TOYO HAKKO

A ferment filtrate material with anti-inflammatory, antioxidant, and moisturizing properties that protects the skin from various external stimuli

Black rice fermented with *Monascus purpureus*

RICETECT®

RICETECT® is an original product made by fermenting *kitanomurasaki*, the only black rice variety originating in Hokkaido, Japan, with soybean cheese-derived *Monascus purpureus*.

With its anti-inflammatory, antioxidant, moisturizing, and barrier-function-improving functions, it protects the skin from various stress as caused by external stimuli.

Characteristics of RICETECT®

Protects the skin from external stimuli, leading to healthy, young skin!

- Made by fermenting *kitanomurasaki* black rice with soybean cheese-derived *Monascus purpureus*
- Has a natural index of 1.0 (compliant with ISO16128)

＜Overview of functions＞

● Anti-inflammatory

Inhibits excess production of NO and IL-6 during inflammation (patent pending)

● Antioxidant

Eliminates active oxygen and protects the cells from active oxygen and UVB

● Moisturizing

Promotes expression of the hyaluronan synthase 3 (HAS3) gene

● Barrier function improvement

Promotes the production of involucrin

● Anti-wrinkle and skin resilience improvement

Promotes collagen production in fibroblasts

● Turnover function improvement

Promotes cell proliferation in epidermal keratinocytes

Product Name		RICETECT	
Cosmetics	INCI	Chinese INCI	Component ratio (%)
	Monascus/Rice Ferment	红曲（MONASCUS）/大米发酵产物	70
	Propanediol	1,3-丙二醇	30
Standard packing		20 kg/Bag in box	
Storage		Store in a dark place at room temperature	

Standard item	Standards	Test method
Appearance	clear to yellowish brown liquid with characteristic odor	Sensory test
pH	4.0～5.5	JSQI general test method
Specific gravity	Actual measurement value	Vibration-type density meter
Heavy metals (as Pb)	≤20ppm	JSQI general test method
Arsenic (as As2O3)	≤2.0ppm	ICP emission spectrometry
Viable molds and yeasts count	≤100 cfu/mL	SCDLP agar culture
Coliform organisms	Negative	AOAC (Petrifilm method)
Viable molds and yeasts count	≤100cfu/ml	AOAC (Petrifilm method)

Various external stimuli: Cause of decreased barrier function and skin roughness

In recent years, environmental issues are becoming more serious around the world, and in addition to impact on the natural environment, impacts on humans are also being reported. In particular, air pollution and UV rays have major impact on the human skin. They are known to cause oxidative stress and to decrease barrier functions, and they have been reported to cause skin roughness, spots, and wrinkles.

Other external stimuli include wearing a face mask for protection from viruses or other reasons. Masks have been reported to cause skin trouble such as acne, severe dryness, and redness. These troubles are caused by oxidation, inflammation, and dryness of the skin, resulting in decreased skin barrier function.

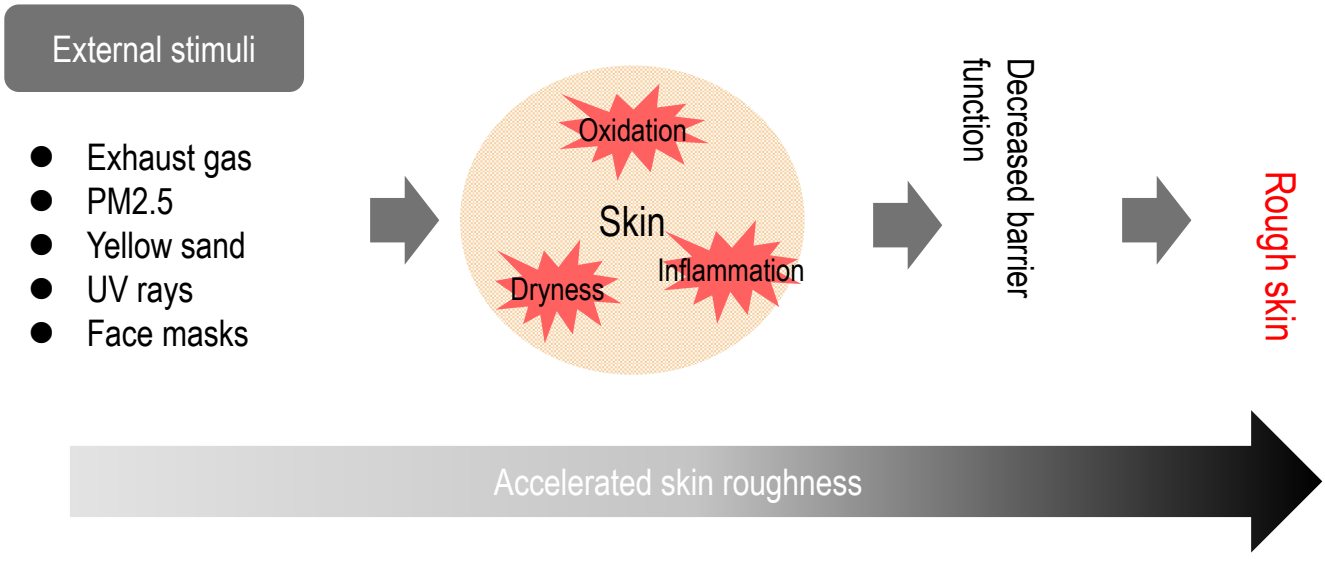
Mechanism of skin roughness and aging caused by air pollution
1. Polycyclic aromatic hydrocarbons (PAHs) such as benzopyrene found in exhaust gas are oleophilic, enabling then to penetrate the cell membrane, and when they bind to the aryl hydrocarbon receptor (AhR) in skin cells, they promote CYP1A1 expression, which induces the production of reactive oxygen species (ROS).
2. Excessive production of ROS by epidermal cells induces the production of inflammation causing inflammatory cytokines and wrinkles causing collagenolytic enzyme MMP-1.
3. As a result, the skin becomes rough, and wrinkles are formed.

Causes of skin roughness from wearing a face mask
1. Excessive sebum secretion: Wearing a face mask for a long period of time makes the area inside the mask hot and humid, causing the excessive secretion of sebum. The sebum become oxidized to form keratotic plugs in the pores and acne, leading to decreased skin barrier function.
2. Friction: Physical stimulation caused by friction between the mask fibers and skin causes inflammation, leading to decreased barrier function.
3. High temperature and humidity: Wearing a face mask for a long period of time makes the area inside the mask hot and humid. This causes the skin to become soft, leading to rapid water evaporation from inside the skin when the mask is removed. As a result, the skin becomes dry, and the barrier function decreases.

Skin with decreased barrier functions is more easily affected by the above, causing the skin to become rougher and aged.

Decreased barrier functions: Cause of accelerated skin roughness!

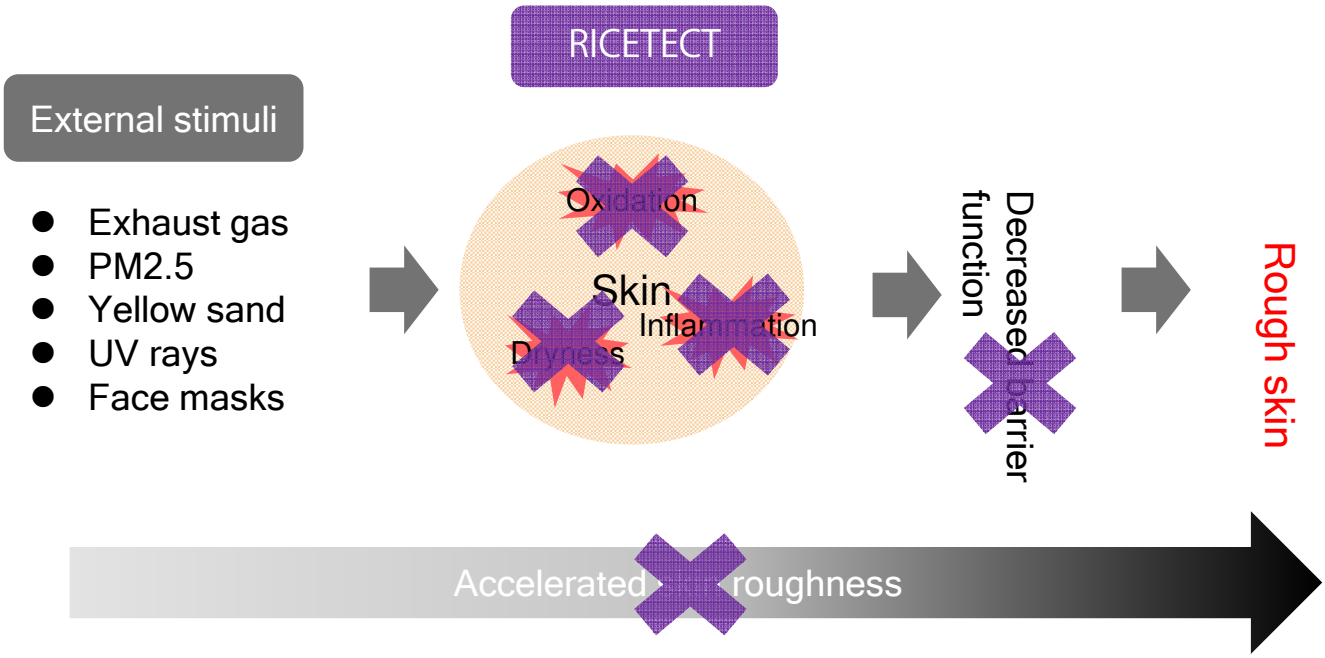
When the skin barrier function decreases due to the stress from external stimuli, it becomes more easily affected by such stimuli. As a result, skin roughness accelerates, leading to more-severe skin-related trouble.



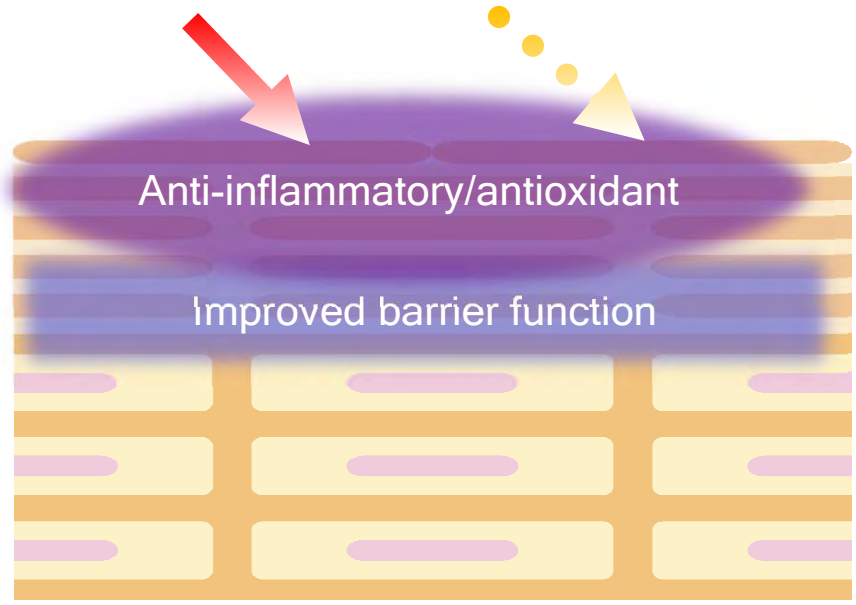
Effects of RICETECT: Protecting the skin from stress caused by external stimuli

Comprehensively “protecting” the skin from various skin stress

The results of a cell test revealed that RICETECT effectively mitigates the causes of skin trouble and aging, including oxidation, inflammation, and dryness as caused by external stimuli. As an anti-inflammatory effect, RICETECT inhibits the production and expression of NO and IL-6 in skin cells during inflammation. As an antioxidant effect, it protects the cells from oxidative stress (H_2O_2 and UV). As a moisturizing effect, it promotes the expression of the hyaluronan synthase 3 (HAS3) gene. Also, as for the barrier function improvement effect, it promotes involucrin production. Furthermore, as for a turnover function improvement effect, it promotes cell proliferation. Finally, as for its anti-wrinkle and resilience improvement effect, it promotes cellular collagen production.
RICETECT thus has the power to comprehensively “protect” the skin from skin stress.



External stimuli (air pollution, UV rays, face masks, etc.)



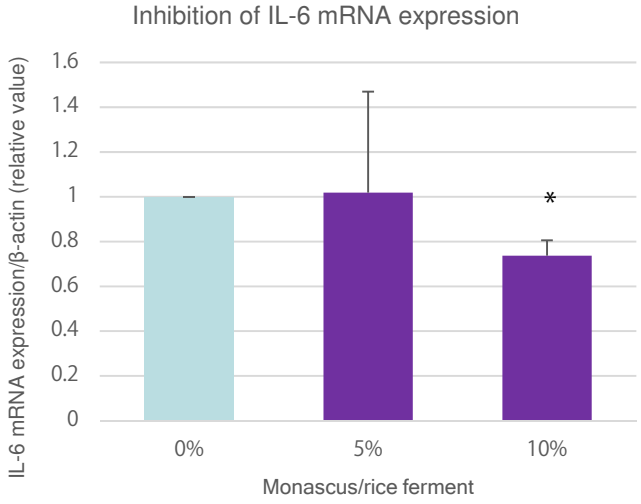
Coating the skin with a ferment filtrate “protects” the skin from various external stimuli.

(1) Anti-inflammatory effects: Inhibiting the production of inflammatory cytokines and NO

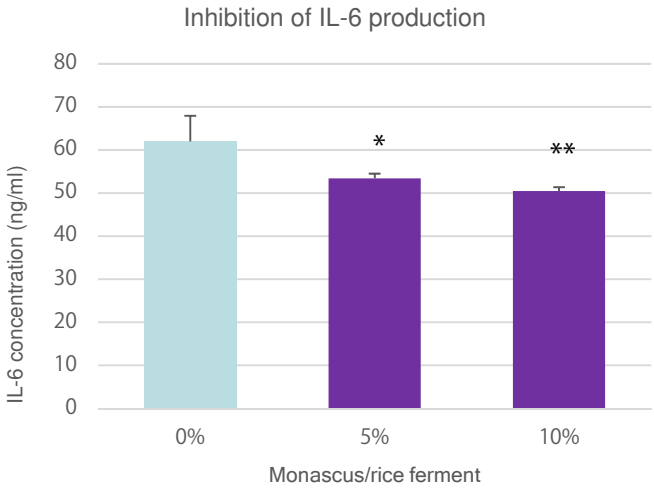
1. Inhibited production of inflammatory cytokine IL-6

When the skin is exposed to UV rays for a long period of time, IL-1 activation in epidermal cells increases. Stimulation by IL-1 induces the production of inflammatory cytokine IL-6. The production of IL-6 is known to be related to excessive inflammatory reactions and chronic inflammatory diseases.
As a test, monascus/rice ferment was added to macrophage-like cells RAW264, along with inflammatory LPS derived from *Escherichia coli*, and was incubated for 24 hours. Then, RNA was extracted from the cells and gene expression was analyzed via real-time PCR.
The results, as shown in the graphs below, confirm that monascus/rice ferment inhibits IL-6 gene expression.

Also, lipopolysaccharide and monascus/rice ferment were added to similarly cultivated macrophage-like cells RAW264 and incubated for 24 hours. Then, the culture supernatant was collected, and the IL-6 concentration was measured using a commercially available Mouse IL-6 kit.
As a result, monascus/rice ferment was confirmed to inhibit concentration-dependent IL-6 production. Thus, monascus/rice ferment is expected to inhibit skin inflammation.



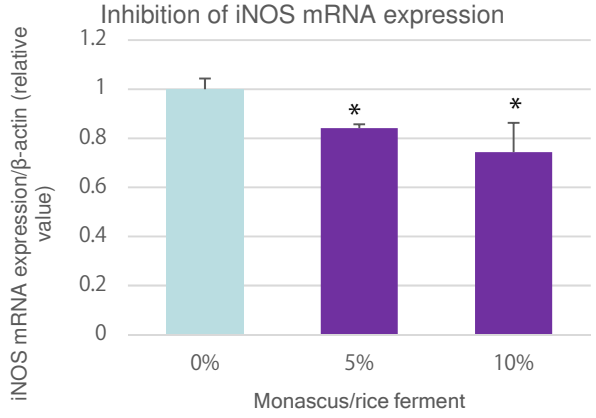
* P<0.05 (vs. control)



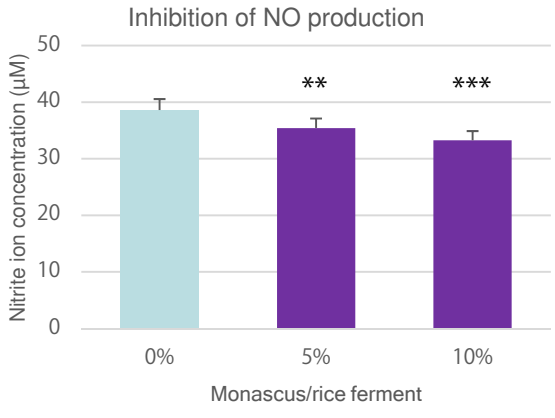
* P<0.05
** P<0.01 (vs. control)

2. Inhibited production of NO

While nitric oxide (NO) produced from macrophage-like cells, etc., exhibits bactericidal properties as a radical and the elimination of foreign substances as an immune signal, excessive NO production damages cells and worsens inflammation. Thus, inhibiting excessive NO production is believed to lead to reduced inflammation.
In the test, macrophage-like cells RAW264 were used to evaluate the ability of monascus/rice ferment to inhibit the expression of the inducible nitric oxide synthase (iNOS) gene, which induces NO production, and to inhibit the production of NO concerning iNOS gene expression, and monascus/rice ferment was added to a culture of macrophage-like cells RAW264 along with inflammatory LPS derived from *Escherichia coli* and incubated for 24 hours. Then, RNA was extracted from the cells and gene expression was analyzed via real-time PCR. Concerning NO production, Griess reagent was added to the supernatant collected from a similarly incubated culture, and after allowing the reagent to react for 10 minutes at room temperature, a measurement of absorbance was made at OD530/700 nm. The NO concentration was calculated using the sodium nitrite standard curve.
As a result, monascus/rice ferment was confirmed to inhibit concentration-dependent iNOS gene expression and NO production. Thus, monascus/rice ferment is expected to inhibit skin roughness caused by inflammation.



* P<0.05 (vs. control)



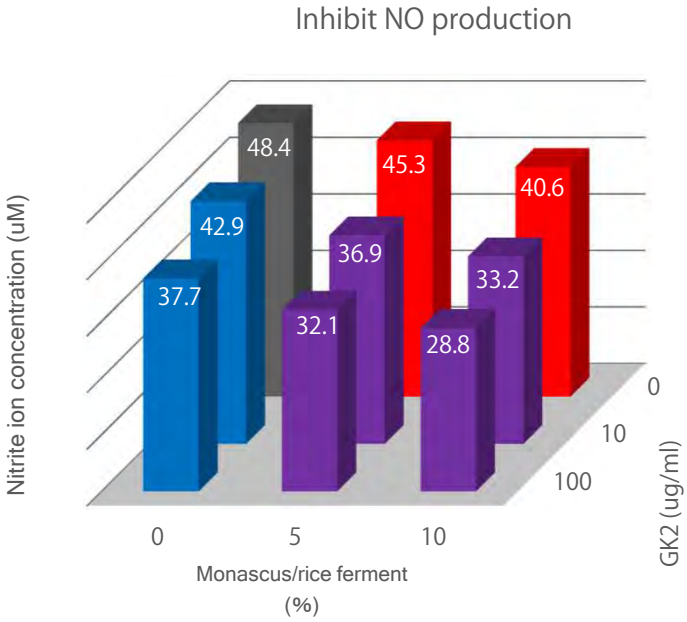
** P<0.01
*** P<0.001 (vs. control)

(1) Anti-inflammatory effects: Synergetic inhibition of NO production in combination with dipotassium glycyrrhizinate

While nitric oxide (NO) produced from macrophage-like cells, etc., exhibits bactericidal properties as a radical and the elimination of foreign substances as an immune signal, excessive NO production causes angiectasis, damages cells, and worsens inflammation. Thus, inhibiting excessive NO production is believed to lead to reduced inflammation.
In this test, through combined use with dipotassium glycyrrhizinate (GK2), which is reported to have anti-inflammation effects, the synergetic effects for inhibiting NO production were evaluated. The evaluation was conducted using macrophage-like cells RAW264. The sodium nitrite standard curve was used to calculate the NO concentration, and the scale factor of the synergetic effect was calculated using the formula below.

Scale factor of the synergetic effect = (NO concentration actually inhibited when mixed / Sum of NO concentration inhibited by monascus/rice ferment alone + Dipotassium glycyrrhizinate alone). When the resulting number exceeds one, a synergetic effect exists.

As a result, the synergetic NO production inhibition effect was confirmed. Monascus/rice ferment is expected to further inhibit skin roughness caused by inflammation via combined use with dipotassium glycyrrhizinate.



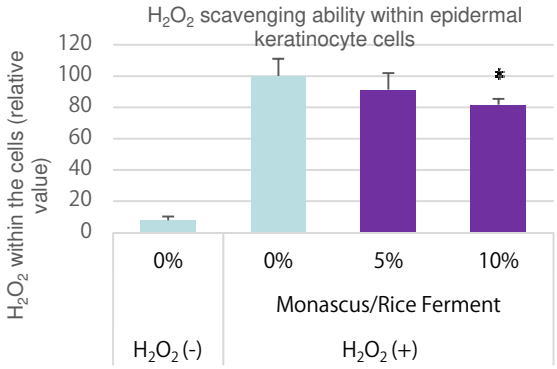
Monascus/rice ferment (%)	GK2 (ug/ml)	Scale factor of the synergetic inhibition of NO production*
5.0	10.0	1.4
5.0	100.0	1.2
10.0	10.0	1.2
10.0	100.0	1.1

* Scale factor when the value of monascus/rice ferment and GK2 added individually is 1

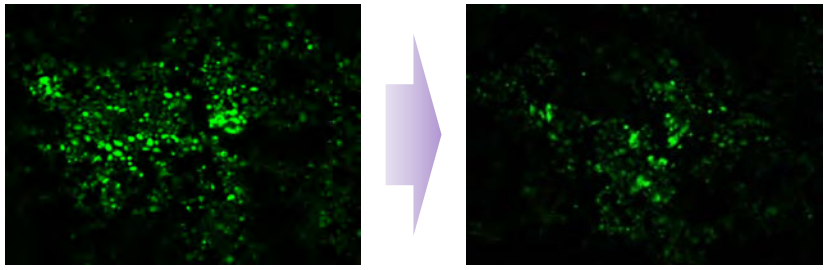
(2) Antioxidant effects: Eliminating active oxygen and protects cells

1. Ability to scavenge reactive oxygen species (ROS): Effects in epidermal keratinocyte cells

In this test, the ability of monascus/rice ferment to scavenge and eliminate active oxygen was evaluated using normal human epidermal keratinocytes (NHEK).
After adding a fluorescent probe to epidermal keratinocyte cells cultured using monascus/rice ferment, the epidermal keratinocytes were exposed to hydrogen peroxide (H_2O_2) as a reactive oxygen species in the presence of monascus/rice ferment, and after the cells took in the H_2O_2, the ability of the ferment to scavenge and eliminate the H_2O_2 in the cells was evaluated. When cells are exposed to H_2O_2, H_2O_2 is quickly absorbed into the cells, and the H_2O_2 concentration within the cells rises. The H_2O_2 absorbed into the cells was evaluated by fluorometry. In addition to the fact that H_2O_2 is the longest-surviving active oxygen species that occurs within cells, it generates hydroxyl radicals that possess extremely high cytotoxicity. As shown in the graph below, monascus/rice ferment eliminates H_2O_2 on a concentration-dependent basis and inhibits oxidative damage to epidermal cells.



* P<0.05 (vs. control)

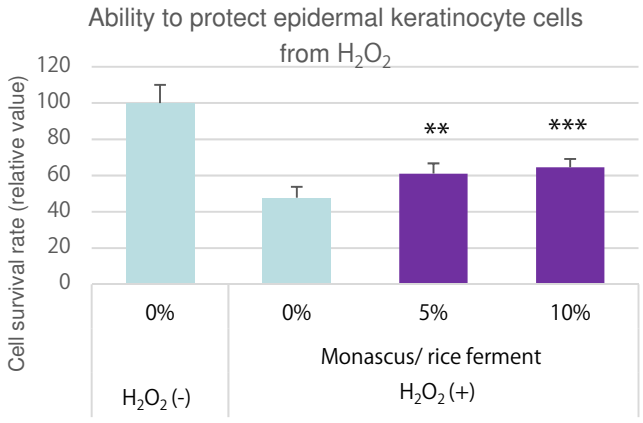


Control

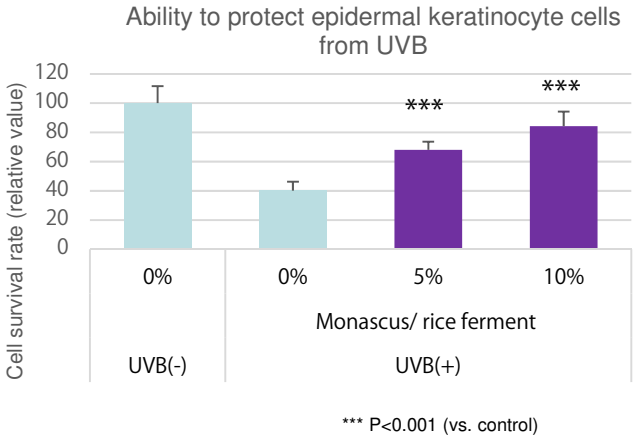
10% monascus/rice ferment added

2. Protecting the cells from reactive oxygen species (ROS) and UV rays: Effects in epidermal keratinocyte cells

In another test, the ability to protect cells from active oxygen (H_2O_2) was evaluated based on the survival rate of normal human epidermal keratinocytes when monascus/rice ferment was added. As shown in the graph, it was confirmed that the cell survival rate increased on a concentration-dependent basis in accordance with the amount of monascus/rice ferment added.
Also, the ability to protect cells from ultraviolet light (UVB) was evaluated using the same test method. When cells are bombarded with UVB rays, active oxygen forms and increases within the epidermal keratinocyte cells, resulting in oxidative damage to the cells and a lower cell survival rate.
As a result of the test, it was confirmed that monascus/rice ferment protects the cells by inhibiting the decline of the cell survival rate due to oxidative damage on an added concentration-dependent basis, even when exposed to UVB.



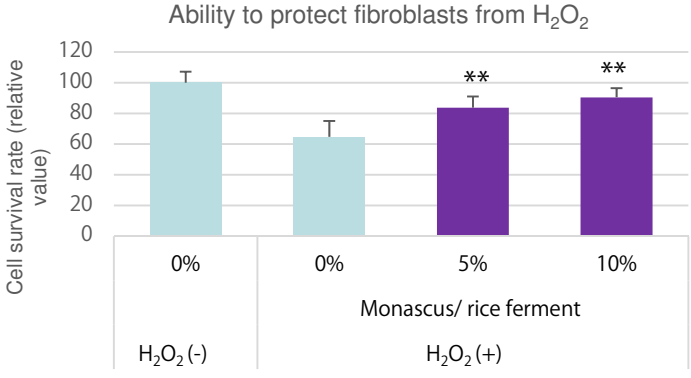
** P<0.01
*** P<0.001 (vs. control)



*** P<0.001 (vs. control)

3. Protecting cells from reactive oxygen species (ROS): Effects in dermal fibroblasts

The cell survival rate when active oxygen (H_2O_2) was added was similarly evaluated in normal human dermal fibroblasts. As a result, it was confirmed that monascus/rice ferment also increases the survival rate on an added concentration-dependent basis in dermal fibroblasts.
Based on the above results, monascus/rice ferment is expected to inhibit the active oxygen that forms and increases in skin cells when exposed to oxidative stress and thereby protects the skin from oxidative damage.

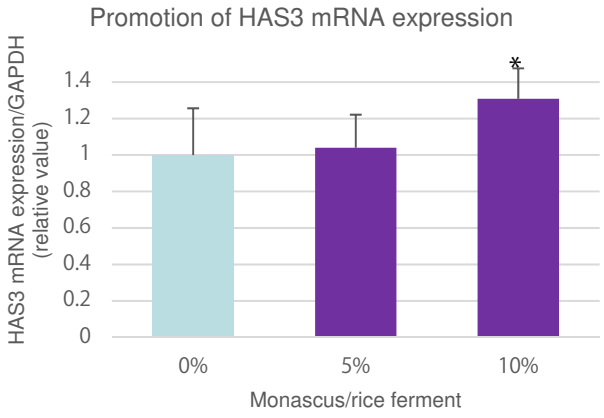


** P<0.01 (vs. control)

(3) Moisturizing effects: Promoting expression of the hyaluronan synthase 3 (HAS3) gene

Epidermal hyaluronic acid keeps moisture within the epidermal layer and maintains the skin's moisture-retention function. Epidermal hyaluronic acid productivity declines due to aging, UV rays, and active oxygen, and it is one of the causes of skin dryness.

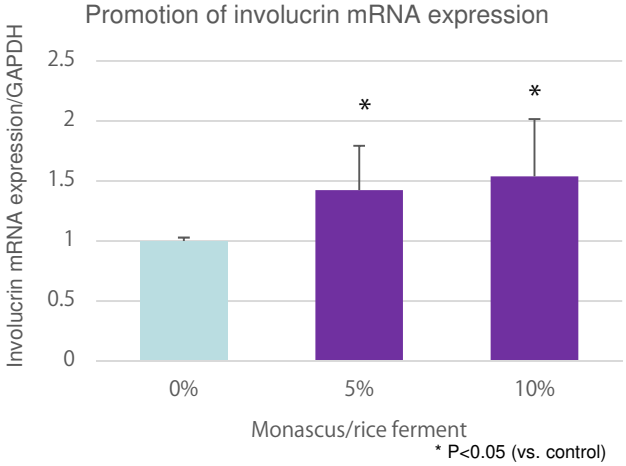
As a result of the hyaluronic acid production test conducted using normal human epidermal keratinocyte cells, it was confirmed that monascus/rice ferment promotes the expression of the hyaluronan synthase 3 (HAS3) mRNA.
Based on these results, monascus/rice ferment is expected to promote epidermal hyaluronic acid productivity in epidermal keratinocyte cells and to increase the skin's moisture-retention function.



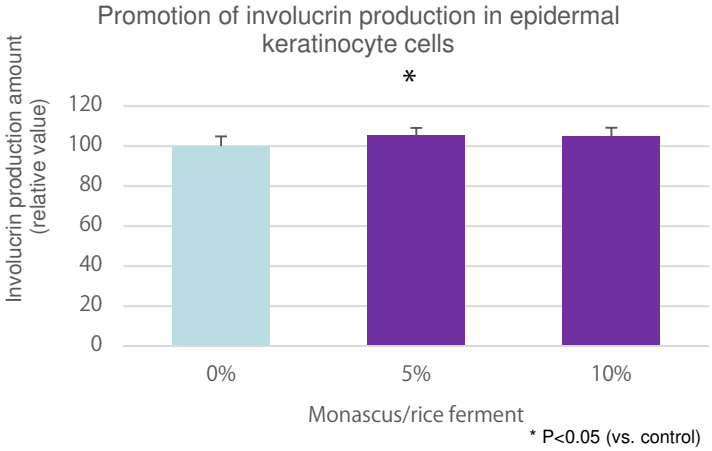
* P<0.05 (vs. control)

(4) Barrier function improvement: Promoting the production of involucrin

Involucrin is a protein that forms the cornified envelope (CE), which is an exterior wall of the horny layer cells that form the basis of the skin's barrier function. Based on this CE foundation, intercellular lipids including ceramide form a lamellar structure and enhance the skin's barrier function.
As a result of an involucrin production test conducted using normal human epidermal keratinocyte cells (NHEK), it was confirmed that monascus/rice ferment promotes the expression of involucrin mRNA and increases the amount of involucrin produced.
Based on these results, monascus/rice ferment is expected to promote involucrin productivity in epidermal keratinocyte cells and to increase the amount of involucrin, thereby increasing the barrier functions of the skin.



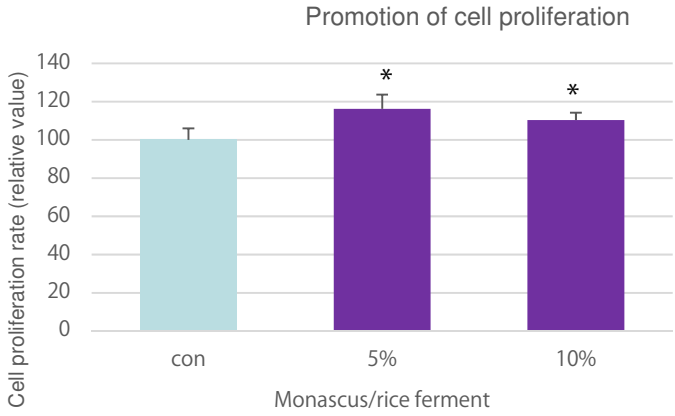
* P<0.05 (vs. control)



* P<0.05 (vs. control)

(5) Turnover function improvement: Promoting cell proliferation

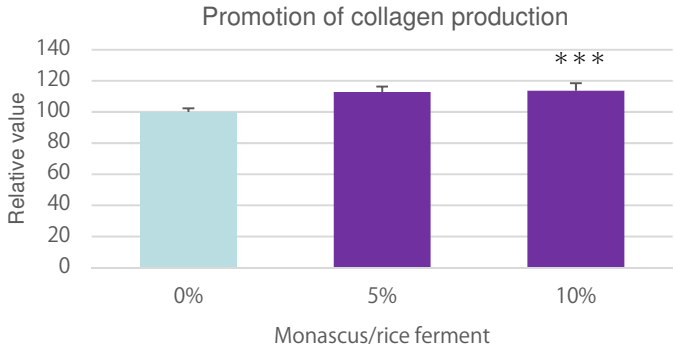
The epidermal keratinocyte cells divided from the basal layer move upward as they differentiate and mature. After reaching the horny layer, they are shed. This repeated cellular turnover forms the epidermis. When the metabolism of epidermal keratinocyte cells decreases due to aging, it leads to fine wrinkles, pigmentation, and reduced expression of natural moisturizing factors in the horny layer.
As a result of a cell proliferation tests conducted using normal human epidermal keratinocyte cells, it was confirmed that monascus/rice ferment significantly activated the cells.
Based on these results, monascus/rice ferment is expected to restore skin metabolism and thereby improve the conditions caused by skin aging, such as fine wrinkles, pigmentation, and reduced expression of natural moisturizing factors in the horny layer.



* P<0.05 (vs. control)

(6) Wrinkle/resilience improvement: Ability to synthesize collagen

Collagen is produced by dermal fibroblasts and gives the skin resilience. As a result of a collagen production test conducted using normal human fibroblasts, the ferment filtrate was confirmed to significantly promote collagen production. Based on these results, monascus/rice ferment is expected to enhance the collagen productivity of fibroblasts and to improve wrinkles and skin sagging.



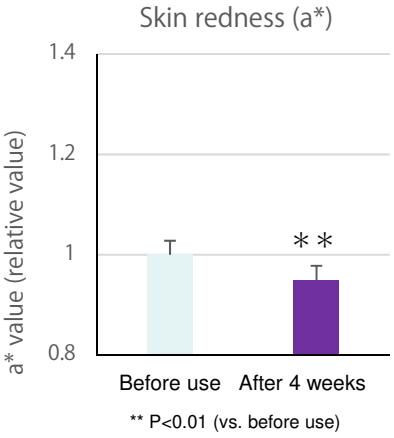
*** P<0.001 (vs. control)

(7) Human test data using cosmetic items containing RICETECT

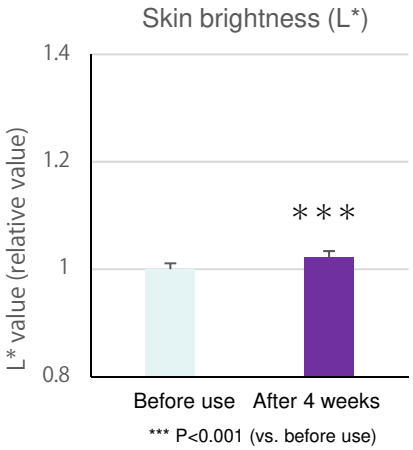
Test method:
An evaluation test was conducted with 17 adult women aged 34 to 54 as the test subjects (average age: 42.3±5.3 years old) and involved the use of cosmetic items containing 15% RICETECT (skin toner, beauty essence, and cream) applied twice a day in the morning and at night for a period of 4 weeks during October and November. The skin conditions before use and after 4 weeks of use were compared using VISIA and a Corneometer.

1. Analysis of changes in skin condition using VISIA and a Corneometer

For the evaluation, facial images were taken using VISIA on the test start date and after 4 weeks of application, and the amount of moisture in the horny layer on both cheeks was measured.
When measuring the amount of moisture in the horny layer, five measurements were made on each cheek at the point where the line drawn down from the corner of the eye meets the horizontal line drawn from the bottom of the nose.
The skin color value L* (brightness) and a* value (redness) were analyzed using L*a*b* analysis software based on the face images obtained with VISIA. The graph shows the average of the values on both cheeks.
As a result, for skin redness (a* value), which indicates the level of skin roughness and inflammation, significant improvement was observed, confirming that the ongoing application of cosmetic items containing RICETECT improves skin redness, which is an indicator of skin roughness.
Also, significant improvements were also observed in multiple VISIA items, as well as in the moisture levels in the horny layer measured using the Corneometer.



** P<0.01 (vs. before use)



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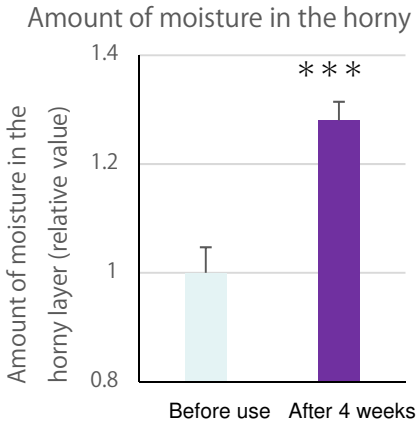

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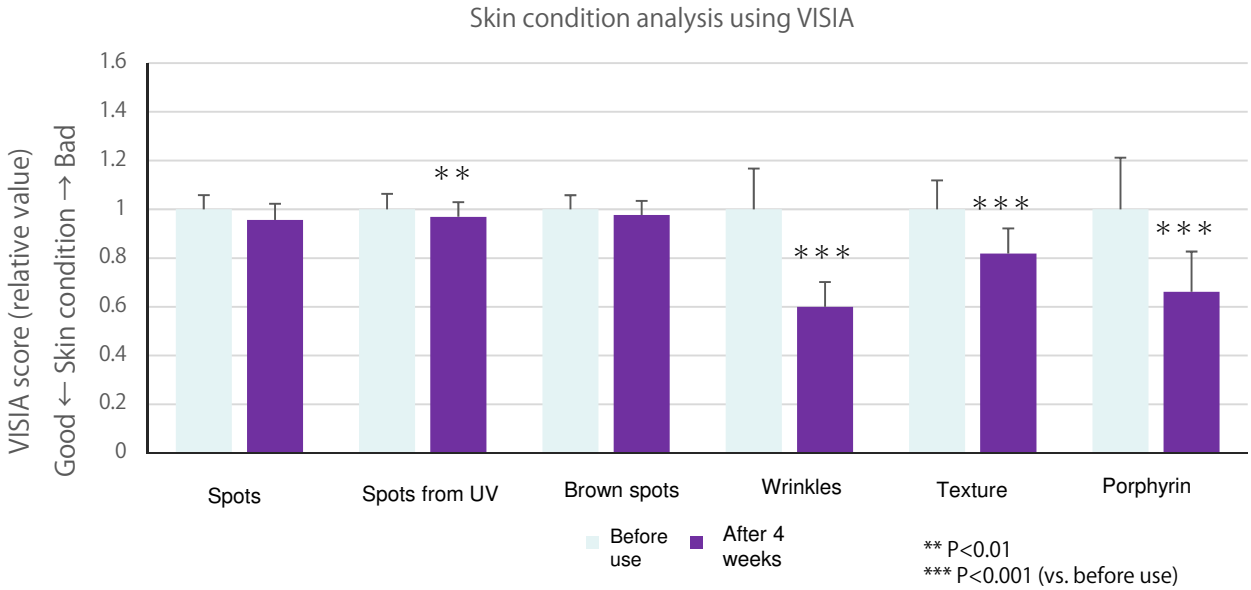
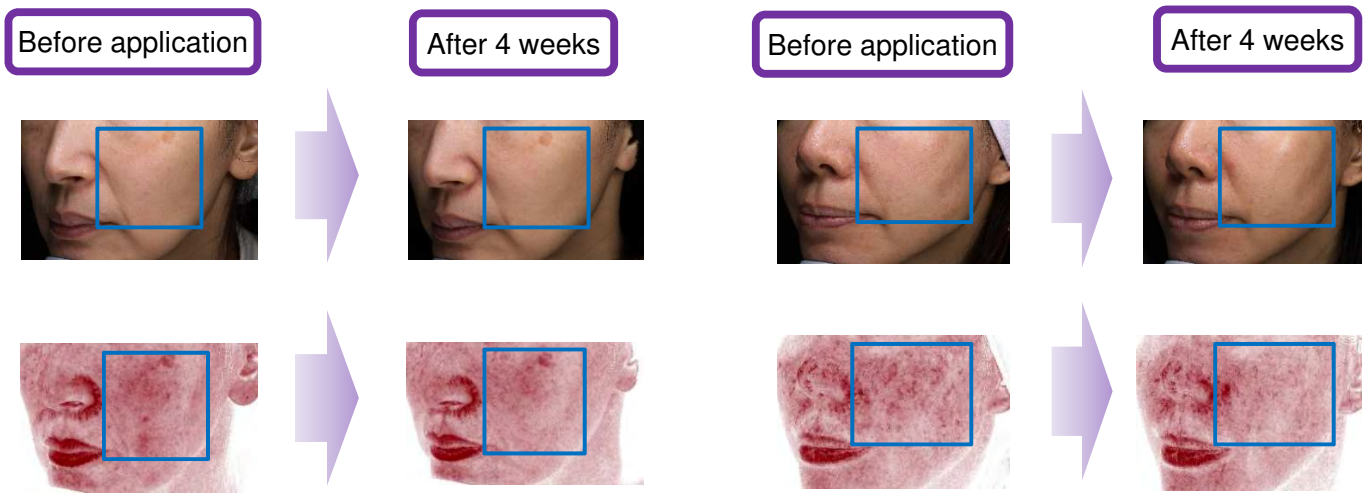


** P<0.01
*** P<0.001 (vs. before use)

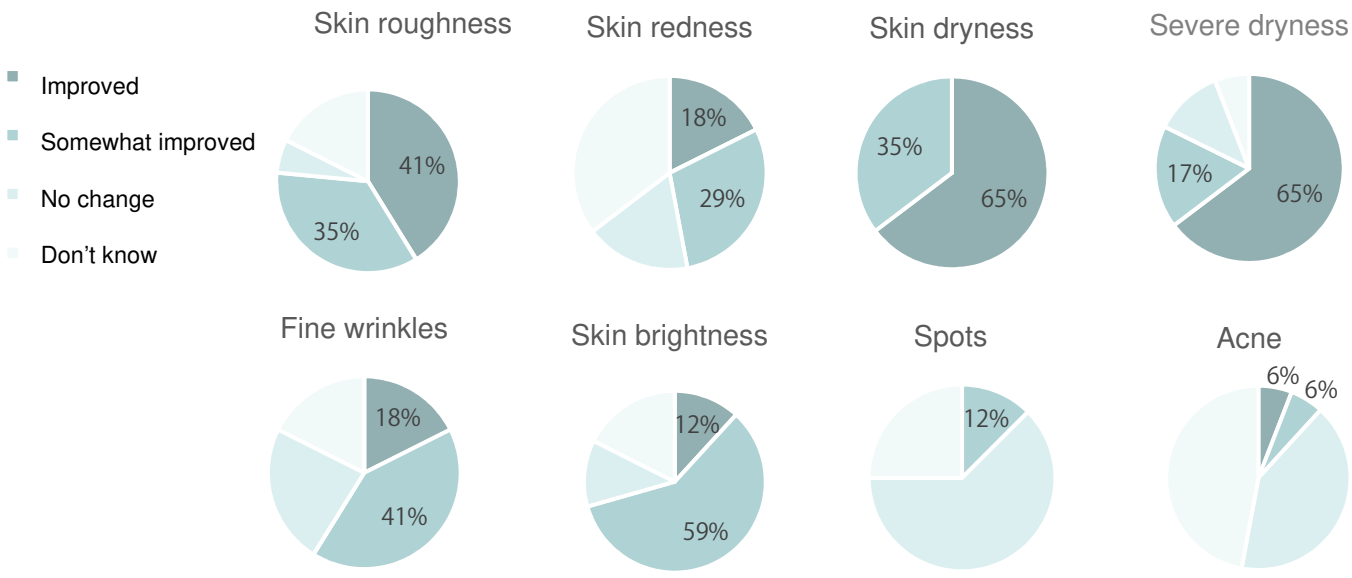
2. Comparison of skin redness improvement before and after application using VISIA

The a* value (redness) of facial images obtained using VISIA were compared before and after 4-week application of a cosmetic item containing RICETECT. Compared to before application, it was confirmed from the images that skin redness was reduced after 4-week application.



3. Evaluation of actual feel via questionnaire survey

A multiple-item questionnaire survey was conducted to investigate the changes actually felt after a 4-week application of a cosmetic item containing RICETECT.
The results show that the subjects actually felt RICETECT's comprehensive functions for protecting the skin, including anti-inflammation, antioxidant and moisturizing effects, and its ability to improve skin roughness, dryness, and fine wrinkles. This confirms that using cosmetic items containing RICETECT will make it easy for users to feel improvement in skin conditions such as skin roughness and dryness.



(8) SAFETY TEST

ITEM	RESULT
Ames TEST	Negative
Skin irritation test (OECD TG 439)	Non-irritant
Eye irritation test (OECD TG 492)	Non-irritant
Phototoxicity test (OECD TG 495)	Negative
Human patch test (24 hours occlusion 20 human)	Safety
Repeat insult patch test (50 human)	Non-irritant and non-sensitizer

※Concentration:25% as Monascus/Rice ferment