Anti-aging vitamin C

Amitose 3GA

Vitamin C derivative having multiple anti-aging effect

8th edition September 13, 2018

1. Vitamin C and Glyceryl Ascorbate

Benefits/Problems of vitamin C

Vitamin C has some problems as cosmetic ingredient, whereas it has excellent physiological effects.

Vitamin C 

The biggest challenge, improve the stability

Benefits!!

Multiple physiological effects

- Antioxidant effect
- Suppression of melanin production
- Enhancement of collagen production

Problems!!

Time degradation
Destabilization of formulae

- Discoloration and bad-smell caused by degradation
- Difficult to feel good effect
- Destabilization of cosmetic products

1. Vitamin C and Glyceryl Ascorbate

The birth of Amitose VC series

Vitamin C

Multiple physiological effects

Glycerin

Moisturizing and skin-conditioning effects

Combined

Amitose VC series

Glyceryl Ascorbate

- High stability
- Excellent sensory texture
- Multiple physiological effects

1. Vitamin C and Glyceryl Ascorbate

Amitose 3GA

Anti-aging vitamin C

Amitose 3GA is a new vitamin C derivative, having balanced-advantages.

1 Keep stability in wide-range formulae

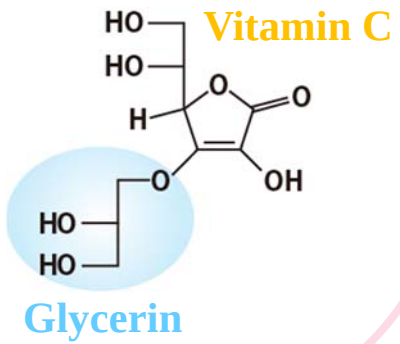
High stability

2 Multiple approach including antioxidant effect

Excellent anti-aging effect

3 Reasonable price of product

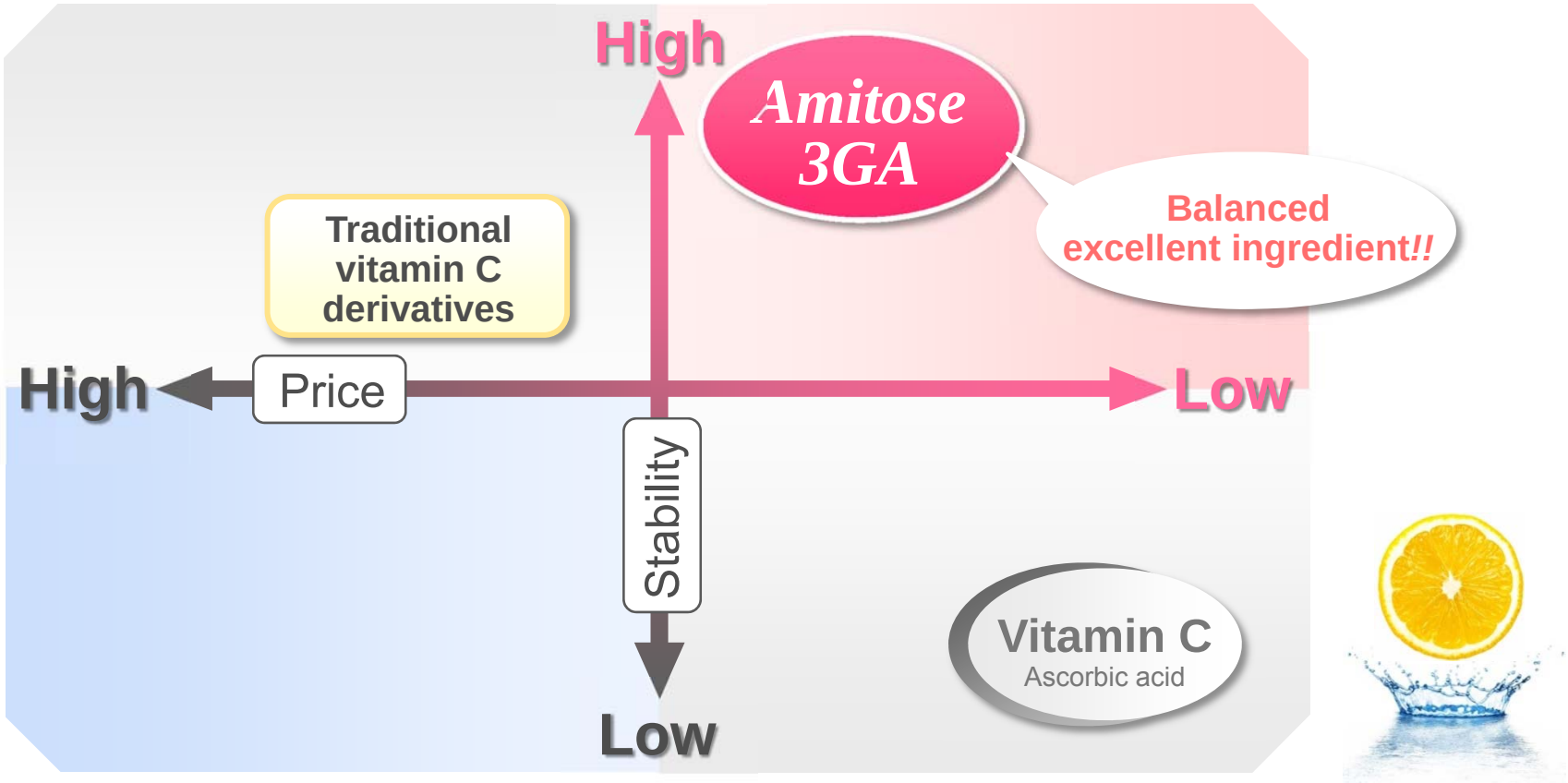
Cost benefit



1. Vitamin C and Glyceryl Ascorbate

Feature of Amitose 3GA

- Positioning map of vitamin C and its derivatives in stability and price



2. Stability of cosmetics

Stability of VC-cosmetics

Vitamin C

Difficult to feel effects in use.

Unstable

Vitamin C deteriorates easily. Formulae containing vitamin C are unstable because of ionic character.

VC-cosmetics

Amitose 3GA

Stable

Easy to feel effects in use.

Amitose 3GA is a stable compound. Formulae containing Amitose 3GA are also stable.

2. Stability of cosmetics

Stable formulation into cosmetics

Amitose 3GA can be formulated in emulsion-typed cosmetics such as skin milk and cream easily.

Stability test of skin cream

(w/w %)

Ingredient	Amitose 3GA	Bench mark
Amitose 3GA	3.3	–
Asc or Asc derivatives	–	1.0
Cetearyl Glucoside, Cetearyl Alcohol	5.0	5.0
Squalane	10.0	10.0
Polyacrylamide, C13-14 Isoparaffin, Laureth-7, Water	0.5	0.5
Preservatives	0.3	0.3
Potassium Hydroxide	adequate amount	adequate amount
Water	up to 100	up to 100

[pH conditions] pH4.5: Amitose 3GA formula,
pH7.0: Benchmark formulae
Each formulae were adjusted to be adequate pH according to the stable pH of individual vitamin C derivatives.

Stable !!

Amitose 3GA

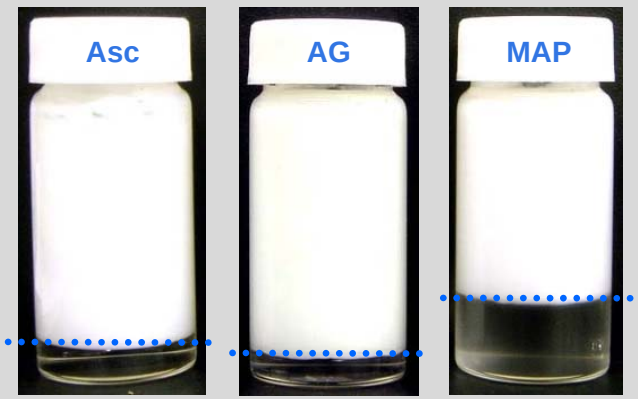
After 4 weeks at 50 deg C

Unstable !!

Bench mark

Asc : Ascorbic acid
AG: Ascorbyl Glucoside
MAP: Magnesium Ascorbyl Phosphate

After 4 weeks at 50 deg C



Separation

2. Stability of cosmetics

Stable formulation into cosmetics

Amitose 3GA can be formulated in gel-typed cosmetics such as beauty serum stably.

Stability test of gel-typed serum

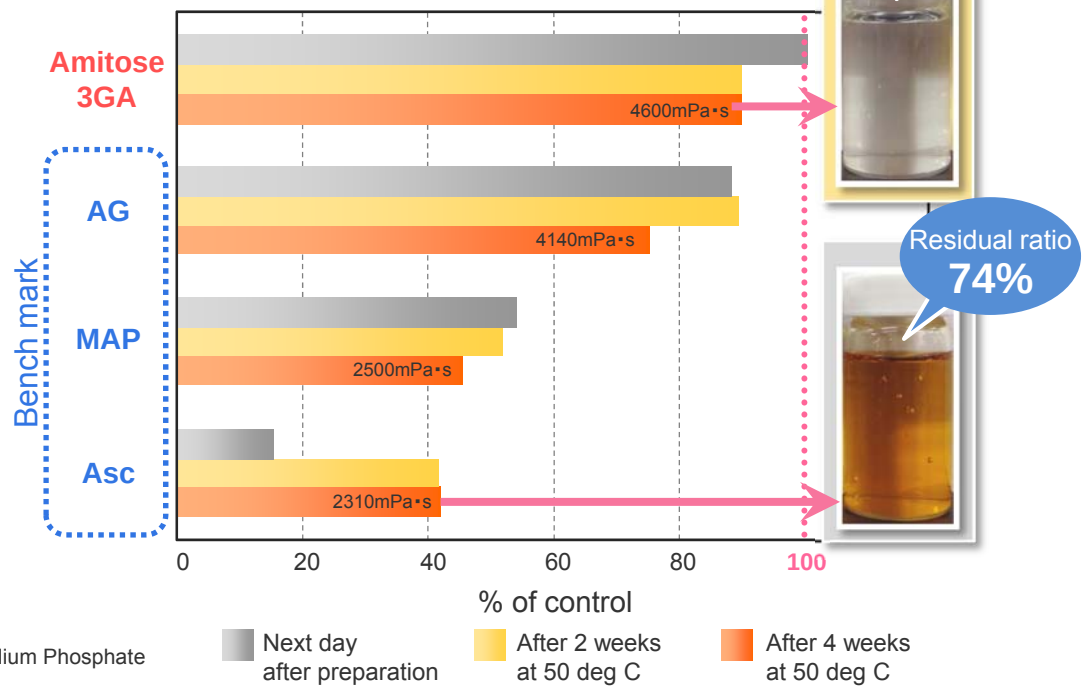
(w/w %)

Ingredient	Control	Amitose 3GA	Bench mark
Amitose 3GA	–	3.3	–
Asc or Asc derivatives	–	–	1.0
Carbomer *1	1.0	1.0	1.0
Glycerin	3.0	3.0	3.0
Butylene Glycol	3.0	3.0	3.0
Alcohol	5.0	5.0	5.0
Ethylhexylglycerin (10%), Phenoxyethanol (90%)	0.5	0.5	0.5
Buffer *2	0.5	0.5	0.5
Potassium Hydroxide	adequate amount	adequate amount	adequate amount
Water	up to 100	up to 100	up to 100

*1 CARBOPOL 941 (Lubrizol)
*2 pH4.5: Citric Acid/ Sodium Citrate, pH7.0: Sodium Phosphate/ Disodium Phosphate
[pH conditions] pH4.5: Amitose 3GA and its Control formulae,
pH7.0: Benchmark and their Control formulae
Each formulae were adjusted to be adequate pH according to the stable pH of individual vitamin C derivatives.

Viscosity variation of each formula

(Relative change rate based on the viscosity of Control formula)



Relative viscosity was calculated assuming the viscosity of Control was 100%.
Viscosity was measured by a rotary viscometer with No.4 rotor at 30 rpm and 25 deg C.

2. Stability of cosmetics

Stable formulation into cosmetics

Amitose 3GA enables to develop stable vitamin C (VC)-rich serum, thanks to its high stability.

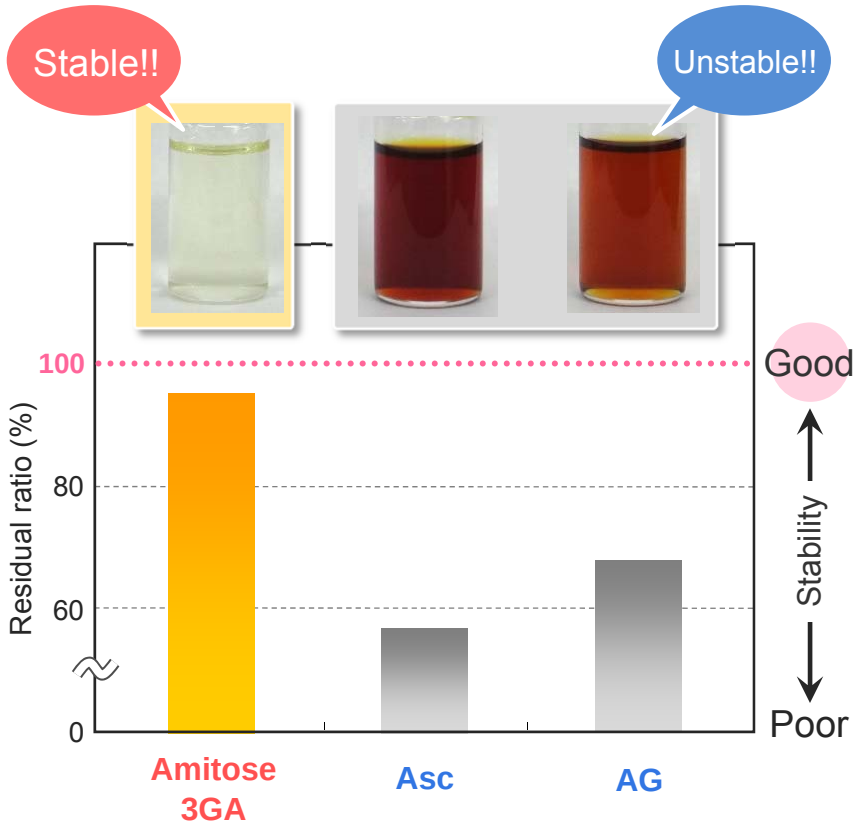
Stability test of VC-rich serum

Formulation No. 0181 (w/w %)

Ingredient	Amitose 3GA	Asc	AG
Amitose 3GA	71.4*	–	–
Asc	–	15.0	–
Asc derivative	–	–	29.0*
Glycerin	–	21.4	21.4
Ethoxydiglycol	25.0	25.0	25.0
Potassium Hydroxide	adequate amount	adequate amount	adequate amount
Water	up to 100	up to 100	up to 100

* Each serum contains Asc derivatives correspond to 15.0% Asc.
[pH conditions] pH3.0-4.0

[Experimental method]
Testing VC-rich serums were stored at 50 deg C for 3 month and stability of those formulae were evaluated by observing the change of appearance and residual ratio of Asc and Asc derivatives.



2. Stability of cosmetics

Stable formulation into cosmetics

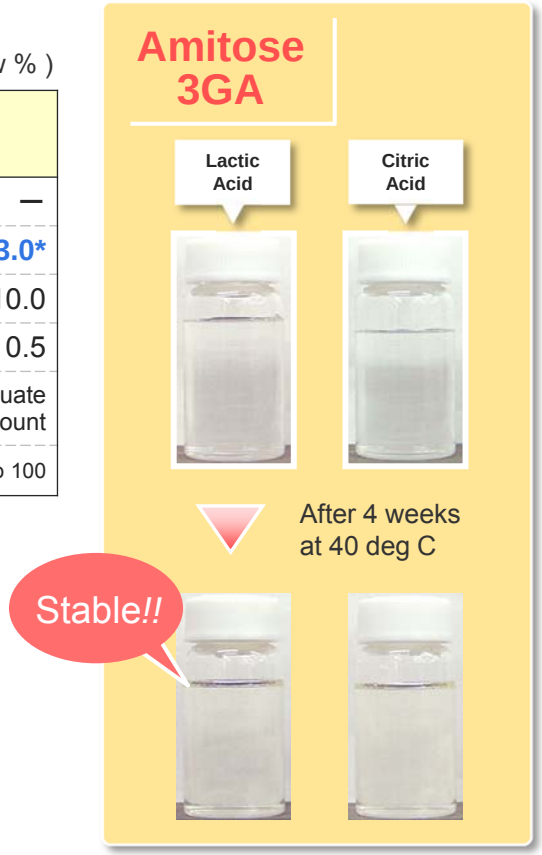
Amitose 3GA can be formulated in mildly-acidic cosmetics stably.

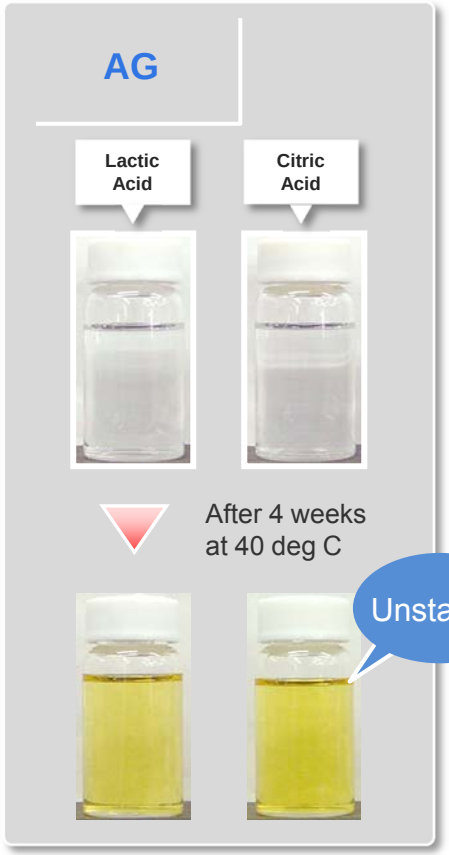
Stability test in mildly-acidic condition

(w/w %)

Ingredient	Amitose 3GA	AG
Amitose 3GA	10.0*	–
Asc derivative	–	3.0*
Alpha Hydroxy Acids	10.0	10.0
Phenoxyethanol	0.5	0.5
Potassium Hydroxide	adequate amount	adequate amount
Water	up to 100	up to 100

[pH conditions]
pH3.0





2. Stability of cosmetics

Good sensory texture

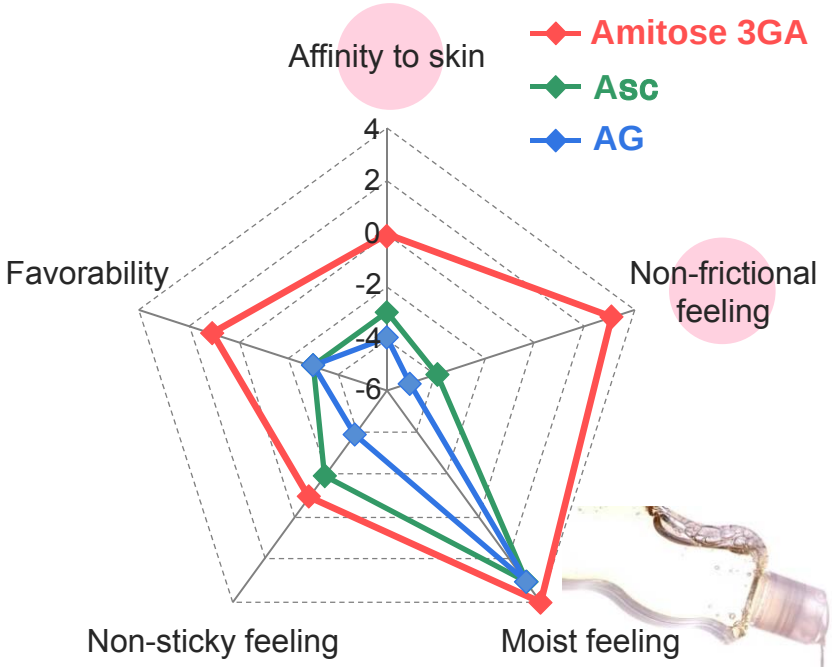
Amitose 3GA-skin toner has an excellent sensory texture without sticky-feeling specific to Asc.

Sensory evaluation test of skin toner

(w/w %)

Ingredient	Control	Amitose 3GA	Bench mark
Amitose 3GA	–	3.3	–
Asc or Asc derivative	–	–	1.0
Glycerin	3.0	3.0	3.0
Ethylhexylglycerin (10%), Phenoxyethanol (90%)	0.5	0.5	0.5
Alcohol	5.0	5.0	5.0
Citric Acid/ Sodium Citrate	0.5	0.5	0.5
Potassium Hydroxide	adequate amount	adequate amount	adequate amount
Water	up to 100	up to 100	up to 100

[pH conditions] pH4.5: Amitose 3GA and its Control formulae,
pH7.0: Benchmark and their Control formulae
Each formulae were adjusted to be adequate pH according to the stable pH of individual vitamin C derivatives.



[Experimental method]
Sensory evaluations were conducted by 8 panels. Control formulation was assigned to be 0 point base, and for evaluating items, -1~+1 points were given. The total evaluation points were summed.

2. Stability of cosmetics

Application for cosmetics

Amitose 3GA

for Cosmetics

Skin-milk, Cream, Serum

Emulsion/gel typed-formulae

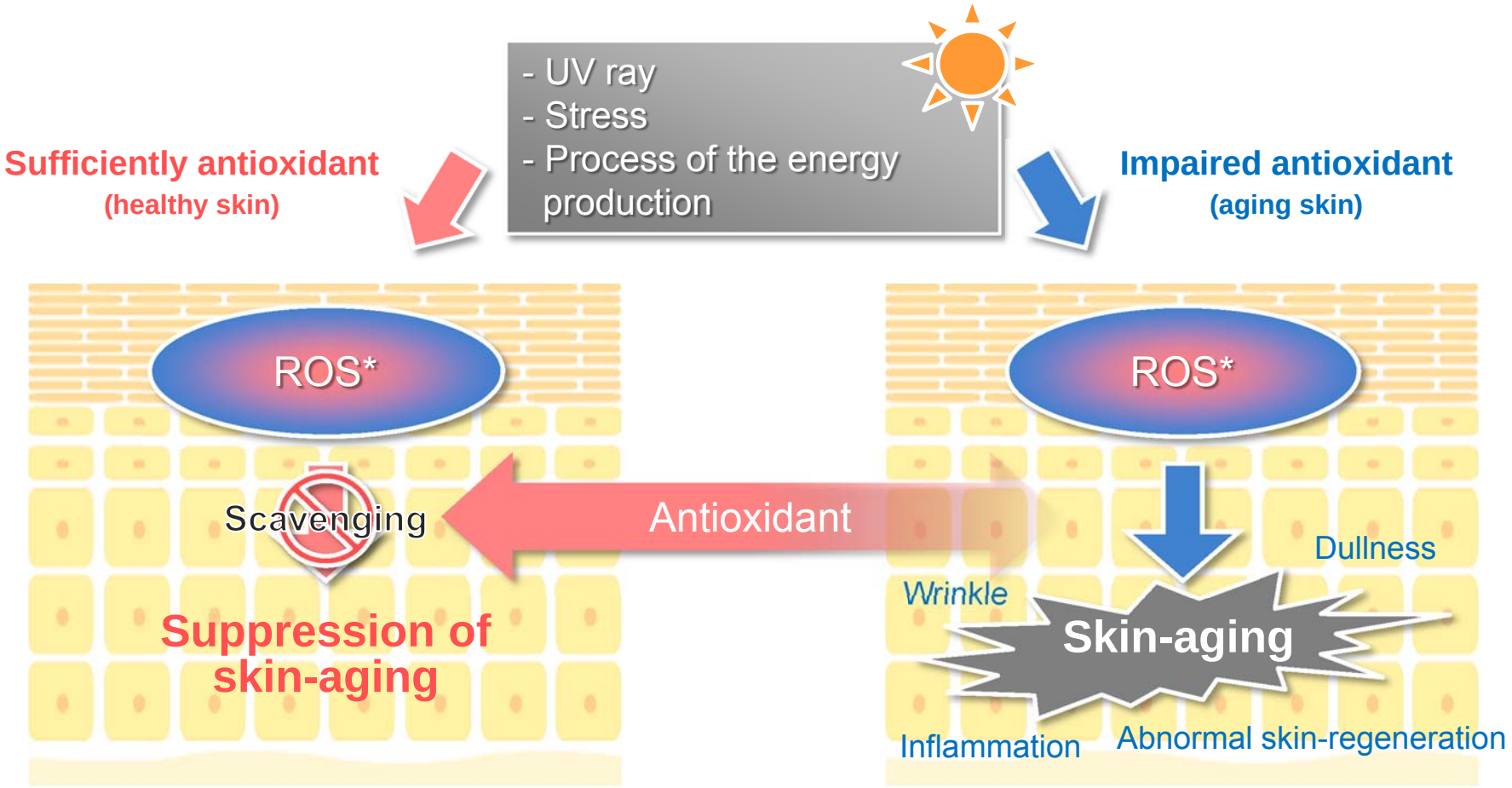
Easy to make stable formulae over time

VC-rich serum

Achieved by high-stability and reasonable price

3. Anti-aging effects

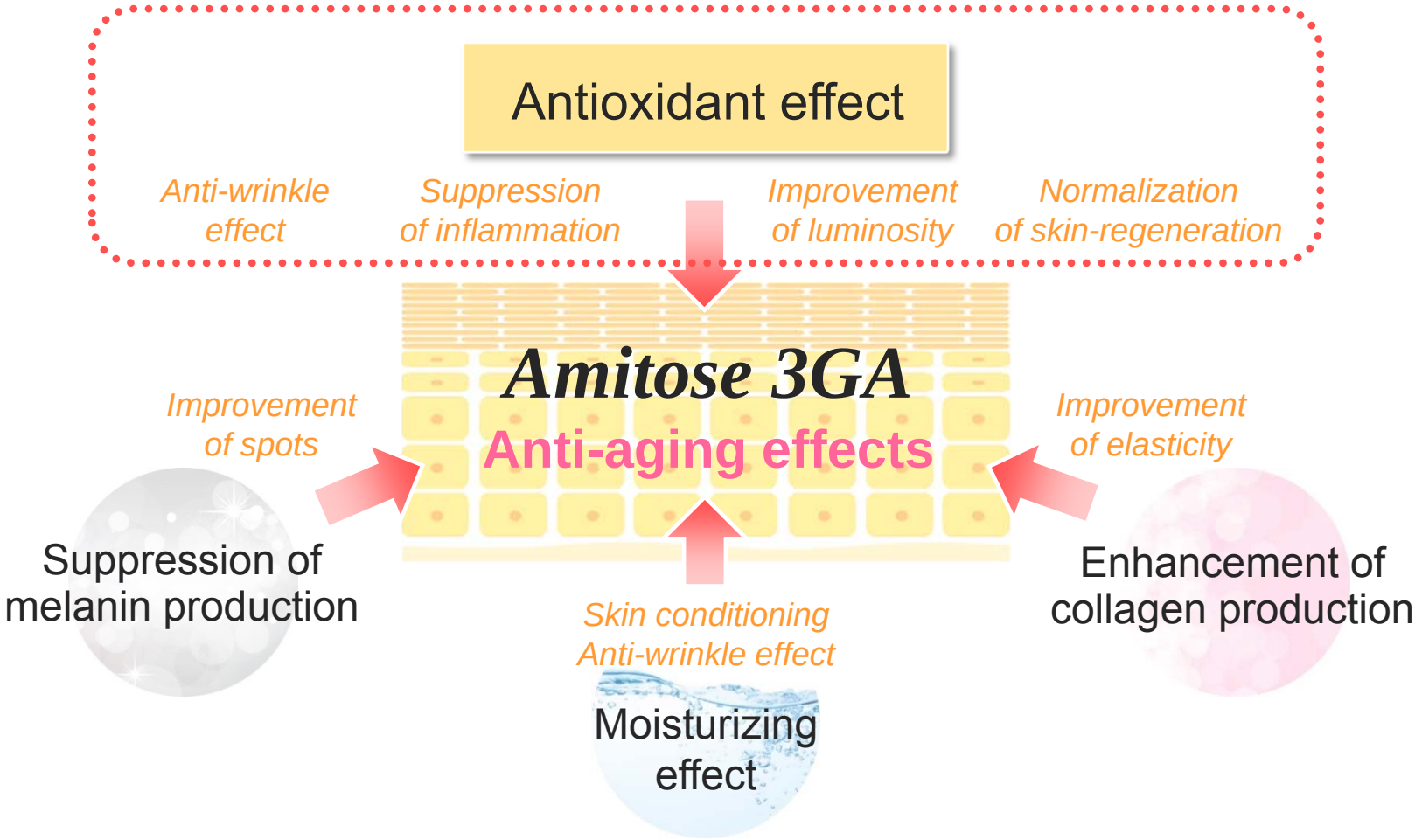
Anti-aging effects by antioxidant effect



* ROS; reactive oxygen species

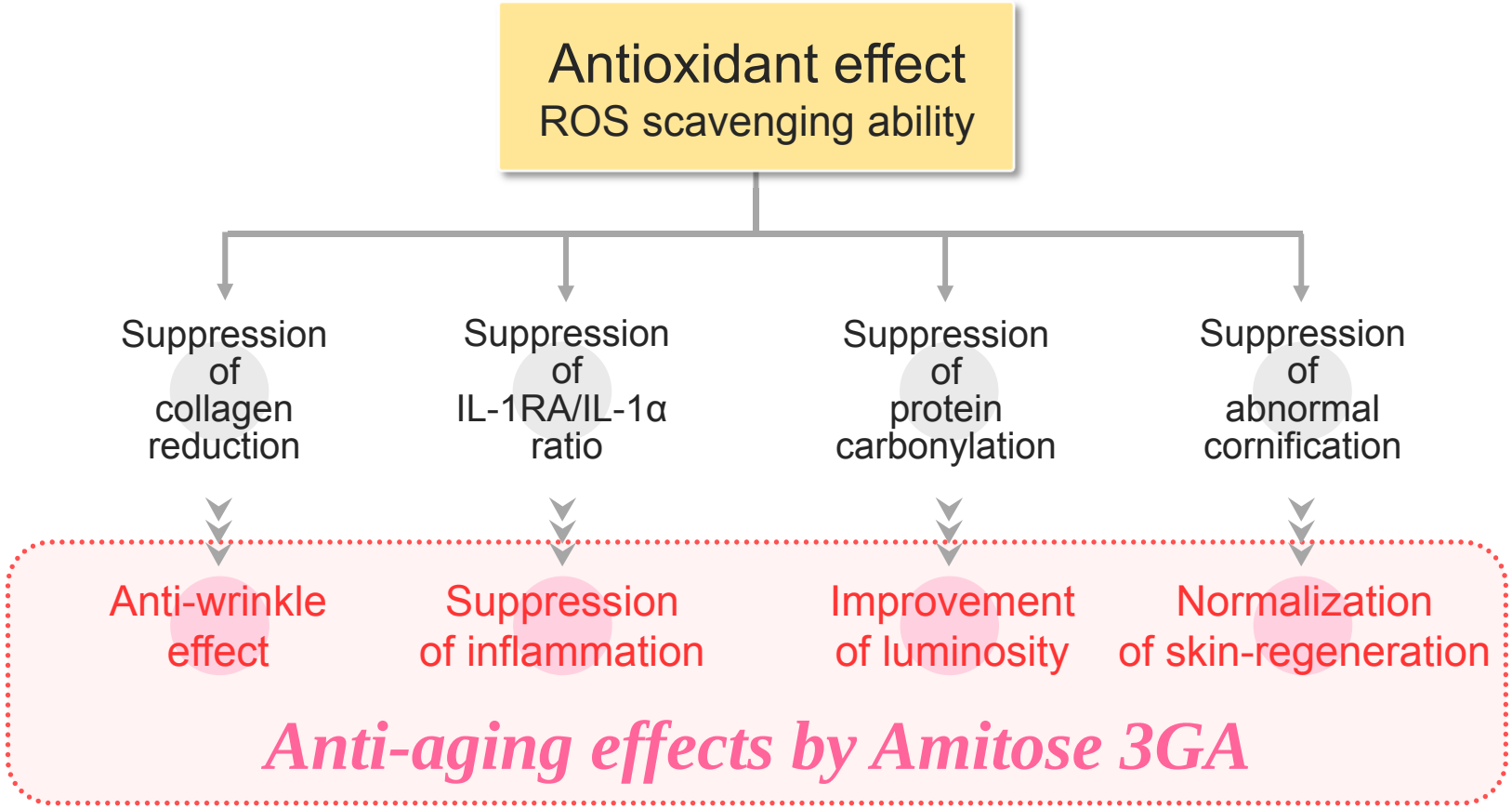
3. Anti-aging effects

Multiple anti-aging effect



3. Anti-aging effects : Approach from antioxidant effect

Anti-aging effects by antioxidant effect

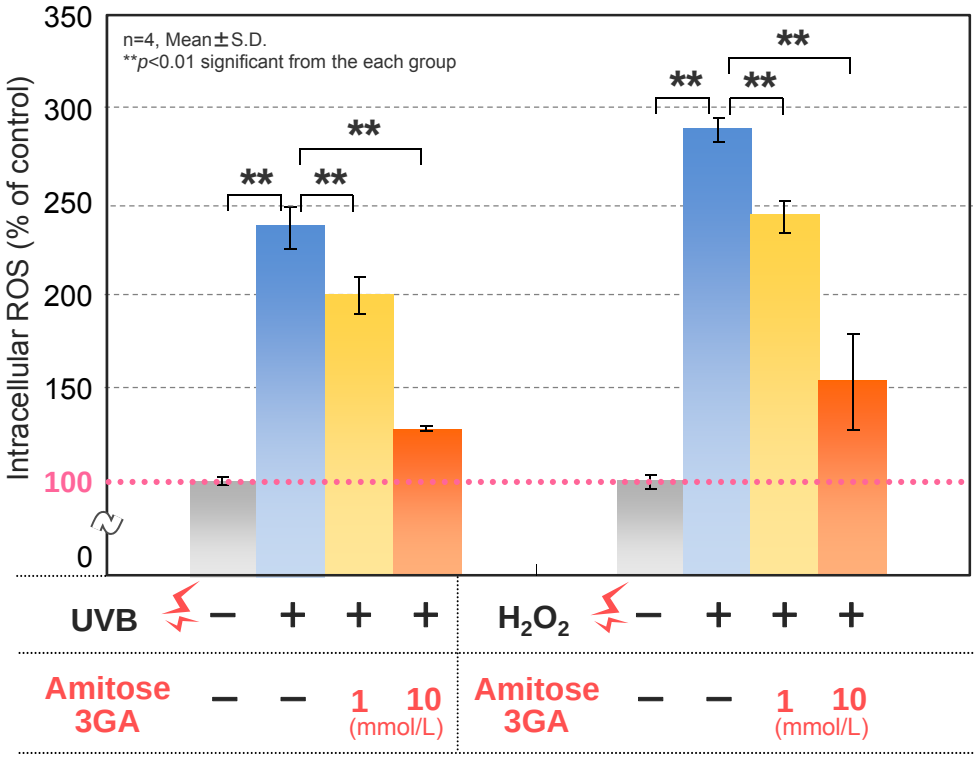


3. Anti-aging effects : Approach from antioxidant effect

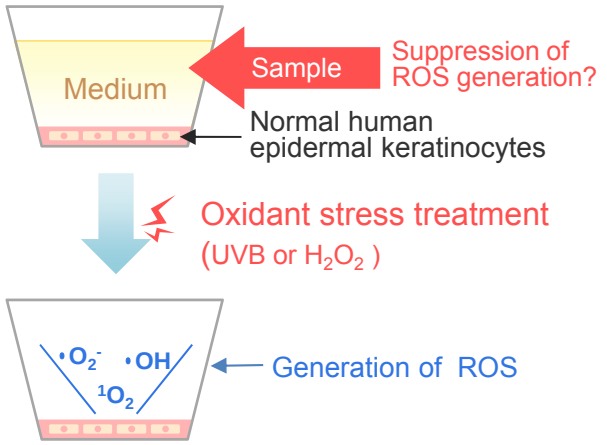
Antioxidant effect

Amitose 3GA
suppresses generation of ROS by oxidant stress.

Evaluation of suppressing effect of ROS using normal human epidermal keratinocytes



[Experimental method]
Normal human epidermal keratinocytes were seeded into 96-well plates and incubated for 1 day in medium containing sample. The medium was removed, and loaded DCFHDA as the probe for intracellular ROS in the cells. Fluorescence intensity was measured after oxidant stress treatment by UVB irradiation (50mJ/cm^2) or H_2O_2 (0.25mmol/L).

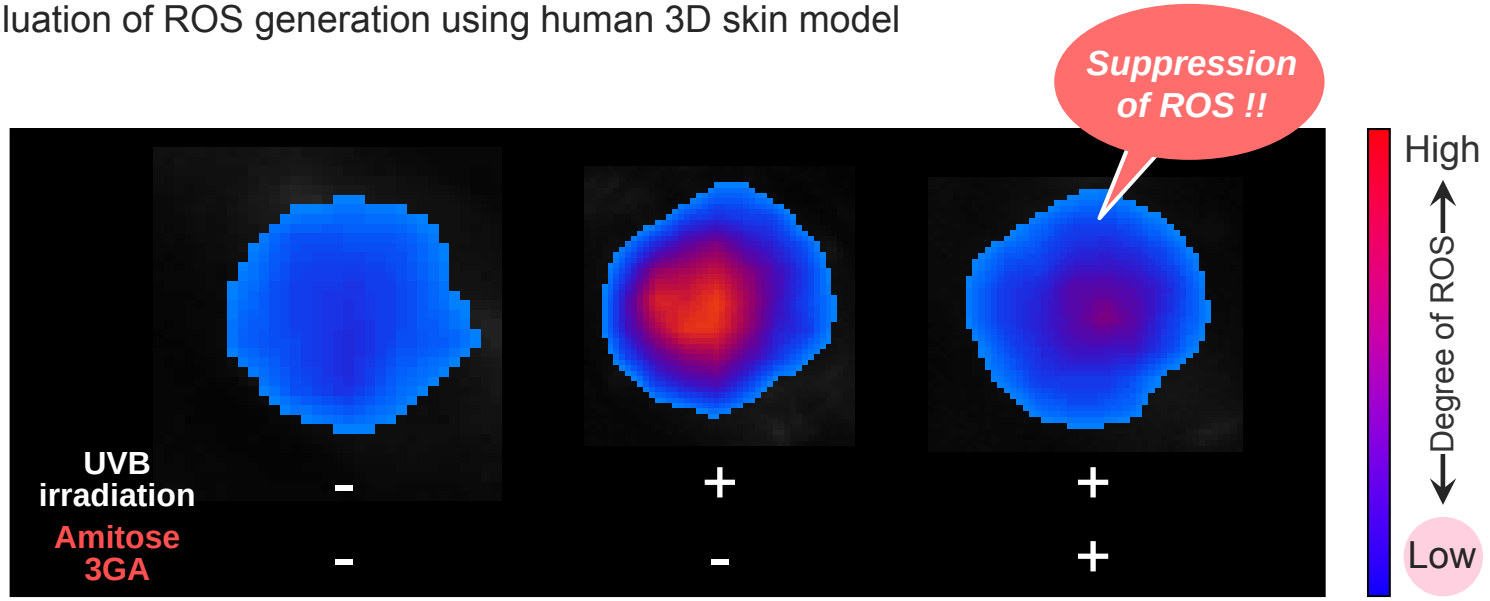


3. Anti-aging effects : Approach from antioxidant effect

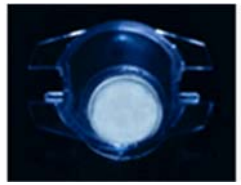
Antioxidant effect

Amitose 3GA penetrates into the epidermis, and suppresses ROS generation.

Evaluation of ROS generation using human 3D skin model



[Experimental method]
Sample* was applied to the surface of human 3D skin model (LabCyte EPI-MODEL 24 (Japan Tissue Engineering Co., Ltd., Japan) and was incubated under the condition of 37 deg C and 5% of CO_2 for 1 day. After the sample was removed, cells were exposed to UVB (150mJ/cm^2). Furthermore, MCLA as the probe for intracellular ROS was applied in order to visualization of generated ROS. After the probe was allowed to enter cells, ROS in cell was monitered by NightOWL (Berthold Technologies GmbH).
* 0.5% water solution of active component of Amitose 3GA, 3-Glyceryl Ascorbate.

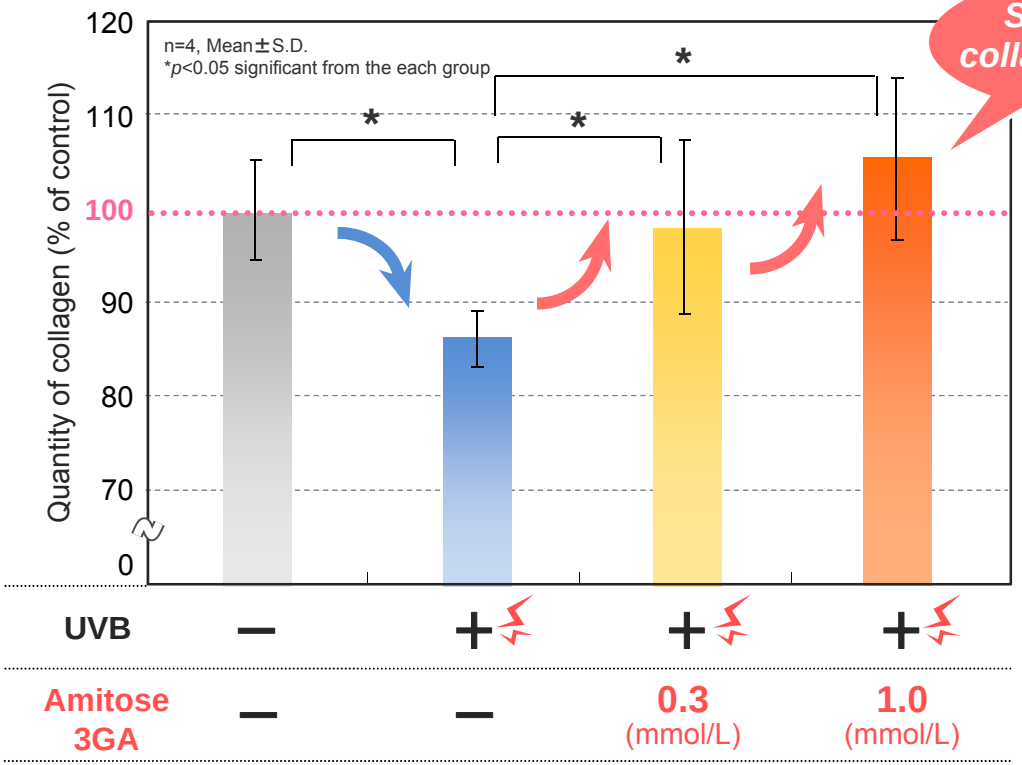

LabCyte EPI-MODEL 24

3. Anti-aging effects : Approach from antioxidant effect

Anti-wrinkle effect

Amitose 3GA
suppresses collagen reduction caused by ROS.

Evaluation of suppressing effect of collagen reduction



Suppression of collagen reduction !!

If amount of collagen in skin is reduced...
- Loss of skin elasticity !!
- Generation of wrinkle !!

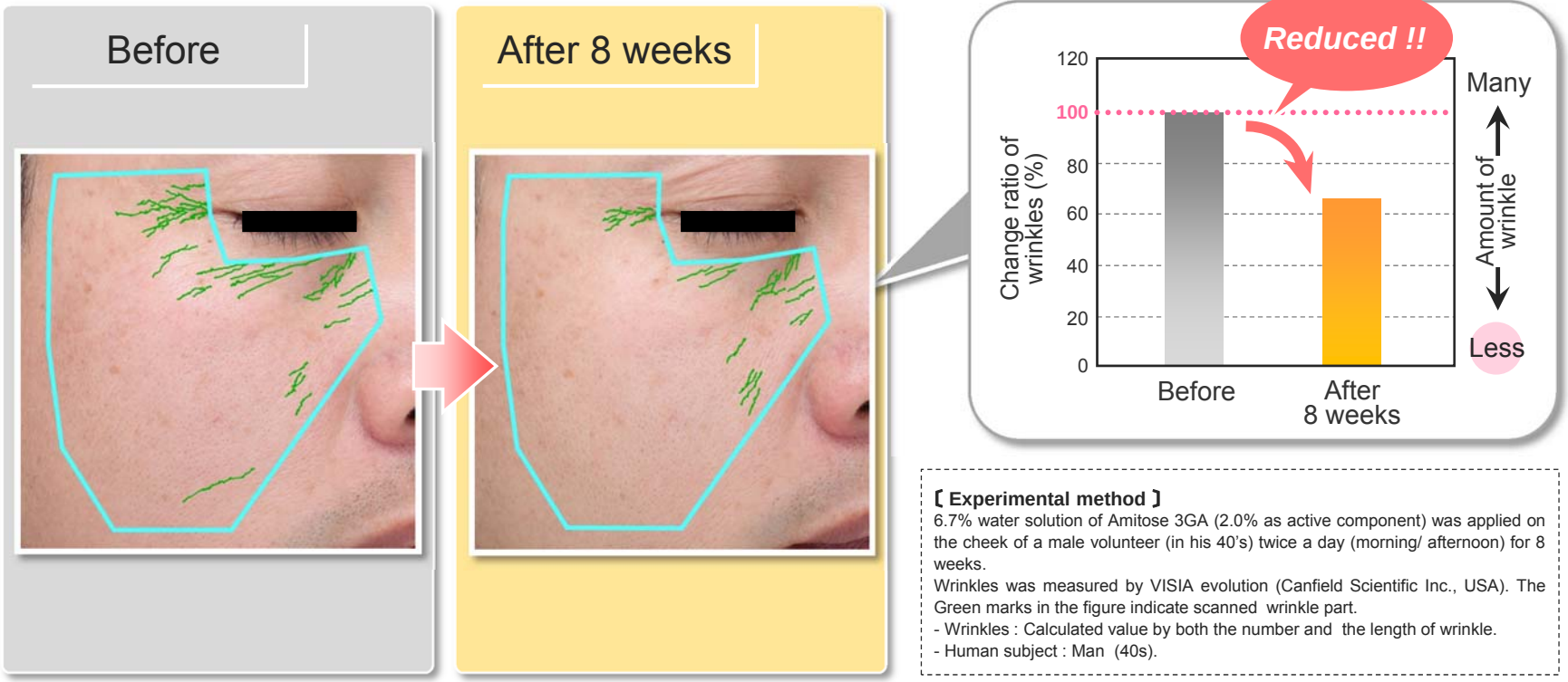
[Experimental method]
Normal human epidermal keratinocytes were seeded into 96-well plates and incubated for 1 day in medium containing sample. The medium was removed and UVB was irradiated (50mJ/cm^2). After keratinocytes were incubated for another 1day in fresh medium, the medium was collected and used for the following assay.
On the other hand, Normal human dermal fibroblasts were seeded into 96-well plates and were incubated for 1 day with the collected medium . After that, quantity of type I collagen in the medium was measured by ELISA assay.

3. Anti-aging effects : Approach from antioxidant effect

Anti-wrinkle effect

Amitose 3GA expects anti-wrinkle effect when used on a daily basis.

Evaluation of anti-wrinkle effect by VISIA; *in vivo* test



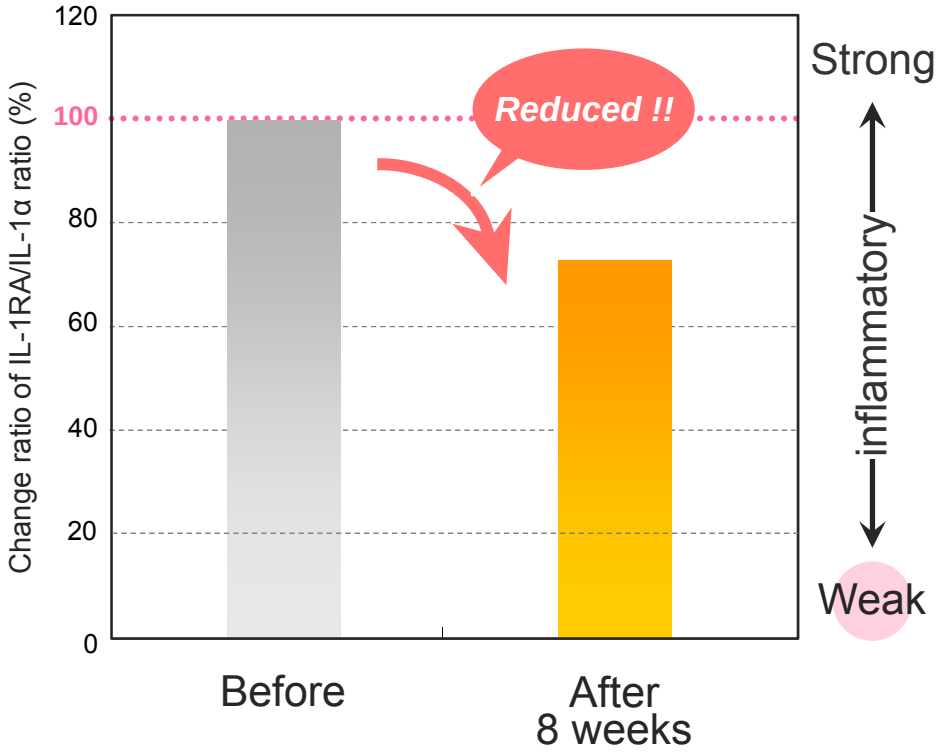
【 **Experimental method** 】
6.7% water solution of Amitose 3GA (2.0% as active component) was applied on the cheek of a male volunteer (in his 40's) twice a day (morning/ afternoon) for 8 weeks.
Wrinkles was measured by VISIA evolution (Canfield Scientific Inc., USA). The Green marks in the figure indicate scanned wrinkle part.
- Wrinkles : Calculated value by both the number and the length of wrinkle.
- Human subject : Man (40s).

3. Anti-aging effects : Approach from antioxidant effect

Suppression of inflammation

Amitose 3GA expects to suppress the inflammation when used on a daily basis.

Evaluation of IL*-1RA/IL-1α ratio



"IL-1RA/IL-1α ratio" is …

Value for evaluating the degree of potential inflammation, skin roughness.

*IL; Interleukin

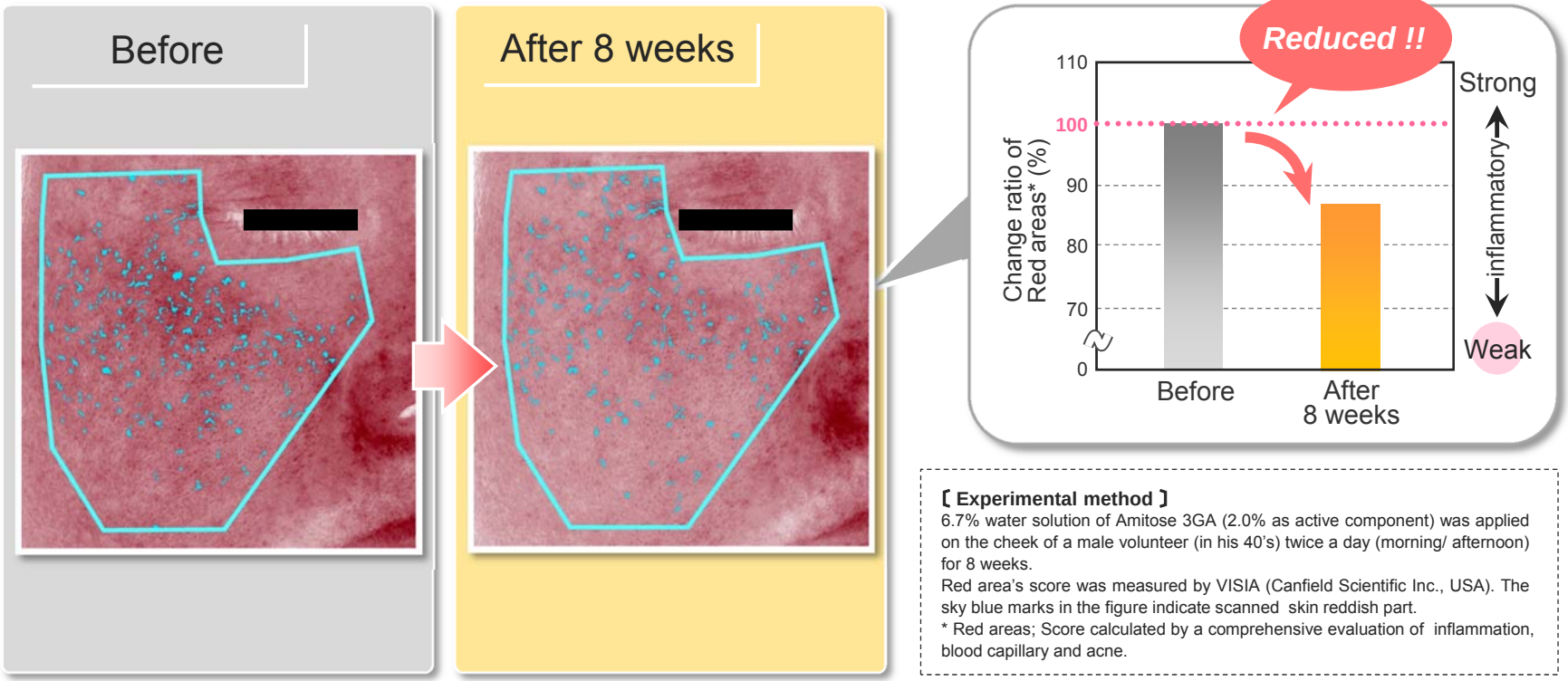
[Experimental method]
6.7% water solution of Amitose 3GA (2.0% as active component) was applied on the cheek of a male volunteer (in his 40's) twice a day (morning/ afternoon) for 8 weeks. Then, the stratum corneum (SC) on the cheek was collected by tape-stripping.
Interleukin-1 receptor antagonist (IL-1RA) and IL-1α in extracts of SC with 0.05% Triton-X 100 were quantified by ELISA assay.

3. Anti-aging effects : Approach from antioxidant effect

Suppression of inflammation

Amitose 3GA is expected to suppress the inflammation when used on a daily basis.

Evaluation of inflammation of cheek by VISIA; *in vivo* test



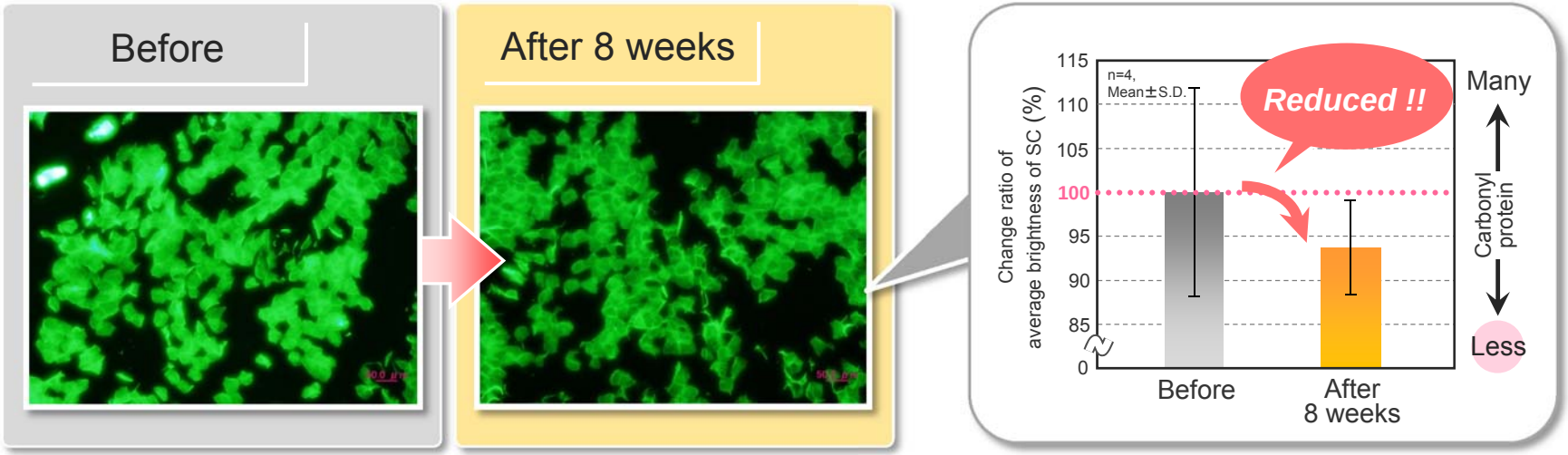
【 **Experimental method** 】
6.7% water solution of Amitose 3GA (2.0% as active component) was applied on the cheek of a male volunteer (in his 40's) twice a day (morning/ afternoon) for 8 weeks.
Red area's score was measured by VISIA (Canfield Scientific Inc., USA). The sky blue marks in the figure indicate scanned skin reddish part.
* Red areas; Score calculated by a comprehensive evaluation of inflammation, blood capillary and acne.

3. Anti-aging effects : Approach from antioxidant effect

Improvement of luminosity

Amitose 3GA suppresses protein carbonylation, and leads to the bright skin when used on a daily basis.

Evaluation of suppressing effect of protein carbonylation



"Carbonyl protein" is …

Oxidative modified protein generated by ROS.

It causes skin dullness because of yellow discoloration of denatured protein.

【 **Experimental method** 】

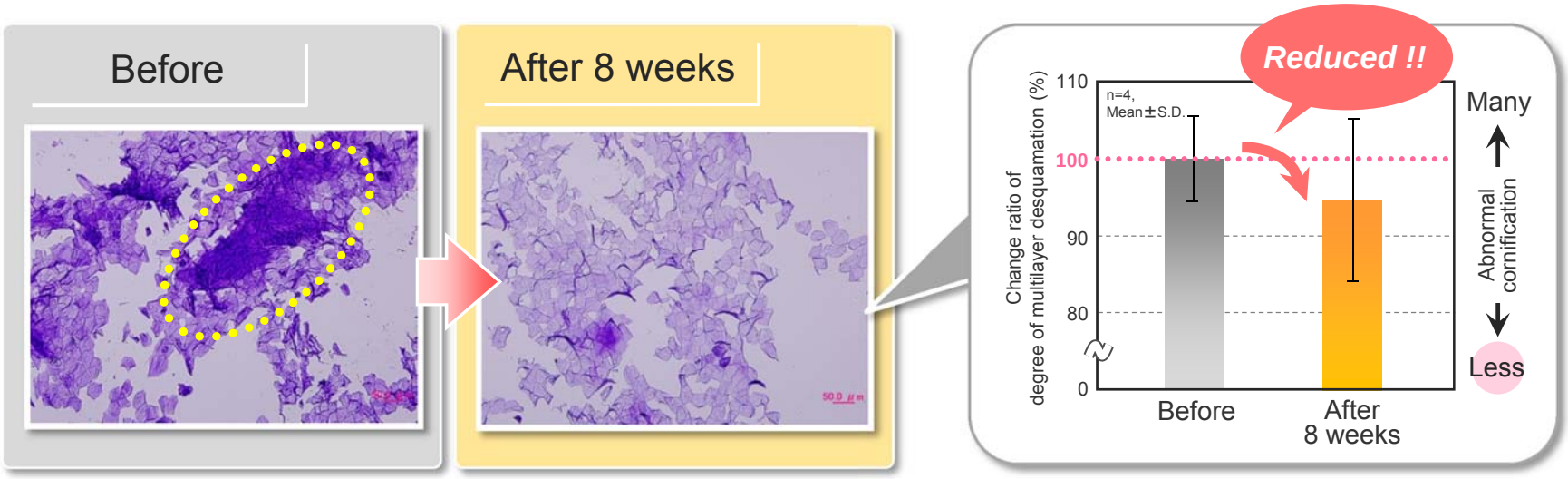
6.7% water solution of Amitose 3GA (2.0% as active component) was applied on the cheek of a male volunteer (in his 40's) twice a day (morning/ afternoon) for 8 weeks. Then, the stratum corneum (SC) on the cheek was collected by tape-stripping. SC transferred onto a glass slide was stained with 20mmol/L fluorescein-5-thiosemicarbazide for 1 hour at room temperature. After washing with PBS, stained SC was analyzed for images observed with an optical microscope (BX-51-FL, Olympus, Japan). Average brightness was calculated using the SC measuring program; corneocytometry (CIEL Co., Ltd., Japan).

3. Anti-aging effects : Approach from antioxidant effect

Normalization of skin-regeneration

Amitose 3GA suppresses abnormal cornification, and normalizes the skin-regeneration used on a daily basis.

Evaluation of suppressing effect of multilayer desquamation of the stratum corneum



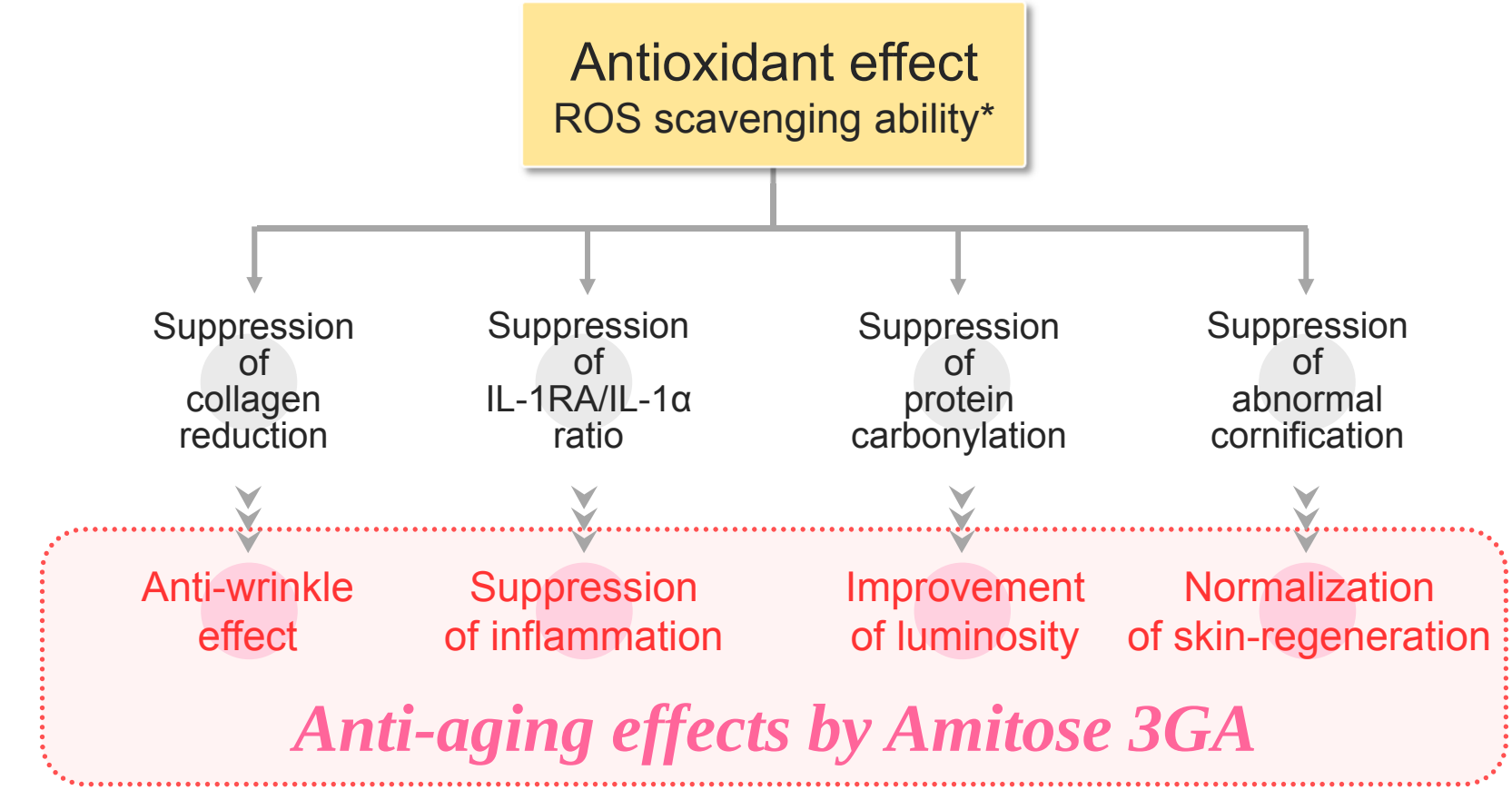
"Multilayer desquamation of the stratum corneum" is …

A phenomenon that stratum corneum is peeled off in folds through the tape-stripping process. It is one of the index standard of skin problems.

【 **Experimental method** 】
6.7% water solution of Amitose 3GA (2.0% as active component) was applied on the cheek of a male volunteer (in his 40's) twice a day (morning/ afternoon) for 8 weeks. Then, the stratum corneum (SC) on the cheek was collected by tape-stripping. SC transferred onto a glass slide was stained with 0.5% brilliant green and 1% gentian violet for 10 min. After washing with water, the ratio of multilayer desquamation was analyzed for images observed with an optical microscope (BX-51-FL, Olympus, Japan). Degree of multilayer desquamation was calculated by the ratio of area in multilayer desquamation and area in the total SC using the SC measuring program; corneocytometry (CIEL Co., Ltd., Japan)

3. Anti-aging effects : Approach from antioxidant effect

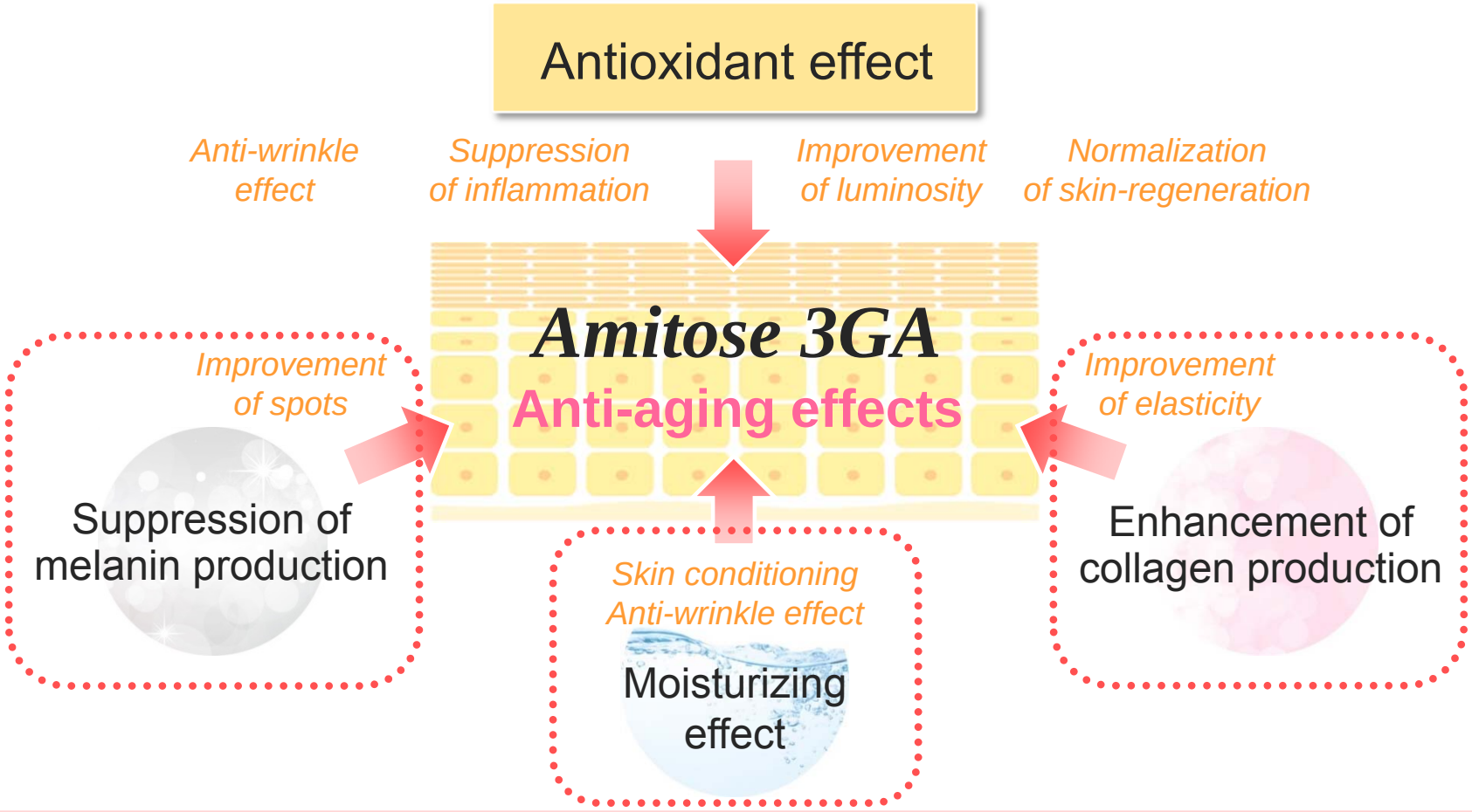
Anti-aging effects by antioxidant effect



* ROS; reactive oxygen species

4. Anti-aging effects : Other approaches

Multiple anti-aging effect

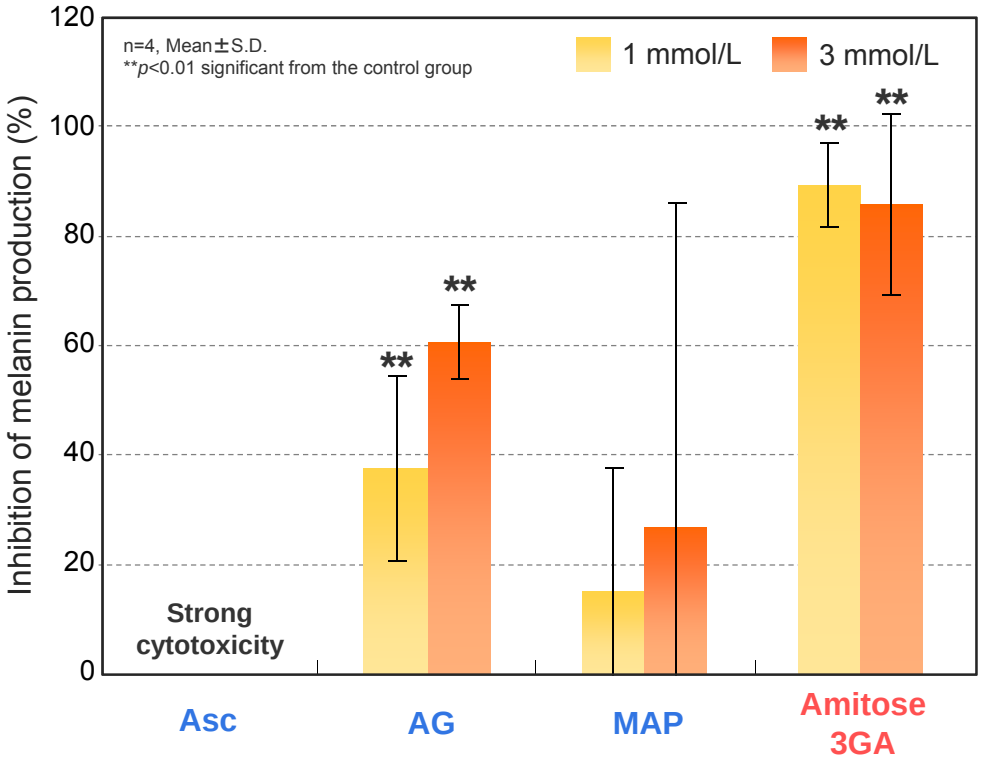


4. Anti-aging effects : Other approaches

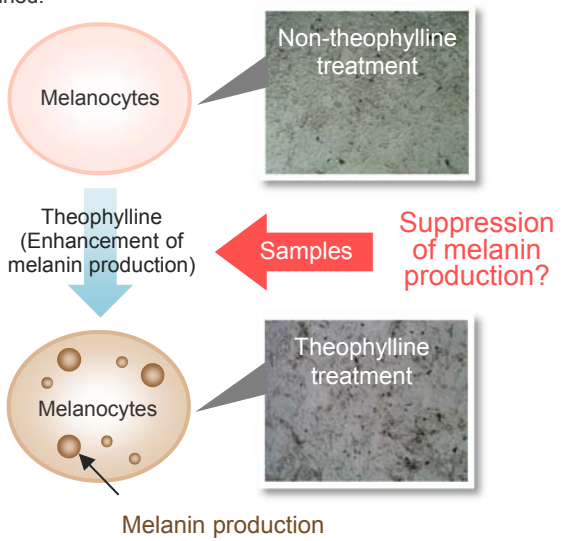
Suppression of melanin production

Amitose 3GA suppresses the melanin production of melanocytes.

Suppressing effect on the melanin production of Melanocytes



[Experimental method]
Melanocytes were cultured in the wells of 48-well plate for 1 day, the medium was replaced with medium containing sample and melanin production enhancing agent (theophylline), and the culture was continued. At 3 days after the medium exchange, the medium was removed, and the produced amounts of melanin in the cell were determined.

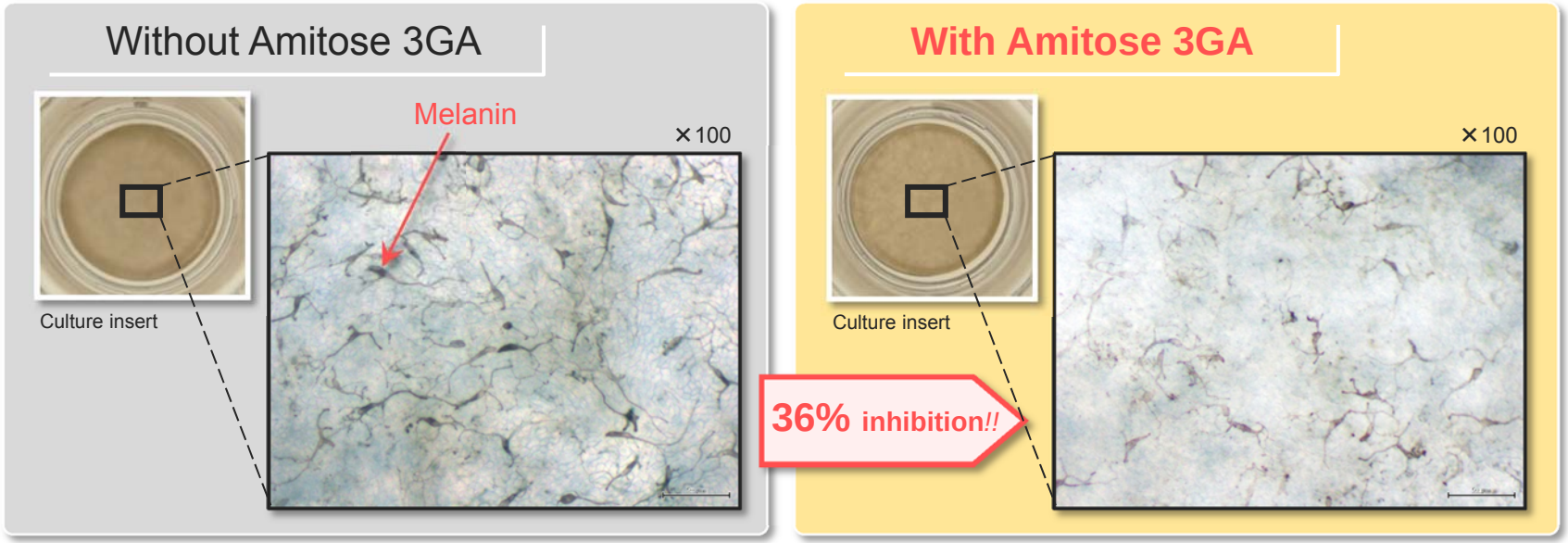


4. Anti-aging effects : Other approaches

Suppression of melanin production

Amitose 3GA suppresses the melanin production on human 3D skin model.

Suppression of melanin production on human 3D skin model



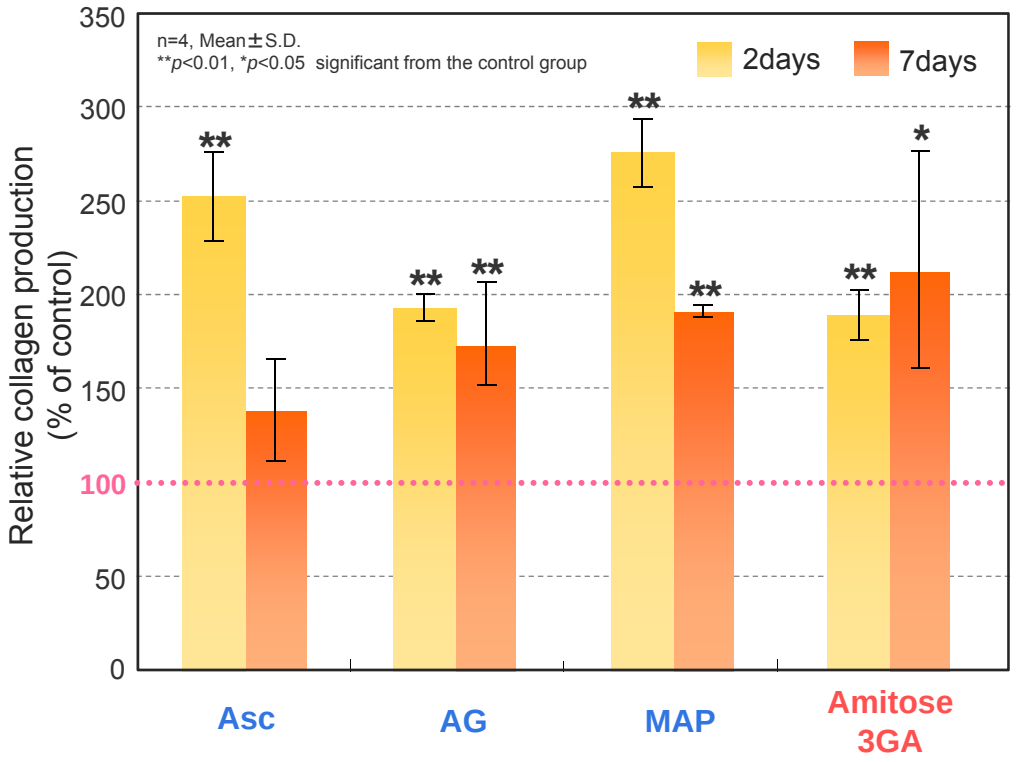
[Experimental method]
The culture inserts with human 3D skin model, "MEL-300 Asian (donor) (MatTek)" were set in the wells of 6-well plate, applied medium, and cultured under the condition of 37 deg C and 5% CO_2 for 3 weeks. The addition of sample* on the surface of the cells and the exchange of culture medium were conducted every second day.
After 3 weeks, the medium was removed, the produced amounts of melanin in the cell were determined by absorbance determination and the appearances of melanocytes were observed by optical microscope.
* 10% solution of active component of Amitose 3GA, 3-Glyceryl Ascorbate.

4. Anti-aging effects : Other approaches

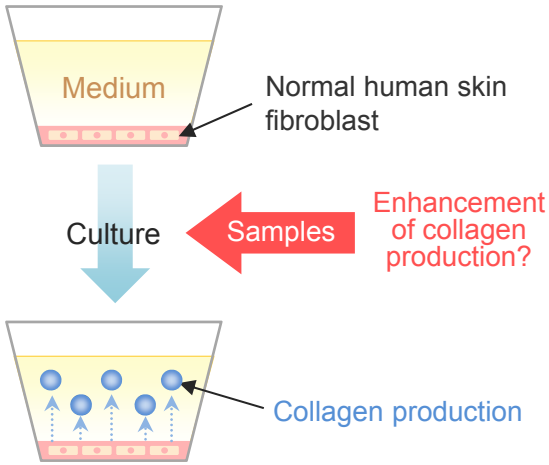
Enhancement of collagen production

Amitose 3GA enhances the collagen production of normal human dermal fibroblast, and the long-term effect is expected.

Enhancing effect on the collagen production of normal human skin fibroblast



[Experimental method]
Normal human skin fibroblasts were cultured in the wells of 96-well plate for 1 day, the medium was replaced with medium containing sample (500 μmol/L), and the culture was continued.
At 2 and 7 days after the exchange of medium, culture supernatants were taken for determining the collagen content of medium.

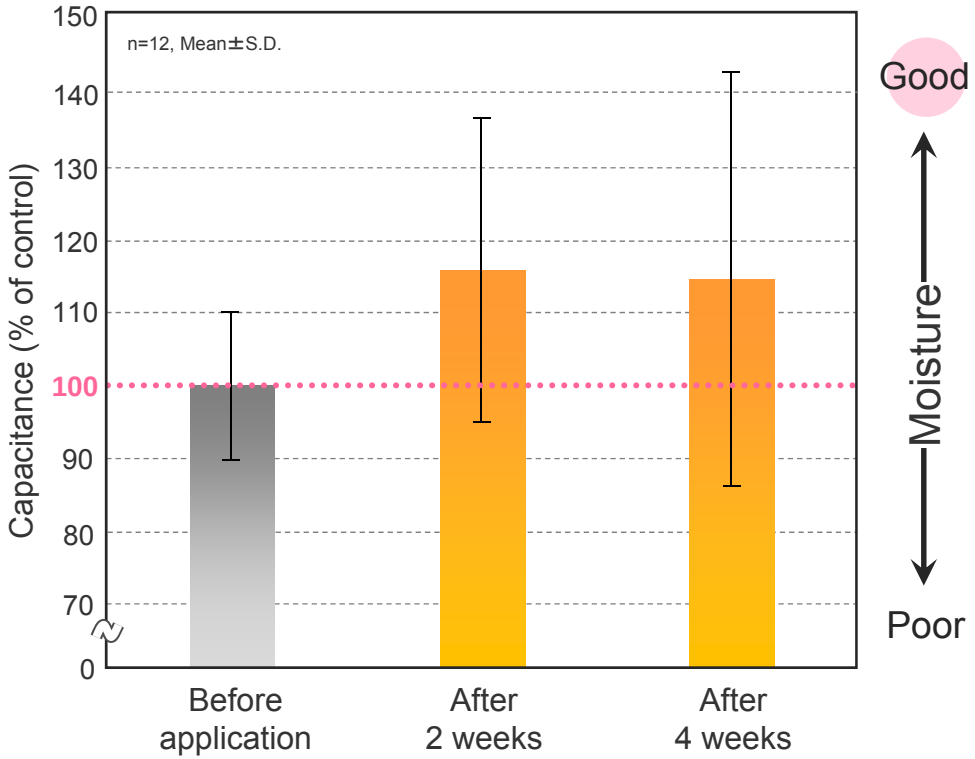


4. Anti-aging effects : Other approaches

Moisturizing effect

Amitose 3GA gives moisture to skin.

Measurement of epidermal water content; continuous *in vivo* test



[Experimental method]
6.7% water solution of Amitose 3GA (2.0% as active component) was applied on palm side of the forearm twice a day in the morning and evening for 4 weeks.
The amount of moisture in stratum corneum was measured and the skin surface was observed by microscope.

Control: apply ion-exchanged water.
Acclimating condition: 18-22 deg C, 40-60% degree of humidity for 20 minutes.
Instrument: Corneometer CM-825 (Courage+Khazaka electronic GmbH)

5. Conclusion

Amitose 3GA

Anti-aging vitamin C, simple to apply

Reliable effect

Stable in wide-range of formulae
Easy to feel certain anti-aging effect

Reasonable price of product

Application for wide-variety of cosmetics

- Enable to apply various cosmetics because of reasonable price and high stability
- Gentle anti-aging effect in daily-use

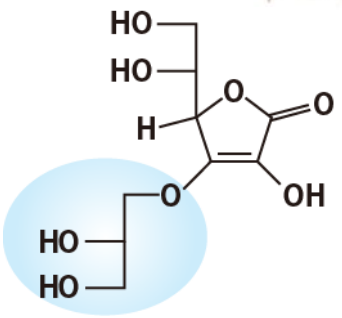
Development of highly-concentrated cosmetics

- Resolve the problem on stability caused by high concentration.
- Cost-saving of formulae
- Excellent anti-aging effect thanks to high concentration.

Product information

Amitose 3GA

Anti-aging vitamin C



Composition

INCI name	Composition
3-Glyceryl Ascorbate	30%
Glycerin	30%
Water	40%

Preservative free

* Recommended storage conditions: Keep refrigerated