice induced by compound 48/80 via mast cell degranulation histamine release in skin [20].



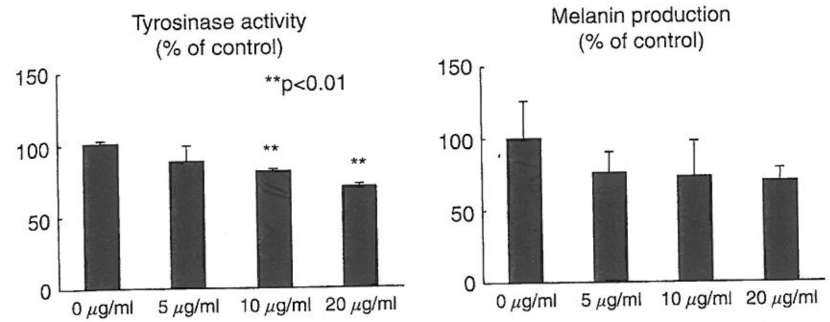


**Figure 16.8** Effects of rice glucosphingolipid on tyrosinase activity (left) and melanin production (right) in melanoma. Each column represents mean with the SE. For determination of tyrosinase activity, mouse melan-a cells ($1\times10^4$ cells/well) were incubated for 24 hours in RPMI 1640 medium containing 200 nM phorbor-12-myristate-13-acetate. The medium was replaced with new medium containing ceramide (glucosphingolipids: >95% of purity) and cultured for 2 days. Cells were lysed with PBS and the tyrosinase activity of lysate was determined using L-DOPA as a substrate. For evaluation of melanin production ability, melan-a cells ($3\times10^5$ cells/well) were cultured with ceramide under the same culture condition described above. Cells were lysed in 1 N NaOH, and absorbance was measured at 405 nm.

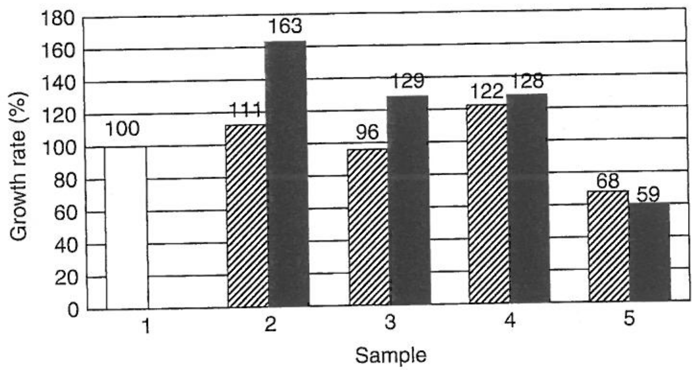


**Figure 16.9** Proliferative effect of various glucosphingolipids on human dermal fibroblasts. (1) Control, (2) rice ceramide, (3) elephant foot ceramide, (4) corn ceramide, (5) wheat ceramide. All ceramides contain more than 95% glucosphingolipids. Hatched and solid column represent 100 μg/ml and 300 μg/ml, respectively. Human dermal fibroblasts (HS-K, $1\times10^5$ cells) were cultured with various ceramides for 72 hours. Cell growth was determined by MTT assay.

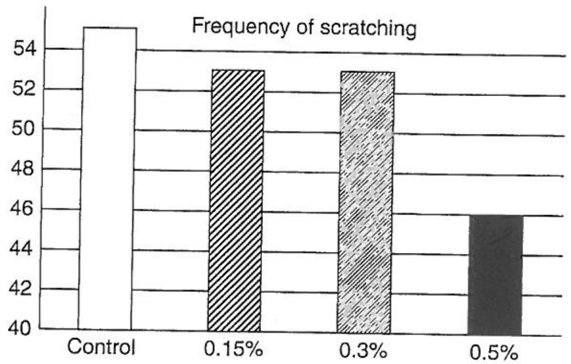


**Figure 16.10** Effect of rice ceramide on scratching behavior in mice induced by compound 48/80. Mice (ddY strain, male) were fed with rice ceramide (0.15–0.5%) freely for 3 days. Compound 48/80 solution (3%) was injected into the cervical skin. The scratching action was counted for 30 minutes after they started to scratch.

**Table 16.1 Inhibitory Effect of Ceramide on Degranulation from RBL-2H Cells**

| Origin | Inhibition (%) |
|---|---|
| Rice | 87.3 ± 2.2 |
| Wheat | 82.2 ± 5.9 |
| Elephant foot | 70.8 ± 5.9 |
| Corn | 64.2 ± 5.4 |

Sample concentration: 1 mg/ml, mean ± S.E., n = 6.

In the model, continuous oral administration of rice ceramide decreased scratching action in mice in a dose-dependent manner (Fig. 16.10), suggesting an antiallergic effect of rice ceramide. We then examined the effect of plant-derived ceramides on degranulation in sensitized mast cells (RBL-2H3). All of the plant ceramides inhibited mast cell degranulation at 1 mg/ml, with rice ceramide being the most potent (Table 16.1). The inhibitory mechanism of ceramides on mast cell degranulation likely involves suppression of phosphorylation of ERK1/2 and p38 mitogen-activation of protein kinase [21]. In contrast, several glycosphingolipids are reported to be mast cell activators. Prieschl et al. [22] reported that galactosylsphingosine enhanced relocation of the tyrosine kinases such as Lyn and Syk, leading to tyrosine phosphorylation followed by mast cell activation. Phosphorylated ceramide (ceramide 1-phosphate) has been reported to play an important role in mast



cell degranulation [23]. Masini et al. concluded that ceramide is a pro-inflammatory agent and that reducing ceramide levels is effective against allergic disease [24]. Principal ceramides contained in plants are glucosylsphingolipids, which are different from ceramides in the above studies. Plant ceramides may thus not act as inducers of allergic responses. However, further investigations are required concerning pro- and antiallergic activities of various ceramides.

## 16.3 Other Plant Extracts as Inhibitors for Skin Component Degradation

Strength and elasticity of skin are maintained by a balance of collagen, elastin, and hyaluronic acid [25,26]. Distributed in the entire dermis of the skin, collagen constitutes 90% of the dermis [27,28]. Hyaluronic acid is widely distributed in tissues such as skin, synovial fluid, vitreous body, and ligaments [29]. This skin tissue component is involved in cellular adhesion, in cell protection, and in maintenance of moisture and flexibility of the tissue. Skin loses moisture and tension, developing wrinkles and sagging as the level of hyaluronic acid decreases. Elastin is distributed in the dermis and is essential for maintaining appropriate elasticity and strength of the skin [30]. These skin constituents are degraded by collagenase, hyaluronidase, and elastase, respectively.

We have developed several plant extracts with inhibitory activities on these enzymes (Table 16.2). Litchi seed extract is extracted from crushed seed of *Litchi chinensis* with aqueous ethanol and contains saponins [31],

**Table 16.2 Inhibitory Effects of Plant Extracts on the Enzyme Activities Related to Skin Degradation**

| | Specification | $IC_{50}$ (μg/ml) | | |
|---|---|---|---|---|
| | | Collagenase | Hyaluronidase | Elastase |
| Litchi seed extract | Polyphenols: 24% | 59 | 290 | 45 |
| Purple rice extract | Polyphenols: 30%<br>Anthocyanins: 10% | >1,000 | >2,500 | 180 |
| Grape extract | Polyphenols: 40%<br>Resveratrol: 10% | 130 | 150 | 5 |

tannins [31], flavonoid (leucocyanidin [32]), and anthocyanins (cyanidin 3-*O*-glucoside and malvidin 3-*O*-glucoside). The biological effect of litchi seed extract has not yet been well studied. Extract of seeds and pericarps of red grapes contain polyphenols including flavonoids, anthocyanidins, and resveratrol. Purple rice extract contains anthocyanins (cyaniding 3-*O*-glucoside and malvidin 3-*O*-glucoside) as the major constituents. Whereas the former two extracts inhibit all three skin-component-degrading enzymes [33–35], purple rice extract selectively inhibits elastase.

Litchi seed extract exhibited the highest collagenase inhibitory activity ($IC_{50}$ = 59 μg/ml), which is also higher than that of persimmon leaf extract ($IC_{50}$ < 100 μg/ml) [36] and comparable to that of procyanidins isolated from grape (*Vitis vinifera*) seeds ($IC_{50}$ value = 38 μM) [37].

For hyaluronidase, the extract of seeds and pericarps of red grapes was the most potent inhibitor, with an $IC_{50}$ of 150 μg/ml. Litchi seed extract also inhibits this enzyme with an $IC_{50}$ of 290 μg/ml. For comparison, the $IC_{50}$ of myrrha (oleoresin from the *Commiphora mukul* tree), a traditionally natural product used for treatment of arthritis, for inhibiting hyaluronidase is as high as 1,000 μg/ml [38]. Hederagenin and oleanolic acid, sapogenins isolated from horse chestnut (*Aesculus hippocastanum*), have been reported to inhibit hyaluronidase with $IC_{50}$ values of 280.4 μM and 300.2 μM, respectively [39]. Similar types of saponins contained in litchi seeds are likely the principal compounds for the hyaluronidase inhibitory activity. In the case of grape extract, constitutive polyphenols including flavonoids, anthocyanidins, and resveratrol seem to account for the hyaluronic inhibitory activity because this activity has been reported for flavonoid [40].

All three extracts exhibited inhibitory activity for elastase, but litchi seed extract and grape extract are extremely potent ($IC_{50}$ value: 45 μg/ml and 5 μg/ml). For comparison, the $IC_{50}$ of elastase-inhibitory activity in the extract from black currant (*Ribes nigrum* L.) and lady's mantle (*Alchemilla vulgaris* L.) was 560 μg/ml and 160 μg/ml, respectively, as reported by Jonadet et al. after evaluating a number of plant extracts [41–43]. Hence, each of these three extracts is likely effective for elastin stabilization in skin.

## 16.4 Conclusion

Plant-derived ceramides consist mainly of glucosphingolipids, which are conjugated with glucose. In contrast, skin ceramides distributed in the stratum corneum are mainly sphingolipids. This difference in structures has been the concern for different biological functionalities of plant- and animal-derived and synthetic ceramides. We described the skin-health-promoting effect of rice ceramide in this chapter. In addition to the well-known barrier function and moisturizing effect, we found other novel activities in rice ceramide, such as inhibition of melanin synthesis, promotion of fibroblast proliferation, and anti-inflammatory and antiallergic effects. Supporting evidence for these skin-healthy effects of rice ceramide can also be obtained from a number of studies on various types of sphingolipids. Plant-derived ceramides can improve skin problems by topical and oral application. Further studies on plant ceramides are in progress that will provide more information supporting application of these skin-health-promoting components in development of new cosmetics. We also introduced several other plant extracts that inhibit collagenase, hyaluronidase, and elastase. These plant extracts are expected to be able to improve disturbed skin turnover and suppress excessive degradation of skin components. Finally, as extracts from common crops or cultivated plants, these natural materials are safe, ecological, and environment-friendly. We believe that these plant materials can be widely applied for various skin care products.



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# 17

# Asiaticoside Supports Collagen Production for Firmer Skin

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# Food-Derived Materials Improving Skin Cell Health for Smoother Skin

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## Summary

We have examined a number of plant-derived substances for their effects in protecting skin from damages and in promoting skin health. Oral application

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of rice ceramide significantly reduced dryness and exfoliation, enhanced moisture-retention, and improved smoothness and texture of skin in a placebo-controlled double-blind ingestion study in 33 subjects. Oral and topically applied plant-derived tocotrienol was transferred to skin, which acted as a scavenger of reactive oxygen species and was effective in protecting skin from UV damages. In addition, we introduce several other plant-derived extracts with fibroblast-proliferative, skin-turnover-promoting, and pigmentation-suppressive activities.

## 19.1 Introduction

For protecting skin from damage and for supporting skin functions, various synthetic chemicals and natural substances are added to cosmetics. For example, synthetic and plant-derived ceramides are being used in skin care products, which support barrier function and provide moisture to skin. Human epidermis consists of stratum basale, stratum spinosum, granulosa, and stratum corneum, each with a distinct lipid composition. In stratum basale, phospholipids and cholesterol are the major components. Glucosylceramide gradually increases from stratum basale toward the granulosa layer, where it becomes the major component. In the stratum corneum, glucosylceramide is converted to ceramide, which is the major component of interstitial lipids between corneocytes. Synthetic ceramides have been widely used as a skin-beautifying substance that holds moisture in the skin corneum. In recent years, natural ceramide is attracting more attention, especially because of its safe and healthy image. Plant ceramides are also a functional food for daily ingestion.

Application of antioxidants is regarded as effective for protecting skin from damage induced by reactive oxygen species, and thus preventing sunburn, inflammation, and pigmentation. Hydrophobic compounds are generally known to be effectively absorbed into skin by topical application. So is tocotrienol, a tocopherol-related compound. In this section, we describe cosmeceutical activities of ceramide, tocotrienol, and several other plant-derived substances.

## 19.2 Rice Ceramide Improves Skin Condition

The stratum corneum protects skin mechanically and maintains moisture. The major lipid components in the stratum corneum are ceramides.



Coderch et al. [1] demonstrated that topical supplementation of ceramide was effective for retaining moisture in skin. They swabbed healthy human skin with wool lipid (IWL) extracts with and without synthetic stratum corneum lipid (SSCL) as liposomes for several months. Transdermal water loss was improved and the water-retaining capacity of the skin was increased by mixed IWL/SSCL, but not by IWL alone. SSCL contains a ceramide, and these results thus suggest that topical application of ceramide is effective for maintaining moisture in the skin [2]. As an intracellular messenger in the sphingomyelin cycle, ceramide provides not only a barrier function to the skin but also regulates lipid biosynthesis [3]. Increase in intracellular ceramide induces skin cell differentiation and/or apoptosis, and suppresses cell proliferation.

Knowing the beneficial effects of topical application of ceramide, we studied the efficacy of oral application of ceramide in a placebo-controlled human study. We gave 40 mg rice ceramide to 33 subjects (6 male, 27 female) aged 25.1 ± 7.8 for 6 weeks and compared skin parameters before, during, and after the supplementation period. As shown in Fig. 19.1, skin moisture was increased and index of skin dryness (SE sc index) was reduced significantly compared to the placebo group. Figure 19.2 shows

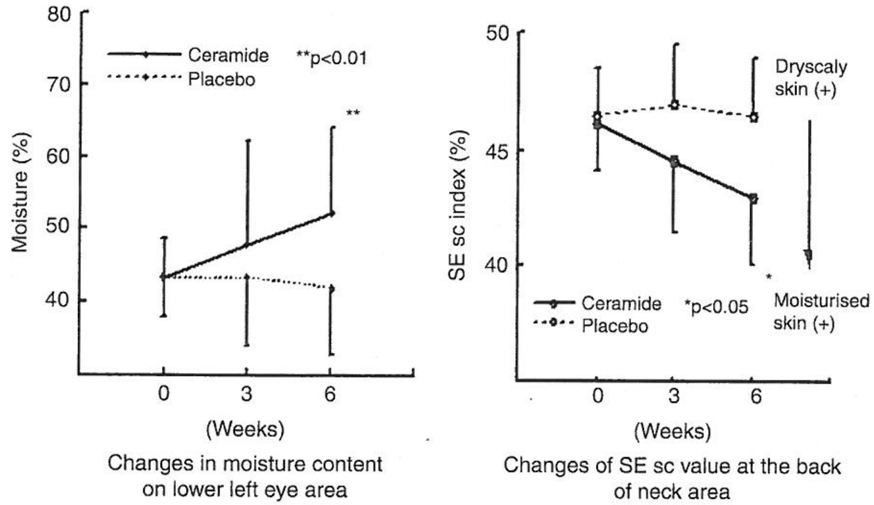


**Figure 19.1** Changes in skin parameters by oral treatment with rice ceramide in human subjects. Each point represents mean with standard deviation (SD). Asterisks denote significant differences from placebo.

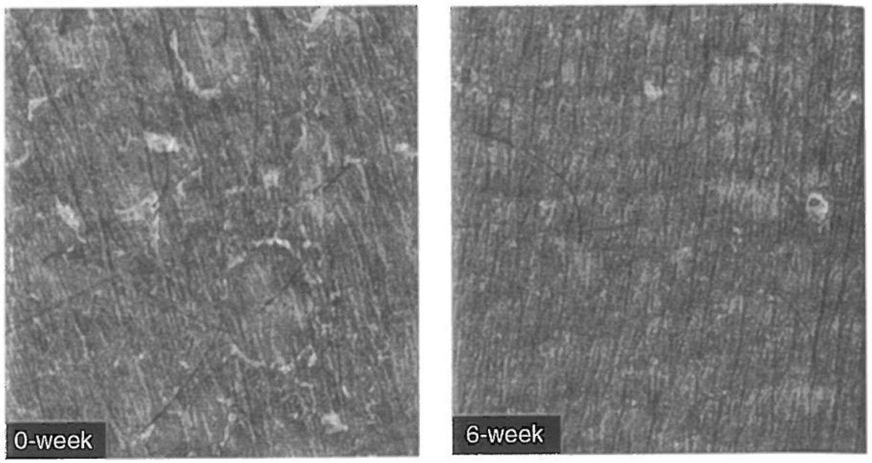


**Figure 19.2** Typical images of three-dimensional microscopic illustration of skin surface in the left eye area of a 23-year old female before and after 6 weeks of treatment with rice ceramide.

light microscope images of the skin of a female subject before and after ingestion of rice ceramide. Wrinkles were visibly reduced after 3 weeks of ingestion.

Other dermatological findings before and after ingestion of rice ceramide are summarized in Table 19.1. In facial skin, significant improvement was observed for "dryness," "flush," and "holding of cosmetic" after 3 weeks and 6 weeks of ingestion in the test group. In the placebo group, "dryness" and "flush" were also significantly improved after ingestion, but "holding of cosmetic" did not show significant improvement. Table 19.2 shows the evaluated improvement scores. Although improvement in "facial dryness," "flush," and "holding of cosmetic" was observed in both the ceramide and the placebo groups, the scores were better in the ceramide group. Regarding general skin parameters, significant improvement in "itching," "dryness," and "flush" was observed in both groups. However, the improvement rates were higher in the ceramide group and the overall improvement (64.7%) was only significant in the ceramide group (Table 19.2).

Water content, oil content, and acidity of the skin before and after ingestion in the ceramide and placebo groups are summarized in Table 19.3. Significant increase in water content was found in the face, neck, and forearm of subjects in the ceramide group after 6 weeks of ceramide ingestion. No



**Table 19.1 Results of Dermatological Diagnosis Before and After Ingestion of the Test Food**

| | | Ceramide Group | | | | Placebo Group | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. of subjects with symptoms | Initial | 3 weeks | 6 weeks | No. of subjects with symptoms | Initial | 3 weeks | 6 weeks |
| Face | Cosmetic rash | 4 | 1.25 | 1.00 | 1.00 | 3 | 1.00 | 1.00 | 1.00 |
| | Dryness | 17 | 2.00 | 1.29** | 1.18** | 16 | 2.13 | 0.69* | 1.63** |
| | Flush | 14 | 1.86 | 1.29** | 1.21* | 15 | 1.93 | 1.53* | 1.47* |
| | Holding of cosmetic | 8 | 1.88 | 1.13* | 1.00* | 5 | 1.40 | 1.20 | 1.20 |
| General | Itching | 17 | 1.65 | 1.24* | 0.94** | 12 | 1.92 | 1.50 | 1.17** |
| | Dryness | 17 | 2.18 | 1.47** | 1.18** | 16 | 2.00 | 1.44 | 1.38* |
| | Flush | 7 | 1.71 | 1.29 | 0.86* | 10 | 1.80 | 1.30* | 1.20* |
| | Erosion | 2 | 2.00 | 1.00 | 0.00 | 3 | 2.33 | 2.00 | 2.00 |
| | Squamation | 4 | 1.50 | 1.25 | 0.75 | 5 | 1.80 | 1.60 | 1.60 |
| | Papules | 3 | 1.33 | 1.33 | 1.33 | 4 | 1.75 | 1.50 | 1.75 |
| | Blebs | 2 | 1.50 | 1.50 | 1.50 | 2 | 1.50 | 1.50 | 2.00 |
| | Swelling | 0 | | | | 3 | 2 | 1.67 | 1.67 |
| | Overall | 17 | 1.71 | 1.24* | 1.00** | 16 | 1.69 | 1.25 | 1.31 |

The value represents the mean in each group. Asterisks denote significant differences from initial value at *$p < 0.05$, **$p < 0.01$, respectively.
0: no symptoms; –1: mild; –2: moderate; –3: severe.

**Table 19.2** Improvement Rate of Each Symptom

| Symptoms | | No. of subjects with symptoms | Improvement rating | | | | | Improvement rate (improved or better) |
|---|---|---|---|---|---|---|---|---|
| | | | Markedly improved | Improved | Unchanged | Aggravated | Markedly aggravated | |
| Facial symptoms | | | | | | | | |
| Cosmetic rash | Ceramide | 4 | 0 | 1 | 3 | 0 | 0 | 25.0% |
| | placebo | 3 | 0 | 0 | 3 | 0 | 0 | 0.0% |
| Facial dryness | Ceramide | 17 | 3 | 8 | 6 | 0 | 0 | 64.7% |
| | placebo | 16 | 0 | 8 | 8 | 0 | 0 | 50.0% |
| Facial flush | Ceramide | 14 | 2 | 8 | 2 | 2 | 0 | 71.4% |
| | placebo | 15 | 2 | 3 | 10 | 0 | 0 | 33.3% |
| Holding of cosmetic | Ceramide | 8 | 2 | 3 | 3 | 0 | 0 | 62.5% |
| | placebo | 11 | 0 | 1 | 4 | 0 | 0 | 9.1% |
| Somatic symptoms | | | | | | | | |
| Itching | Ceramide | 17 | 5 | 4 | 8 | 0 | 0 | 52.9% |
| | placebo | 12 | 4 | 1 | 7 | 0 | 0 | 41.7% |
| Dryness | Ceramide | 17 | 6 | 5 | 6 | 0 | 0 | 64.7% |
| | placebo | 16 | 1 | 8 | 7 | 0 | 0 | 56.3% |
| Flush | Ceramide | 6 | 2 | 2 | 2 | 0 | 0 | 66.7% |
| | placebo | 10 | 2 | 3 | 5 | 0 | 0 | 50.0% |
| Erosion | Ceramide | 2 | 2 | 0 | 0 | 0 | 0 | 100.0% |
| | placebo | 3 | 0 | 1 | 2 | 0 | 0 | 33.3% |
| Squamation | Ceramide | 4 | 2 | 1 | 0 | 1 | 0 | 75.0% |
| | placebo | 5 | 0 | 1 | 4 | 0 | 0 | 20.0% |
| Papules | Ceramide | 3 | 0 | 0 | 3 | 0 | 0 | 0.0% |
| | placebo | 4 | 0 | 1 | 2 | 1 | 0 | 25.0% |
| Blebs | Ceramide | 2 | 0 | 1 | 0 | 1 | 0 | 50.0% |
| | placebo | 2 | 0 | 0 | 1 | 1 | 0 | 0.0% |
| Overall | Ceramide | 17 | 3 | 8 | 5 | 1 | 0 | 64.7% |
| | placebo | 16 | 0 | 7 | 8 | 1 | 0 | 43.8% |

**Table 19.3** Measurement Results of Water Content, pH, and Oil Content Before and After Ingestion of the Test Food

| | | Ceramide group (n = 17) | | |
|---|---|---|---|---|
| | | Initial | 3 weeks | 6 weeks |
| Water content (arbital unit) | Under left eye | 43.2 ± 5.5 | 48.0 ± 14.3 | 52.2 ± 12.1** |
| | Left forearm | 37.0 ± 5.6 | 41.1 ± 11.0 | 43.2 ± 35.7** |
| | Dorsal neck | 43.5 ± 10.8 | 51.2 ± 11.76** | 55.9 ± 11.1** |
| Acidity (pH) | Under left eye | 5.8 ± 0.7 | 5.6 ± 0.6 | 5.8 ± 0.5 |
| | Left forearm | 5.5 ± 0.5 | 5.5 ± 0.6 | 5.8 ± 0.5 |
| | Dorsal neck | 5.9 ± 1.1 | 5.5 ± 0.5 | 5.4 ± 0.4 |
| Oil content (μg/cm$^2$) | Under left eye | 42.3 ± 34.8 | 49.9 ± 35.1 | 38.1 ± 25.9 |
| | | Placebo group (n = 16) | | |
| | | Initial | 3 weeks | 6 weeks |
| Water content (arbital unit) | Under left eye | 43.4 ± 5.4 | 43.2 ± 9.2 | 41.7 ± 9.4 |
| | Left forearm | 35.7 ± 5.6 | 37.7 ± 7.0 | 35.7 ± 9.0 |
| | Dorsal neck | 49.1 ± 8.8 | 51.0 ± 10.4 | 56.1 ± 20.5 |
| Acidity (pH) | Under left eye | 5.9 ± 0.8 | 5.8 ± 0.6 | 5.9 ± 0.7 |
| | Left forearm | 5.5 ± 1.0 | 5.6 ± 0.8 | 5.9 ± 0.5 |
| | Dorsal neck | 5.9 ± 0.8 | 5.5 ± 0.5 | 5.8 ± 0.4 |
| Oil content (μg/cm$^2$) | Under left eye | 58.4 ± 55.8 | 29.5 ± 22.0 | 40.8 ± 33.3 |

Each value represents the mean ± SD. Asterisks denote significant differences from initial value at **$p < 0.01$.



significant change was found in the placebo group. No significant change was found for acidity and oil content in either group (Table 19.3).

Results of imaging analysis of skin using VISIOSCAN are shown in Table 19.4. Significant improvement was obtained for kurtosis (representing smoothness of total skin), SE sm value (an index of skin smoothness calculated from the depth, width, and notch of furrows), and SE r (index of skin roughness) in the ceramide group. No significant improvement was found in the placebo group. SE w (number and width of skin wrinkles) was significantly improved only at the site below the left eye after 3 weeks of ingestion in the ceramide group. These findings show that a dietary supplement of rice-derived ceramide reduces skin dryness and improves general skin health.

## 19.3 Tocotrienol Prevents UV Damages in the Skin

UV photons induce skin damage mainly through two mechanisms [4]. One is the direct absorption of ultraviolet via cellular chromophores that can lead to photo-induced DNA base damage, with the consequence of increased mutation rate [4]. The other mechanism is photosensitization leading to formation of free radicals including reactive oxygen species (ROS) and reactive nitrogen species (RNS) [5]. ROS include singlet oxygen ($^1O_2$), superoxide anion, $H_2O_2$, and hydroxyl radical ($OH^{\cdot}$). ROS and RNS are also constantly generated in keratinocytes and fibroblasts, but rapidly neutralized by nonenzymatic (ascorbic acid, tocopherol, ubiquinol, and glutathione) and enzymatic (glutathione peroxidases, superoxide dismutases, catalase, and quinine reductase) antioxidants *in vivo* [5]. However, UV radiation produces excessive free radicals that cannot be neutralized by endogenous antioxidants. These excessive free radicals are the pathogenesis of a number of skin disorders, allergic reactions, and neoplasms. Moreover, ROS induce expression of activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) [5]. Activation of these factors causes inflammation, flare, and pigmentation. Highly reactive peroxynitrite ($ONOO^-$), which is a reaction product of super oxide anion and nitric oxide (NO), damages DNA and thereby causes point mutations, deletions, and chromosomal rearrangements [5].

Plant-derived polyphenols (e.g., caffeic acid, quercetin, genistein, resveratrol, nordihydroguaiaretic acid, carnosic acid, silymarin, catechins, and procyanidin B1) have antioxidative activity [6] and thus can help neutralize

**Table 19.4** Parameter Values Measured by VISIOSCAN Before and After Ingestion of the Test Food

| | | Ceramide group | | | Placebo group | | |
|---|---|---|---|---|---|---|---|
| | | Initial | 3 weeks | 6 weeks | Initial | 3 weeks | 6 weeks |
| Kurtosis | Under left eye | 0.38 | 0.37 | 0.35 | 0.39 | 0.38 | 0.38 |
| | Left forearm | 0.35 | 0.39 | 0.40 | 0.43 | 0.43 | 0.40 |
| | Dorsal neck | 0.40 | 0.40 | 0.30* | 0.40 | 0.40 | 0.40 |
| SE sm | Under left eye | 377.4 | 364.4 | 342.1 | 368.0 | 354.1 | 347.7 |
| | Left forearm | 339.4 | 304.8 | 308.5 | 326.1 | 317.3 | 334.2 |
| | Dorsal neck | 386.8 | 327.8* | 333.1* | 355.2 | 349.2 | 354.5 |
| SE r | Under left eye | 0.29 | 0.26 | 0.25* | 0.30 | 0.31 | 0.30 |
| | Left forearm | 0.26 | 0.20 | 0.16 | 0.31 | 0.26 | 0.25 |
| | Dorsal neck | 0.18 | 0.15 | 0.14* | 0.31 | 0.30 | 0.30 |
| SE sc | Under left eye | 49.6 | 47.6* | 46.8* | 46.6 | 46.8 | 46.6 |
| | Left forearm | 48.9 | 47.9* | 47.6* | 48.3 | 48.9 | 48.4 |
| | Dorsal neck | 46.1 | 44.5* | 42.9* | 46.4 | 46.9 | 46.4 |
| SE w | Under left eye | 36.1 | 32.3* | 33.9 | 36.0 | 33.0 | 35.5 |
| | Left forearm | 26.7 | 24.7 | 27.1 | 27.5 | 24.5 | 27.7 |
| | Dorsal neck | 28.4 | 24.7 | 26.1 | 28.2 | 25.9 | 30.6 |

The value represents the mean in each group. Asterisks denotes significant difference from initial value at $^*p < 0.05$. The ideal value of kurtosis is 0, and the other SE values are indicated as the ratio of ideal value versus low value.



excessive oxidants and prevent UV damages in the skin. Our laboratory has developed a number of extracts from rice germ and other plants for cosmetics and dietary supplements for supporting skin health. In this section, we introduce functions of tocotrienol, a hydrophobic antioxidant derived from rice bran oil and palm oil.

Rice bran oil is a traditional plant-derived oil widely used as a cooking oil in Southeast Asia. The oil contains large amounts of unsaturated fatty acids, as well as other healthy constituents such as γ-oryzanol, sterols, tocopherols, and tocotrienols. Our product Oryza tocotrienol™ is extracted and refined from rice bran oil. It contains large amounts of tocotrienols and tocopherols. The chemical structure of tocotrienol is similar to that of tocopherols (Fig. 19.3). γ-Tocotrienol is the principal constituent in rice tocotrienols and has been demonstrated to show biological activity in skin. Weber et al. [7] found that topical application of tocopherols and tocotrienols to mice increased content of α-tocopherol and vitamin E derivatives in their skin (Fig. 19.4). Even after UV irradiation, the remaining contents of these components were still significantly higher than that of the control group. The result shows that topically applied tocotrienol and tocopherol can permeate into skin and protect skin from UV damage.

Also, orally applied tocotrienols have been reported to reach skin. Khanna et al. [8] revealed that tocotrienol given orally reached the skin in tocopherol-deficient mice. Ikeda et al. [9,10] reported that continuous supplementation of γ-tocotrienol increased its contents in rat skin. Human epidermis contains 3% tocotrienol and 1% tocopherol [11]. Hence, oral

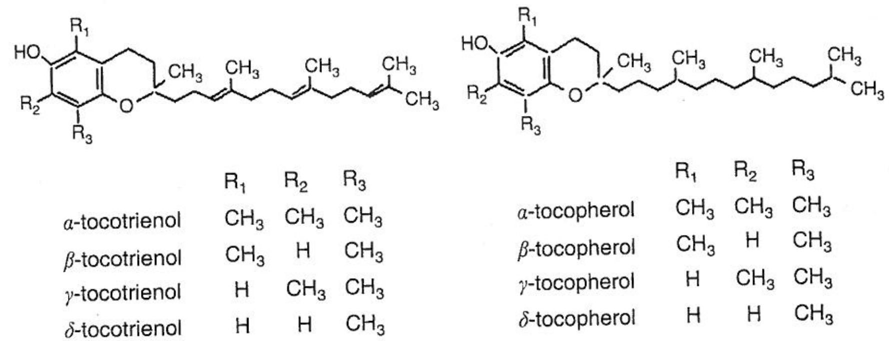


**Figure 19.3** Structures of tocotrienols and tocopherols.

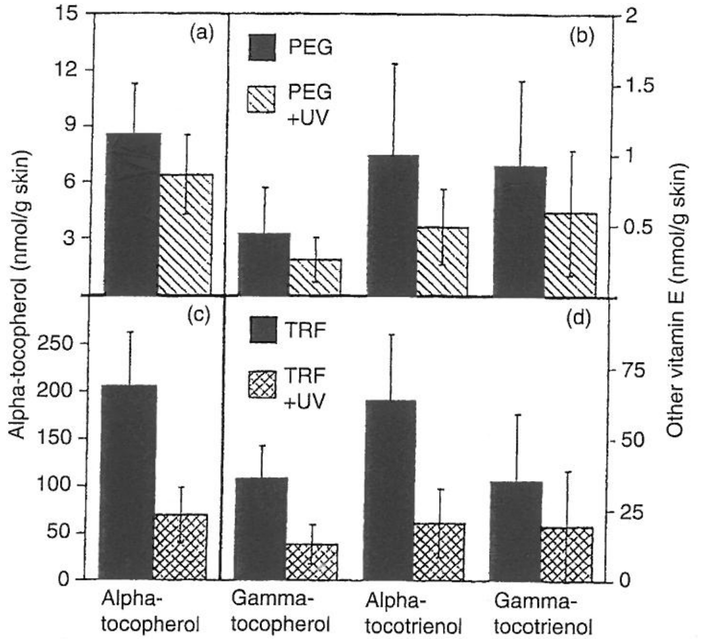


**Figure 19.4** The contents of alpha-tocopherol and other vitamin E derivatives in murine skin treated with tocotrienol rich fraction (TRF) containing alpha-tocopherol, gamma-tocopherol, alpha-tocotrienol, and gamma-tocotrienol [7]. Each column represents mean with SD. Polyethylene glycol (PEG) means control group.

application of tocotrienol is expected to be effective for the protection of skin from UV.

## 19.4 Plant Extracts Enhance Skin Turnover and Inhibit Skin Pigmentation

### 19.4.1 Enhanced Fibroblast Proliferation

Fibroblasts play significant roles in epithelial-mesenchymal interactions, secretion of various growth factors and cytokines, and differentiation and formation of the extracellular matrix [12]. The interaction of fibroblasts and keratinocytes is essential in the wound-healing process [13]. Keratinocytes stimulate fibroblasts to synthesize growth factors, which in return stimulate keratinocyte proliferation in a double-paracrine manner. We found a number of plant extracts that enhanced fibroblast proliferation



**Table 19.5 Proliferative Effect of Food-Derived Materials on Fibroblast Proliferation**

| | Cell name | Concentration (μg/ml) | Proliferative ratio (%) |
|---|---|---|---|
| *Citrus unshiu* peel extract | NB1RGB | 10 | 20 |
| Yuzu seed extract | NB1RGB | 10 | 40 |
| Kiwifruit seed extract | NB1RGB | 10 | 70 |
| Rice ceramide | HSK | 300 | 63 |
| α-Lipoic acid | NB1RGB | 25 | 22 |
| Rice tocotrienol | NHDF | 25 | 22 |

Cells were treated with each sample for 2 days.

*in vitro* (Table 19.5). Kiwifruit seed extract contains flavonoid glycosides such as quercitrin and kaempferol 3-*O*-rhamnoside. The biological activity of this extract has not yet been well studied. Yuzu (*Citrus junos*) is a citrus species that is mainly cultivated in Japan. Yuzu seed extract contains limonoids (triterpenoids) such as limonin and nomilin. *Citrus unshiu* is also a citrus species fruit mainly cultivated in Japan and China. It contains β-cryptoxanthin and flavonoids (hesperidin). Kiwifruit seed, a food-derived material extract, exhibited the most potent proliferative effect for fibroblasts (70% at 10 μg/ml), followed by yuzu seed extract (40% at 10 μg/ml), *Citrus unshiu* peel extract (20% at 10 μg/ml), and rice tocotrienol (22% at 25 μg/ml). The activity of synthetic α-lipoic acid in enhancing fibroblast proliferation was similar to that of rice tocotrienol. In addition to its fibroblast-proliferative effect, yuzu seed extract also inhibited 5α-reductase and lipase. Application of these plant-derived substances in cosmetics may enhance proliferation of skin fibroblasts.

### 19.4.2 Enhanced Skin Turnover

The epidermis is constituted and continuously regenerated by terminally differentiating keratinocytes [14]. In the wound-healing process, an increase in cytokine and growth factor production from keratinocytes and fibroblasts is induced to enhance skin cell proliferation [15]. For evaluation of effects of various compounds on skin turnover, a three-dimensional cell culture system has been established by which skin-composing cells are seeded trilaminarily onto membranes that form epidermis, basal lamina, and dermis layers [16–19]. Using this *in vitro* human skin model, we

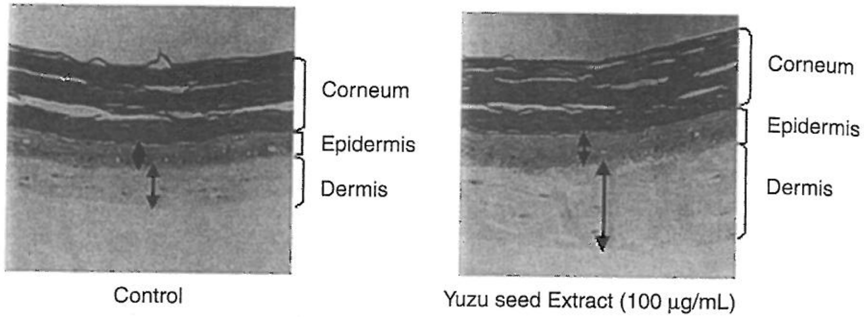


**Figure 19.5** Light microscope photograph (×400) of cultured three-dimensional human skin model (TESTSKINTM, Toyobo Co., Ltd., Japan) with and without supplementation of yuzu seed extract.

evaluated the effect of our plant extracts on skin turnover. Yuzu seed extract was found to enhance thickening of the epidermis and dermis (Fig. 19.5), suggesting its proliferative activity for skin cells in these layers. The result suggests that yuzu seed extract promotes skin turnover and may have a softening effect for the skin, and thus can be used as an additive for skin care products.

### 19.4.3 Inhibited Skin Pigmentation

Melanin is produced from tyrosine by multiple enzymes contained in melanocytes in the basal layer of the epidermis. Because tyrosinase is the rate-limiting enzyme in melanin production, inhibition of this enzyme has been considered as a basic strategy for developing skin care products with whitening effect. We found tyrosinase-inhibiting activity in a number of plant extracts and in α-lipoic acid, as shown in Table 19.6. Litchi seed extract and evening primrose seed extract exhibited the strongest tyrosinase-inhibitory effect, followed by *Citrus unshiu* peel extract, broccoli sprout extract, and kiwifruit seed extract. In addition, melanin formation in melanoma cells was suppressed *in vitro* by most of the extracts we tested to various extents (Table 19.6). Finally, topical or oral application of some of these extracts suppressed UV-induced pigmentation in guinea pigs (Fig. 19.6) [20]. In particular, litchi seed extract, kiwifruit seed extract, and rice ceramide inhibited tyrosinase, suppressed melanin formation *in vitro*, and suppressed UV-induced pigmentation *in vivo*. The former two extracts contain polyphenols: litchi seed extract contains anthocyanins such as cyanidin 3-*O*-glucoside and malvidin 3-*O*-glucoside, and evening primrose



**Table 19.6** Suppressive Effects of Food-Derived Extracts on Various Melanin Formation Assays

| | Tyrosinase activity inhibition (%)/ concentration (μg/ml) | Melanin formation in B16 melanoma inhibition (%)/ concentration (μg/ml) | Pigmentation in UV-irradiated guinea pig recovery[a] (%)/dose (mg/kg or %), administration route |
|---|---|---|---|
| Litchi seed extract | 100/1200 | 35/100 | 63/1, topical |
| Evening primrose seed extract | 80/1000 | – | – |
| *Citrus unshiu* peel extract | 50/1000 | 55/1000 | 61[b]/800, oral |
| Yuzu seed extract | – | 25/100 | – |
| Broccoli seed extract | 49/1000 | 40/100 | – |
| Kiwifruit seed extract | 38/1000 | 30/100 | 100/800, oral |
| Purple rice extract | 85/500[c] | 49/500 | – |
| Rice ceramide | 20/20[d] | 30/100 | 41/1, topical |
| α-Lipoic acid | – | 35/100 | 51/800, oral |
| Rice tocotrienols | – | – | 39/1[e], topical |

[a]Recovery ratio from initial luminosity value; [b]inhibitory ratio; [c]co-treatment with ascorbic acid (5 mg/ml); [d]melan-a cells; [e]as total tocotrienols and tocopherols.

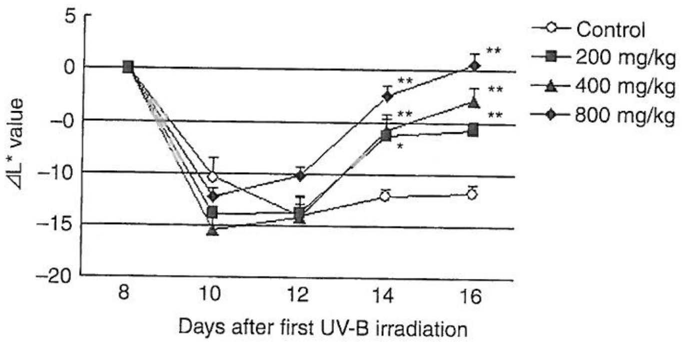


**Figure 19.6** Effect of kiwifruit seed extract on skin hyperpigmentation in guinea pigs induced by UV-B. Each point represents mean with the S.E. (n = 4). Asterisks denote significant differences from the control, *$p < 0.05$, **$p < 0.01$, respectively. Decrease in $\Delta L^*$ value means change in brightness to darker.

seed extract contains condensed tannins and hydrolyzable tannins. Ceramide is a skin component and its content decreases with age and in dermatitis. These extracts provide effective ingredients for skin-whitening products.

## 19.5 Conclusion

We described the effects of several of our extracts derived from plants in preventing skin damage induced by UV, in enhancing fibroblast proliferation and skin turnover, and in suppressing skin pigmentation. Oral and topical application of these substances may thus protect skin and promote skin health.

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# PART 7
# NATURAL SUPPORT FOR A HEALTHIER COMPLEXION