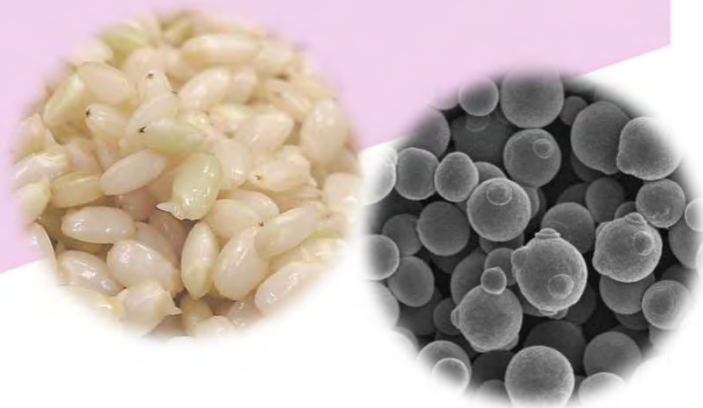


Possesses a skin moisturizing effect; in addition, it improves the barrier function, moisture retention function, and metabolism function, along with reducing wrinkles and restoring skin firmness.

Substance made by fermenting germinated brown rice with yeast

YUKIME®

An original substance developed by fermenting germinated “yukihikari” brown rice, a variety of rice grown in Hokkaido, with *Lachancea kluyveri*, isolated from figs; it moisturizes the skin and prevents skin aging, and also has beauty effects such as improved barrier function, moisture retention function, and metabolism function. It also reduces wrinkles and restores skin firmness.



Features of YUKIME

Protects the skin from dryness so as to create moist, young skin!

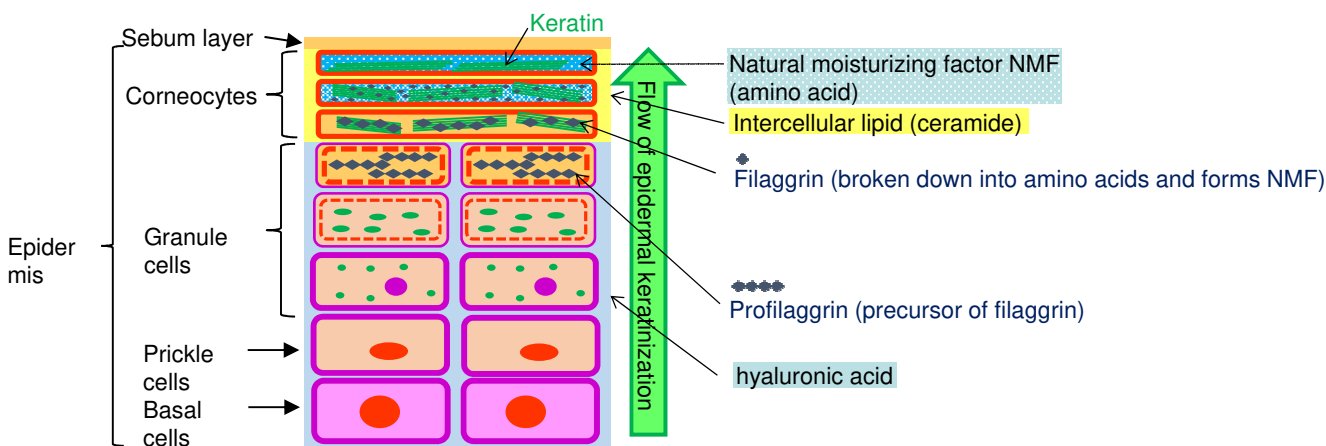
- YUKIME was created by fermenting germinated “yukihikari” brown rice, a variety developed in Hokkaido, with *Lachancea kluyveri*, isolated from figs. (Patent No.7162351)
- It was confirmed that fermentation promotes the gene expression of multiple enzymes involved in the synthesis of ceramide in the epidermal keratinocytes.
- Effect on epidermal keratinocytes: Improved barrier function, moisture retention function and metabolism function; it increases ceramide production, enzymes involved in ceramide synthesis, profilaggrin (a natural moisturizing factor precursor), the gene expression amount of aquaporin (AQP3) (adjusts intercellular moisture), ATP production, and cell propagation.
- Effect on fibroblasts: Reduced wrinkles and improved skin firmness; it promotes the gene expression of hyaluronan synthase (HAS2), hyaluronic acid production, collagen production, and cell propagation.
- It was confirmed in human trials to improve and maintain skin moisture.
- Results of the human trial: Improved moisture retention function of the stratum corneum, improved turnover, maintained a healthier stratum corneum, reduced yellowish dullness, and increased skin translucency; the human trial confirmed the effects of increasing moisture in the stratum corneum, reducing squamae, reducing the separation of the thickened stratum corneum, and reducing carbonylated proteins.

YUKIME		Standard item	Standards	Test method
INCI	Component ratio (%)	Appearance	Light yellow to yellowish brown liquid with characteristic odor	Sensory test
Lachancea/Hydrolyzed Germinated Rice Grain Ferment Lysate Filtrate	95	pH	4.0~5.5	JSQI general test method
Pentylene Glycol	5	Evaporation residue	≥0.5 %	Atmospheric heating drying method
Standard packing	20 kg/Bag in box	Specific gravity	Actual measurement value	Vibration-type density meter
Storage	Store in a dark place at room temperature	Heavy metals (as Pb)	≤20ppm	JSQI general test method
		Arsenic (as As ₂ O ₃)	≤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 method (Petrifilm method)

Works well on the skin's dermis and epidermis • • •

The improvement effect of the barrier function ,moisture retention function and metabolic function

The epidermis is composed of cells that form the basal layer, the prickle layer, the granular layer, and the stratum corneum. The barrier function of the skin prevents moisture that is inside the stratum corneum of the epidermis from transpiring, and also prevents abnormal substances from the external environment (such as allergens, bacteria) from entering the body. An epidermis cell in the skin grows from a basal cell to a prickle cell and then to a granule cell. The granule cells store moisturizing ingredients, such as ceramide and amino acid, in the granules. As the cell grows further, it releases the contents in the granules to the outside of the cell, and the The epidermis is composed of cells outer cell membrane and the nucleus in the cell will disappear. A corneocyte is formed, outside of the cell, intercellular lipids such as ceramide creates the multi-layered lamellar structure. Meanwhile, inside the cell, keratin fibers develop and increase the strength of the cell, and along with resisting the pressure and stimulation from the outside, the natural moisturizing factors (NMF) such as amino acids stored inside the cell retain the moisture to keep the stratum corneum moist. Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate promotes ceramide production in the epidermis, enzymes involved in ceramide synthesis, profilaggrin, mRNA gene expression of aquaporin 3 (AQP3), ATP production, and cell propagation, thereby enhancing skin metabolism and protecting the skin from aging by improving the barrier function and moisture retention function of the skin.

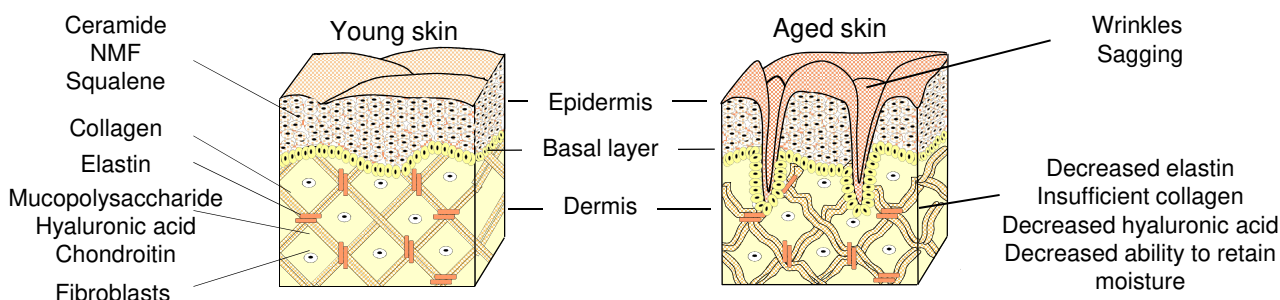


Effects on reducing wrinkles and restoring skin firmness

The dermis, which is located inside the skin's epidermis layer, forms a major part of the skin tissue and can also be said to be the "true skin." The dermis is mostly made of filamentous protein collagen with jelly-like compounds, such as hyaluronic acid, that contain moisture filling in the space in between. The cells that create these components are called "fibroblasts," and forming a proper matrix structure of the skin keeps it in good condition.

Skin aging is caused by the decreased functioning of the fibroblasts, which form the skin, as well as the reduced production capability of the extracellular matrix components such as collagen and hyaluronic acid. As a result, the matrix structure, which supports the skin, breaks down, reducing moisture in the skin and causing wrinkles and sagging.

Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate accelerates the growth of the fibroblasts in the dermis, as well as the production of collagen and hyaluronic acid. It works on the skin functions that have declined with age and promotes improved skin texture, including moisture and skin firmness.



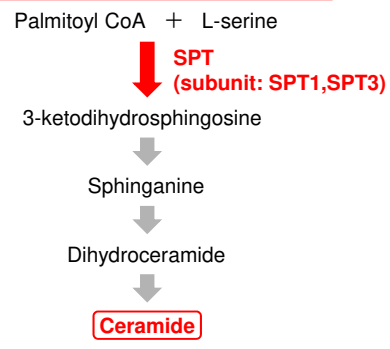
(1)Effects on the epidermis (keratinocyte) : Improves the barrier function and the moisture retention function • Effect of metabolic function improvement

1. Promotes the production of ceramide

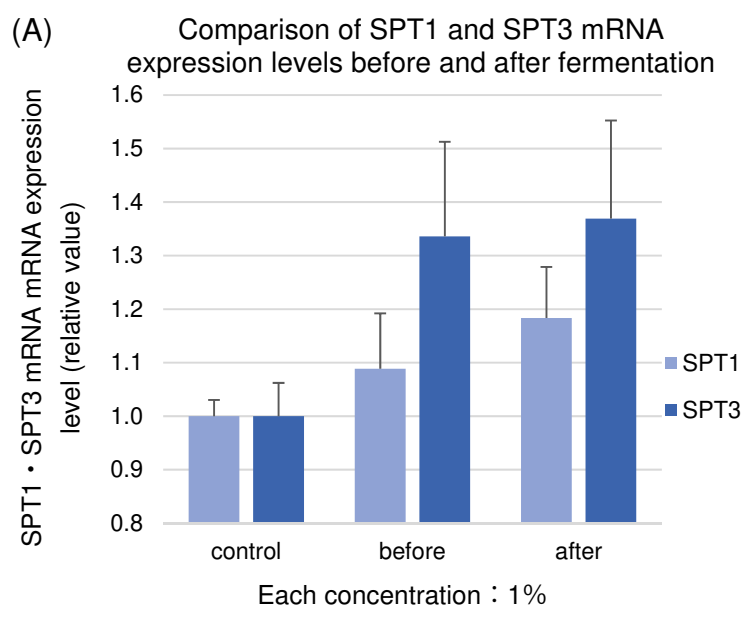
Ceramide forms a lamellar structure as a stratum corneum intercellular lipid. As a result of a ceramide production test conducted using normal human epidermal keratinocytes, it was confirmed that, through fermentation, the mRNA expression promotion effect of serine palmitoyltransferase (SPT1,SPT3), which is an enzyme involved in ceramide synthesis, is increased. (A) Also, it was found that lachancea/hydrolyzed germinated rice grain ferment lysate filtrate concentration is correlated to SPT1 and SPT3 mRNA expression level increase (B), with the same being true for ceramide production (C).

Given this, Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate is expected to promote ceramide productivity of epidermal keratinocytes and reinforce the intercellular lipid lamellar structure through increased ceramide production, thereby improving the barrier function and moisture retention function of the skin .

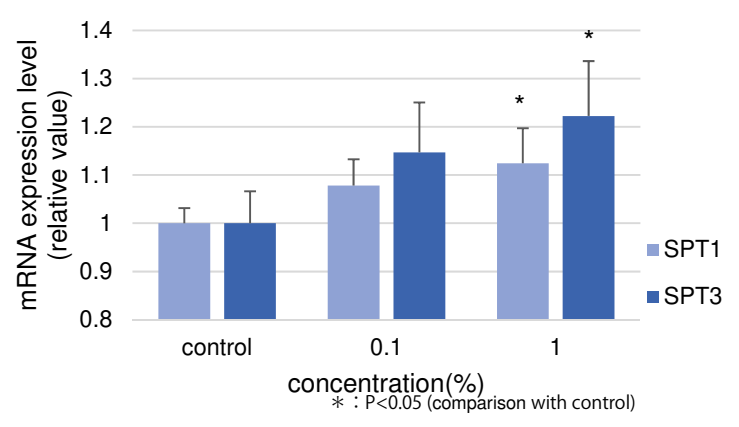
Ceramide biosynthetic pathway



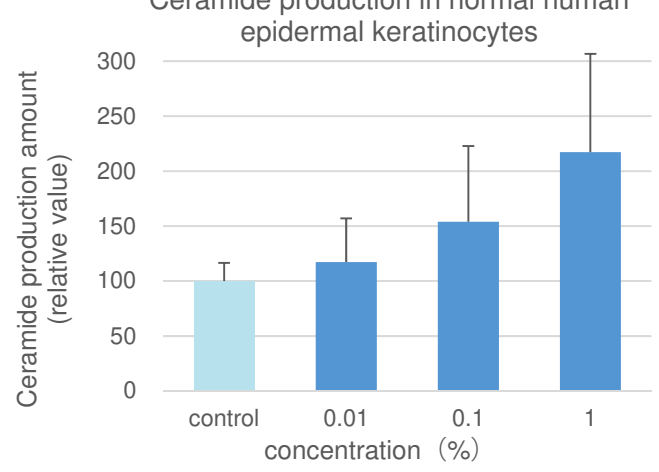
concentration : Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate



(B) SPTLC1,SPTLC3 gene expression promotion



(C) Ceramide production in normal human epidermal keratinocytes

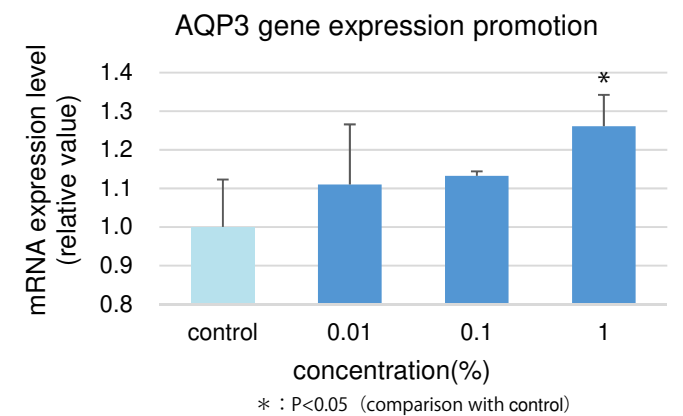


(1)Effects on the epidermis (keratinocyte) : Improves the barrier function and the moisture retention function • Effect of metabolic function improvement

2. Promotes aquaporin 3 (AQP3) gene expression

Aquaporin 3 is a water channel that exists in the keratinocyte of the skin and accelerates the transportation of water molecules and glycerol. Thus, it helps maintain skin moisture and firmness. It is also said to be involved in accelerating wound healing and controlling inflammation. Restoring declining AQP3 expression caused by aging can be expected to rejuvenate skin texture. In an AQP3 gene expression test conducted using normal human epidermal keratinocytes, it was found that lachancea/hydrolyzed germinated rice grain ferment lysate filtrate concentration is correlated to AQP3mRNA expression level increase. From this result, lachancea/hydrolyzed germinated rice grain ferment lysate filtrate is expected to improve the moisture retention function and texture of the skin.

concentration : Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate

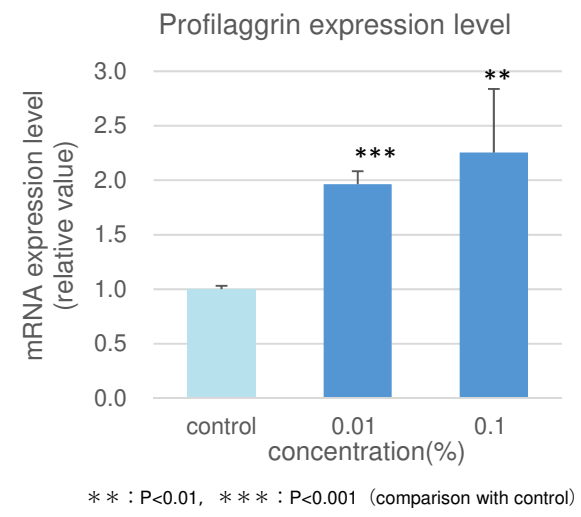
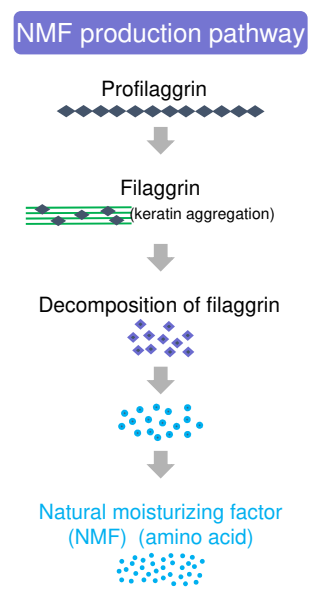


3. Promotes the production of profilaggrin acid

Filaggrin is formed as its precursor profilaggrin in granular cells. Then, as the epidermal cells differentiate, profilaggrin decomposes into filaggrin. The free filaggrin aggregates keratin fibers within the corneocytes and then decomposes into the amino acid natural moisturizing factor (NMF) in the upper layers of the stratum corneum.

As a result of a filaggrin gene expression evaluation test by using normal keratinized human epidermal keratinocytes, it was found that Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate promotes filaggrin mRNA expression.

Given this, Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate is expected to promote the productivity of filaggrin, which becomes NMF (amino acid), thereby increasing the moisture retention function in the stratum corneum along with skin metabolism.



4. Promotes ATP production

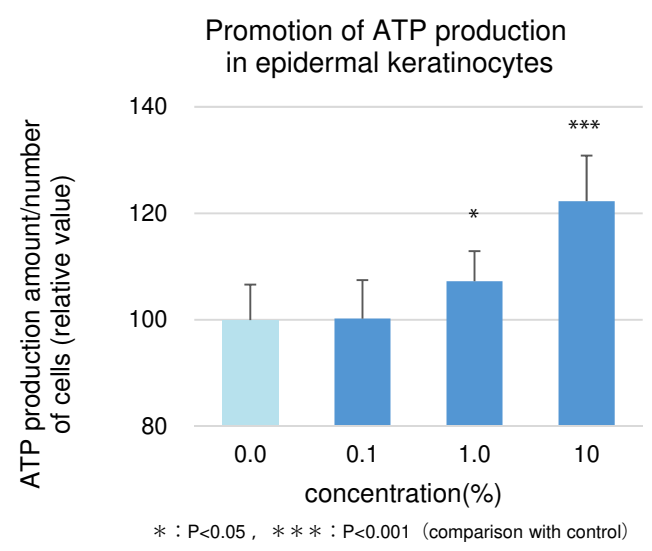
When the activities and propagation capability of epidermal cells decrease due to external environmental factors or aging, epidermis turnover slows, causing differentiation defects such as thinning of the epidermis and thickened keratin. As a result, the moisture retention function and firmness of the skin decrease, and keratin abnormally peels off. In addition, wrinkles, dullness, loss of fine texture, decreased firmness, and other changes occur.

If keratinocytes propagation is accelerated, skin turnover will also be accelerated, and the skin metabolism function will be restored. In order to accelerate cell propagation, it is important to increase the production of ATP, which is the bioenergy required for cell division. Actually, in cells with decreased functions and aged cells, ATP production is lower compared to normal cells.

Therefore, if ATP production in the cells is accelerated, skin turnover will be accelerated, and the skin metabolism function is expected to be restored.

In an ATP production test conducted using normal human epidermal keratinocytes, it was found that lachancea/hydrolyzed germinated rice grain ferment lysate filtrate accelerates ATP production.

From this result, lachancea/hydrolyzed germinated rice grain ferment lysate filtrate is expected to promote ATP production in epidermal keratinocytes and restore the metabolism function of the skin.



(1) Effects on the epidermis (keratinocyte) : Improves the barrier function and the moisture retention function • Effect of metabolic function improvement

5. Promotes the growth of epidermal keratinocytes

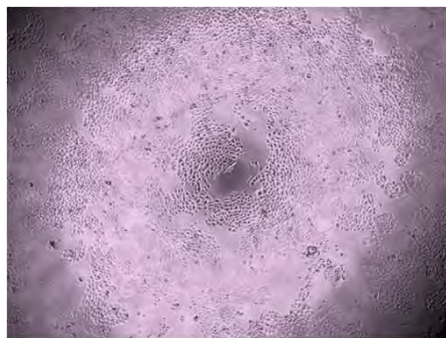
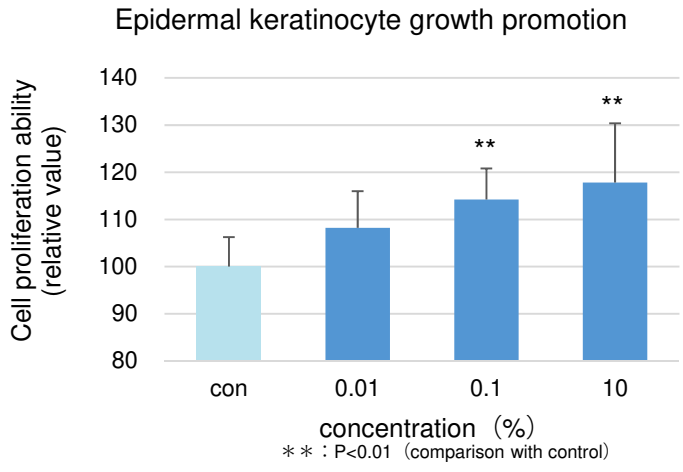
Epidermal keratinocytes that have divided in the basal layer reach the stratum corneum while migrating to the upper layer through differentiation and maturation. After that, epidermal keratinocytes are shed and turnover is repeated to form the epidermis.

When the metabolism in epidermal keratinocytes decreases due to aging, fine wrinkles, pigmentation, and decreased expression of natural moisturizing factors in the stratum corneum occur.

As a result of a cell proliferation test using normal keratinized human epidermal keratinocytes, it was found that the increase of cell activation is concentration-dependent on Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate.

From this result, lachancea/hydrolyzed germinated rice grain ferment lysate filtrate is expected to restore the skin metabolism function to thereby improve signs of aging such as fine wrinkles, pigmentation, and decreased expression of natural moisturizing factors in the stratum corneum.

concentration :
Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate



control



Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate

- Picture of the plate after cultivating normal human epidermal keratinocytes for 48 hours; it was found that lachancea/hydrolyzed germinated rice grain ferment lysate filtrate accelerates the propagation of epidermal keratinocytes.

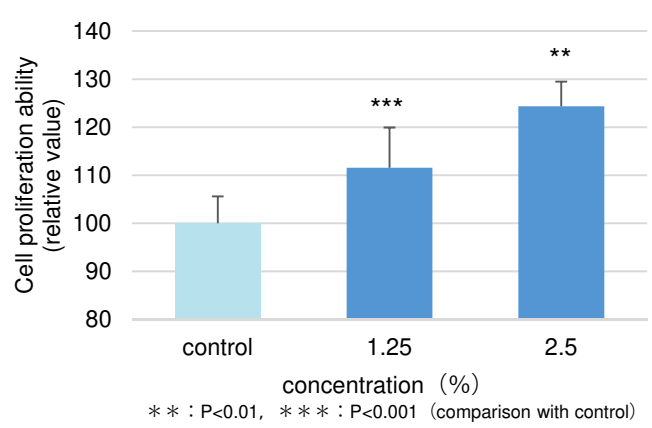
(2) Effects on the dermis (fibroblasts): Improves wrinkles and firmness

1. Promotes cell propagation in fibroblasts

In a cell propagation test conducted using normal human fibroblasts, lachancea/hydrolyzed germinated rice grain ferment lysate filtrate was found to have significantly high activation despite being plant derived.

From this result, lachancea/hydrolyzed germinated rice grain ferment lysate filtrate is expected to accelerate the growth of fibroblasts so as to thereby accelerate the production of components such as collagen, elastin, and hyaluronic acid made by the cells, as well as to prevent skin aging.

Fibroblast activation



Measurement:
• Usage of Cell Counting Kit-8

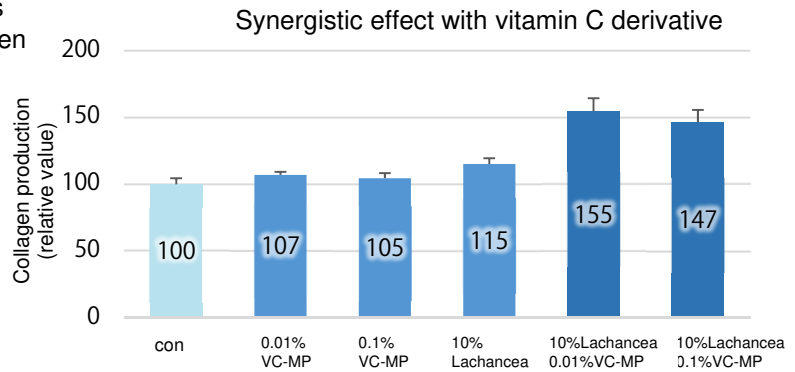
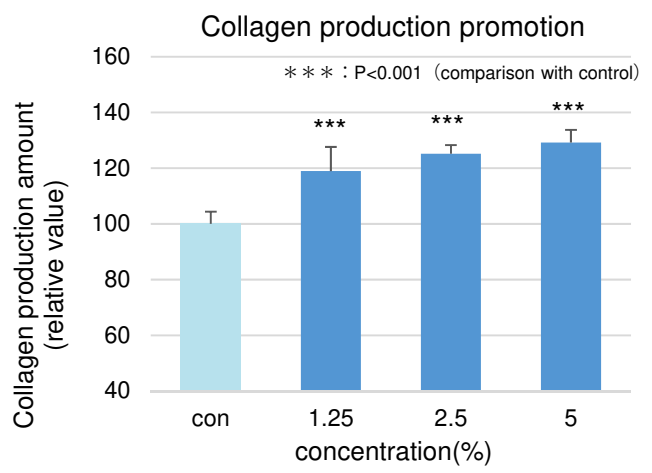
(2) Effects on the dermis (fibroblasts) : Reduces wrinkles and restores skin firmness

2. Promotes the production of collagen

In a collagen production test conducted using normal human fibroblasts, lachancea/hydrolyzed germinated rice grain ferment lysate filtrate was found to significantly accelerate collagen production.

In addition, the synergistic effect was confirmed in the presence of Ascorbic Acid Phosphate Magnesium Salt (VC-MP) in combination with Lachancea/Hydrolyzed germinated rice fermentation solute solution, as the combined effect was higher than the sum of the effects of each on collagen production promotion.

From this result, lachancea/hydrolyzed germinated rice grain ferment lysate filtrate is expected to increase collagen production in fibroblasts and to improve wrinkles and sagging. Synergistic effects were also suggested when used in combination with vitamin C derivatives.



Measurement:
• Usage of Collagen Stain Kit

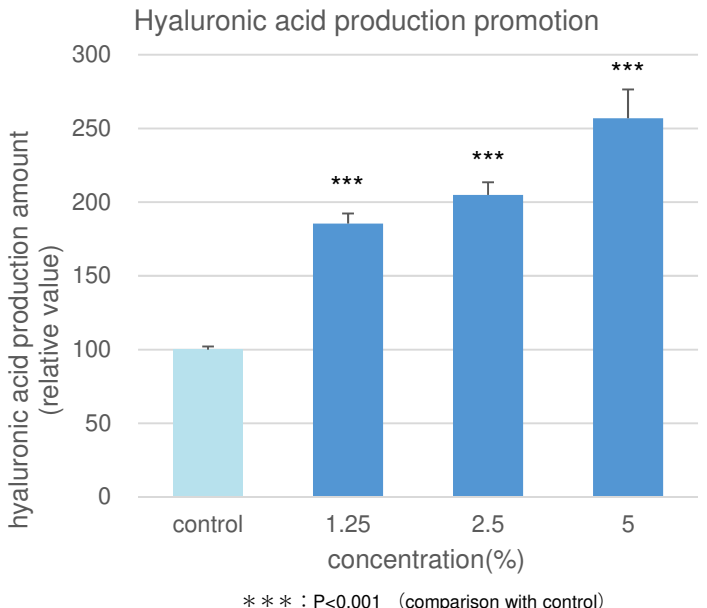
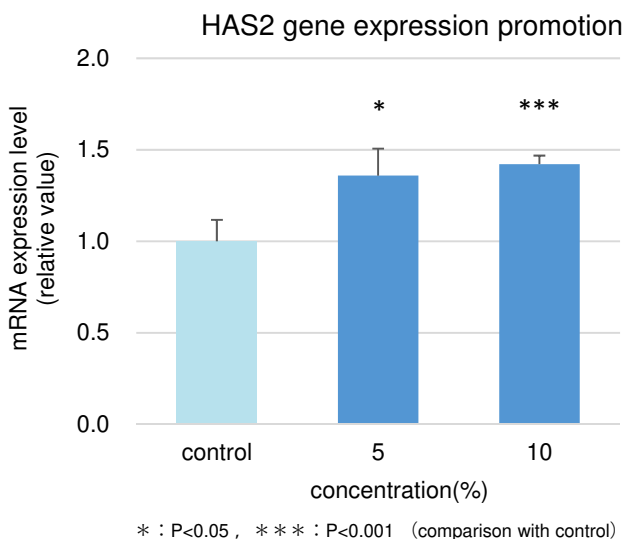
concentration : Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate

3. Promotes the production of hyaluronic acid

In a hyaluronic acid production test conducted using normal human fibroblasts, lachancea/hydrolyzed germinated rice grain ferment lysate filtrate concentration was found to correlate with hyaluronan synthase (HAS2) mRNA expression level increase, with the same being true for hyaluronic acid production.

Because lachancea/hydrolyzed germinated rice grain ferment lysate filtrate accelerates not only fibroblast collagen production but also hyaluronic acid production, it is further expected to improve wrinkles and sagging.

concentration : Lachancea/hydrolyzed germinated rice grain ferment lysate filtrate



Measurement:
• Usage of Quantikine Hyaluronan ELISA Kit

(3) Results of the human trial using lotion containing YUKIME : Improved moisture retention function

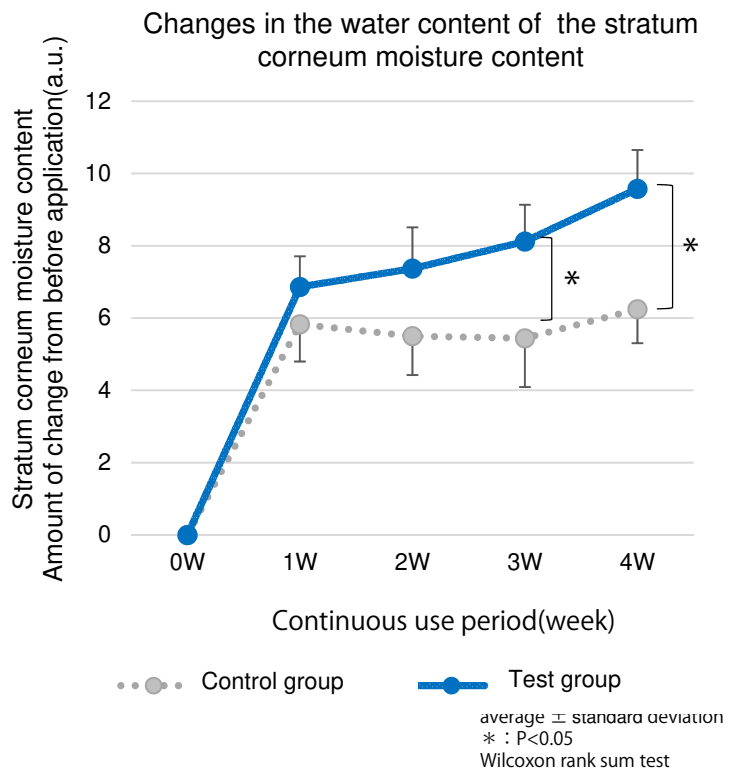
1. Changes in moisture content in the stratum corneum

A trial was conducted with 16 male and female adults (11 female and five male with an average age of 41.4±9.5). The inner side of their front arms was used as the trial site.

The subjects applied the lotion twice a day in the morning and evening for four weeks, and a measurement was made every week from prior to the start of use through to the completion of the four-week trial. On the measurement day, the subjects did not apply the lotion in the morning. The moisture content in the stratum corneum was measured after cleansing the test site with a mild cleanser and acclimating for 20 minutes in a thermostatic chamber at 20–22°C room temperature and at 40–45% humidity. A Corneometer CM825 was used for the measurement. The measurement was conducted at five points at the test site, and the average value of three points, excluding the maximum and minimum values, was recorded. The same amount of the following 2 lotions were applied, respectively, to the test site on each arm.

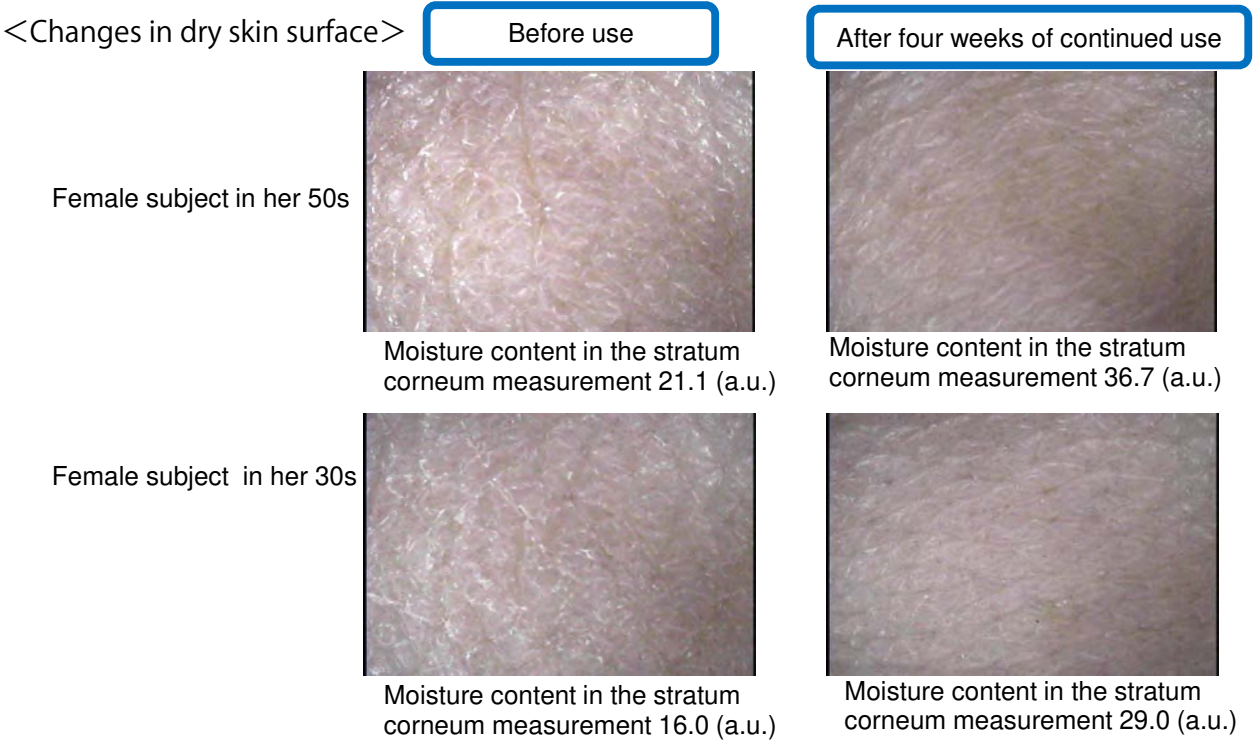
Test group : YUKIME formulated 90%
Control group : Non-formulated YUKIME
(Replaced equivalent amount of YUKIME with water)

Compared to the control lotion, the lotion containing YUKIME™ resulted in higher moisture in the stratum corneum. In addition, that condition was maintained, and a significant difference was observed after three weeks of use. This result clearly shows that YUKIME™ helps maintain the moisture retention capability of the skin.



2. Changes to skin condition resulting from the higher moisture content in the stratum corneum

In the above four-week continued-use trial, the skin condition at the same trial site before use and after using the lotion containing YUKIME™ for four weeks was observed using an MC-50T dry skin microscope (manufactured by Integral Corporation). It was confirmed that, as the moisture content in the stratum corneum rises, the scales observed in dry skin conditions decreased.



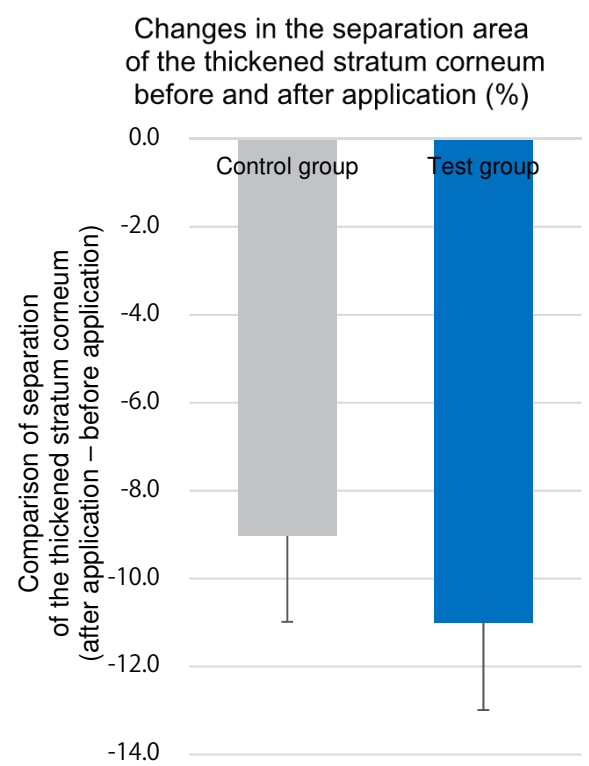
(3) Results of the human trial using lotion containing YUKIME : A healthier stratum corneum

3. Changes to skin conditions as a result of increased moisture in the stratum corneum – Reduced the separation of the thickened stratum corneum –

When the stratum corneum is stripped using an adhesive tape and more than one cell layer is removed, it is called the separation of the thickened stratum corneum. Skin with a high level of separation of the thickened stratum corneum usually has decreased barrier function, rough skin, dryness, and inflammation. When the level of separation is higher, the skin conditions become worse; when the skin conditions improve, the level of separation becomes lower. As part of this mechanism, when the skin is dry and there is little moisture retained in the stratum corneum, the decomposition of desmosome, which adheres stratum corneum cells together, is reduced, and a thicker than usual layer of stratum corneum cells peel off. When skin turnover functions normally, each layer of the stratum corneum cells is neatly positioned behind the layer above.

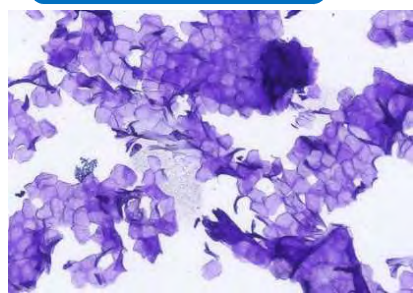
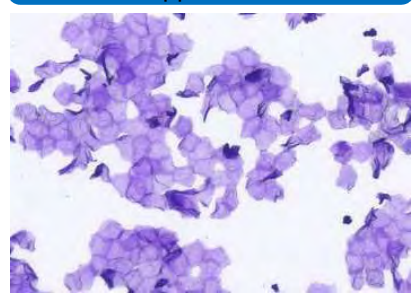
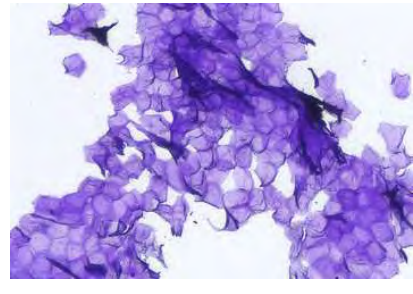
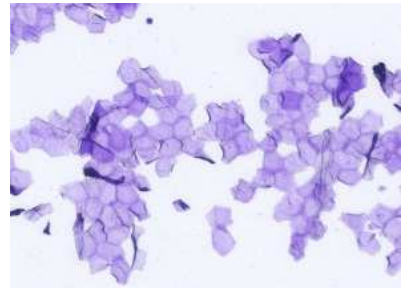
Before lotion application and four weeks after consecutive lotion application during the human trial, the stratum corneum that were stripped using adhesive tape were dyed with gentian violet. Then, the total cell area and the separation area of the thickened stratum corneum were extracted respectively using image analysis, and the average value of the ratio of the separation area of the thickened stratum corneum to total cell area was assessed as the level of separation of the thickened stratum corneum.

As a result of the consecutive application in the human trial, the test group showed greater reduction in the separation area of the thickened stratum corneum after application compared to the control group. Based on this result, YUKIME can be expected to improve skin turnover and maintain a healthier stratum corneum.



Test group: Lotion containing YUKIME
Control group: Lotion not containing YUKIME (water alternative)

<Changes in the condition of separation of the thickened stratum corneum>

	Before application	After 4 weeks of consecutive application
Female test subject in her 40s	 Moisture content in the stratum corneum - Measured value 23.2 (a.u.)	 Moisture content in the stratum corneum - Measured value 39.3 (a.u.)
Female test subject in her 50s	 Moisture content in the stratum corneum - Measured value 21.1 (a.u.)	 Moisture content in the stratum corneum - Measured value 36.7 (a.u.)

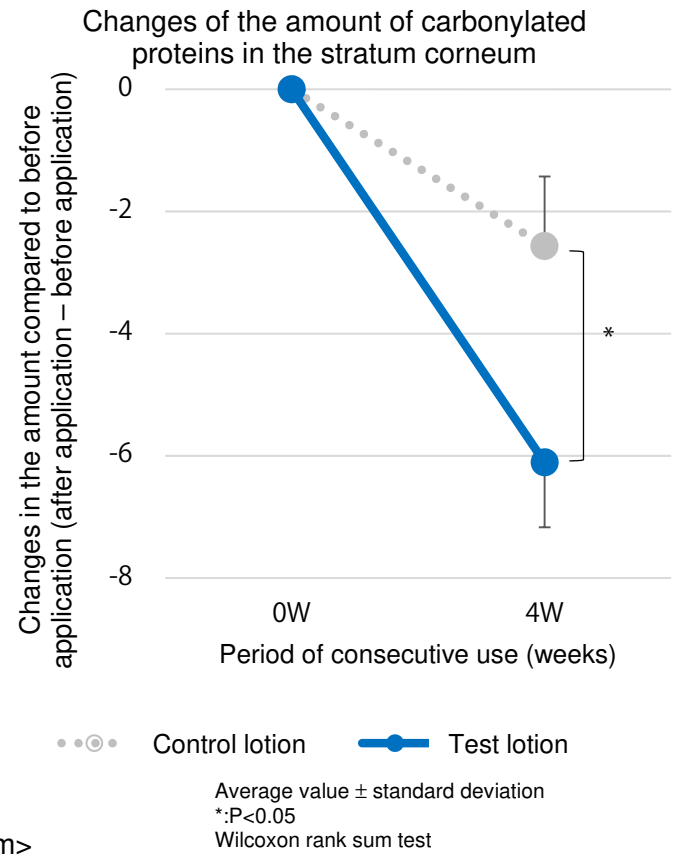
(3) Results of the human trial using lotion containing YUKIME : Improved moisture retention function, reduced yellowish dullness, and better skin translucency

4. Changes to skin conditions as a result of increased moisture in the stratum corneum – Reduced carbonylated proteins –

Carbonylated proteins are produced when aldehyde compounds, which are the end product of lipid peroxide, oxidize proteins. The main cause of this is said to be dryness. In addition, carbonylated proteins reduce the moisture retention function and cause yellowish dullness and decreased translucency of the skin.

Before lotion application and four weeks after consecutive lotion application during the human trial, the horny cell layers that were obtained using adhesive tape were reacted with Fluorescein-5-thiosemicarbazide (5-FTSC) and fluorescent-stained. Then, using fluorescent image analysis, the relative value of the cell area with fluorescent brightness compared to the total cell area was obtained from each image, and the level of carbonylated proteins was assessed.

As a result of the consecutive application in the human trial, the test group showed significant decrease in the amount of carbonylated proteins compared to the control group. This reduction effect is thought to contribute to the increased moisture content in the stratum corneum triggered by YUKIME, and it is also expected to reduce yellowish dullness of the skin and increase translucency.



<Changes of carbonylated proteins in the stratum corneum>

	Before application	After 4 weeks of consecutive application
Female test subject in her 40s	 Moisture content in the stratum corneum - Measured value 25.4 (a.u.)	 Moisture content in the stratum corneum - Measured value 33.7 (a.u.)
Female test subject in her 50s	 Moisture content in the stratum corneum - Measured value 21.1 (a.u.)	 Moisture content in the stratum corneum - Measured value 36.7 (a.u.)

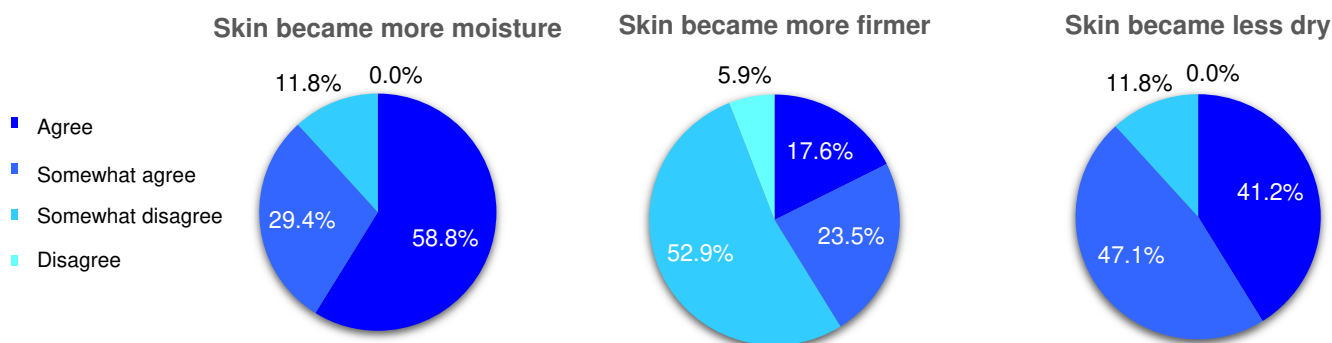
(3) Results of the human trial using lotion containing YUKIME : Questionnaire survey about their experience after usage

5. Results of the actual feel of the skin condition based on a questionnaire survey conducted after four weeks of continuous application of the test lotion and the control lotion

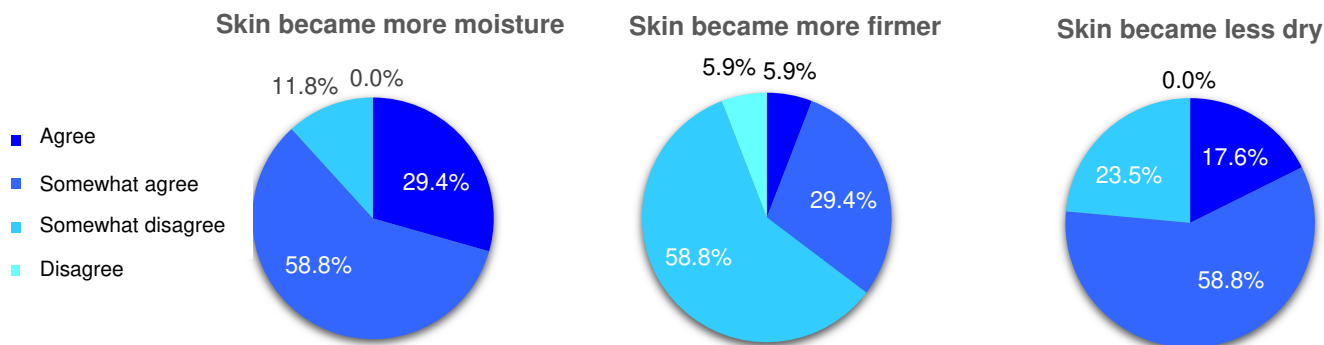
After the four-week continuous-use trial, a questionnaire survey was conducted for the internal company trial subjects regarding the skin condition after four weeks of continuous use. In the results, more of the subjects responded “Agree” for the lotion containing YUKIME™ for the “Skin became more moisture,” “Skin became more firmer,” and “Skin became less dry” items.

It was found that the subjects felt that YUKIME™ makes it easier to achieve improved skin condition.

Test group:YUKIME formulated



Control group:Non-formulated YUKIME (Replaced equivalent amount of YUKIME with water)



(4) 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 432)	Negative
Human patch test (24 hours occlusion 20 human)	Safety
Repeat insult patch test (50 human)	Non-irritant and non-sensitizer