



**G** Rutin  
**Glucosyl Rutin**

# Glucosylated Vitamin P

## GRutin (for cosmetics)

### Introduction

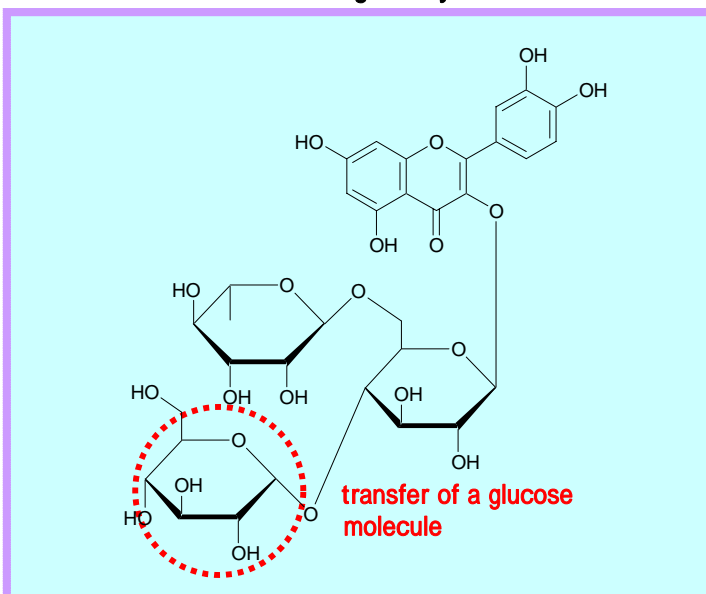
From ancient times, buckwheat has been considered as a cereal very effective in reducing high blood pressure. The effect of this cereal is well known to be attributable to rutin, a flavonoid compound (vitamin P) that improves blood circulation. Rutin has also anti-oxidant and anti-allergy activities. All things considered, rutin has important health benefits.

The poor solubility of rutin not only in water but also in alcohol or oil restricted its commercial use. Hayashibara Biochemical Laboratories, Inc., in collaboration with Toyo Seito Co., Ltd, has succeeded in the development and manufacture of a water-soluble glucosyl rutin marketed for cosmetics applications under the name of GRutin ( for cosmetics ) .

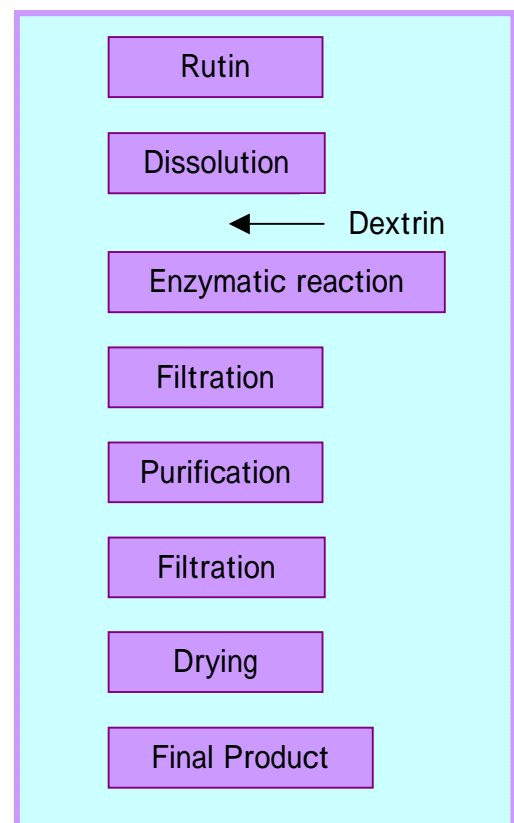
### Chemical structure of glucosyl rutin

Glucosyl rutin is manufactured by transferring from starch to rutin, using an enzyme obtained from a microorganism.

#### Chemical structure of glucosyl rutin



#### Manufacturing process



## Properties of glucosyl rutin

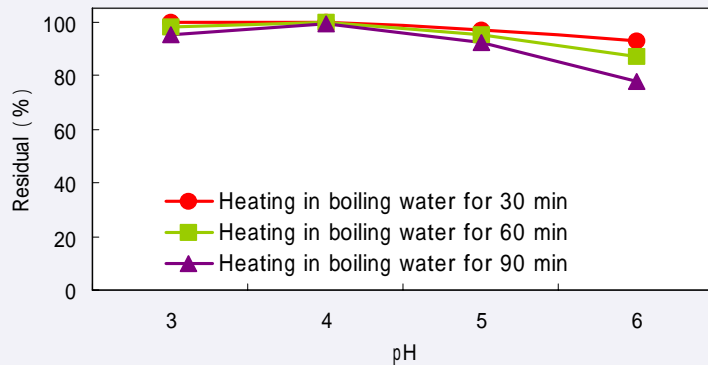
### ■ High water solubility

Glucosylation of rutin significantly raised its solubility in water. Glucosyl rutin is stable to heat within a pH range of 3 to 5.

#### Solubility in water

	Solubility in water <sup>1)</sup> (g / 100g water, room temperature)
Glucosyl rutin	120
Rutin	0.01

#### Heat resistance

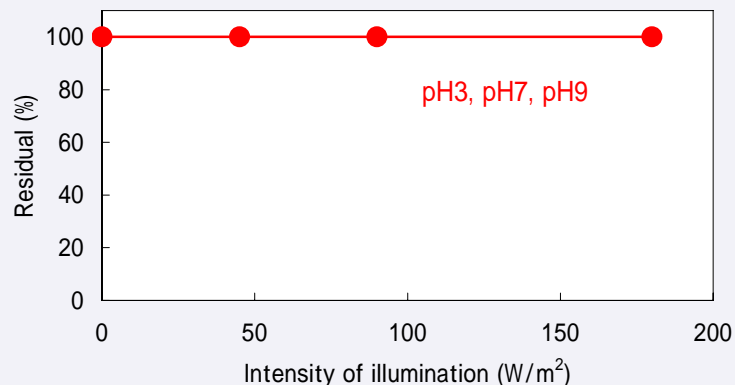


0.1% solutions were prepared by dissolving glucosyl rutin in different pH buffers. Glucosyl rutin was quantified after heating<sup>2)</sup>.

### ■ High photostability

Glucosyl rutin is photostable within a pH range of 3 to 9.

#### Photostability in solution



0.005% solutions were prepared by dissolving glucosyl rutin in different pH buffers.

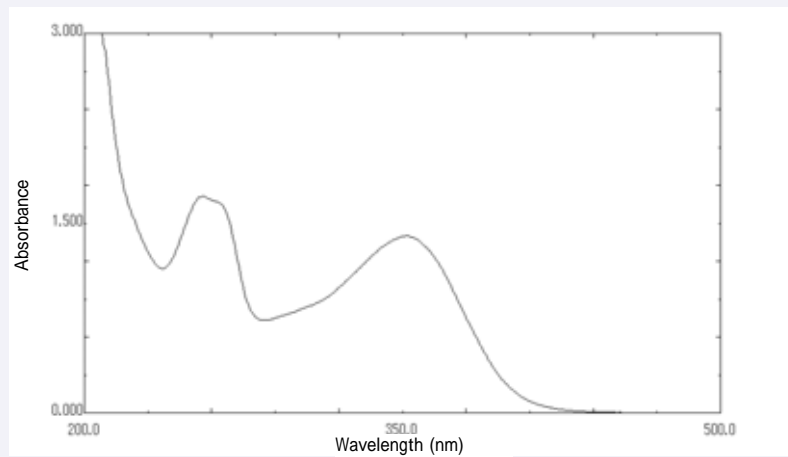
After a 2-hour lighting at 100W/m<sup>2</sup> (25 C, 60%RH), glucosyl rutin was quantified.<sup>4)</sup>

\* The effect of the lighting described above is equivalent to that of an 8-hour exposure to sunlight in summer.

### ■ Ultra violet (UV) absorption

Glucosyl rutin has a peak absorbance at 260 nm and 360 nm, which means it absorbs UV.

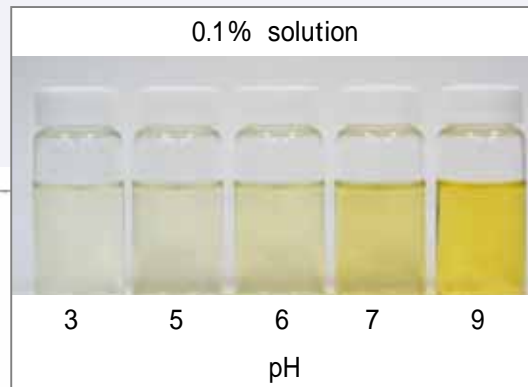
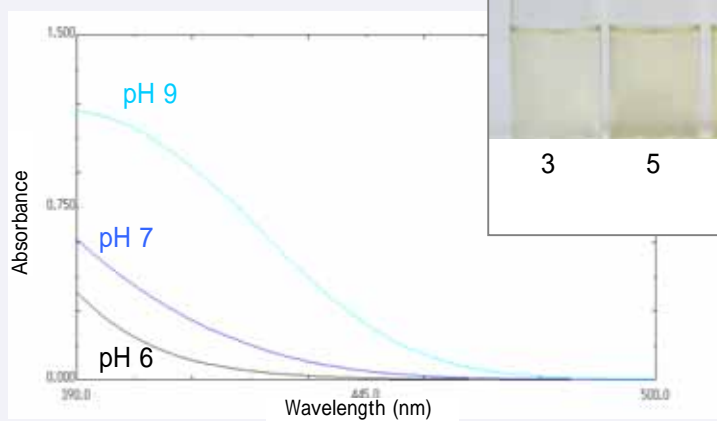
#### Absorption spectrum



A 0.06% solution of glucosyl rutin was used.<sup>4)</sup>

The increase of pH value in glucosyl rutin solution promoted coloration (yellow).

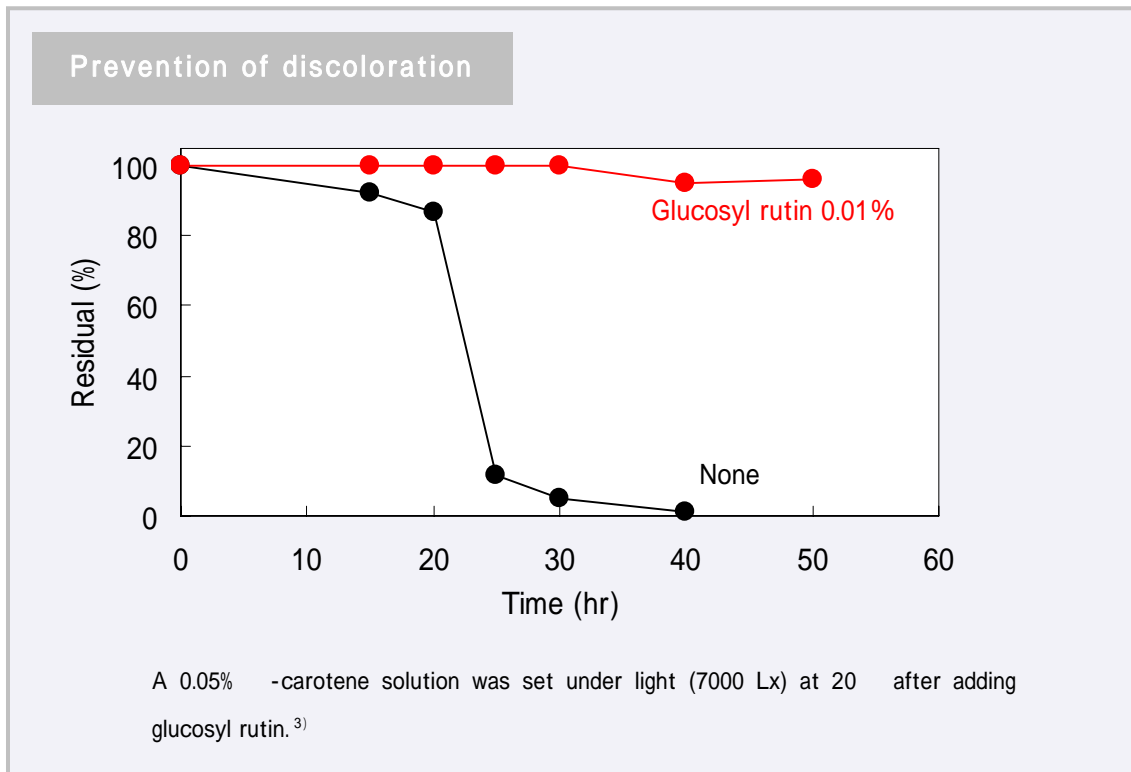
#### Absorption spectrum



A 0.005% solution of glucosyl rutin was used.<sup>4)</sup>

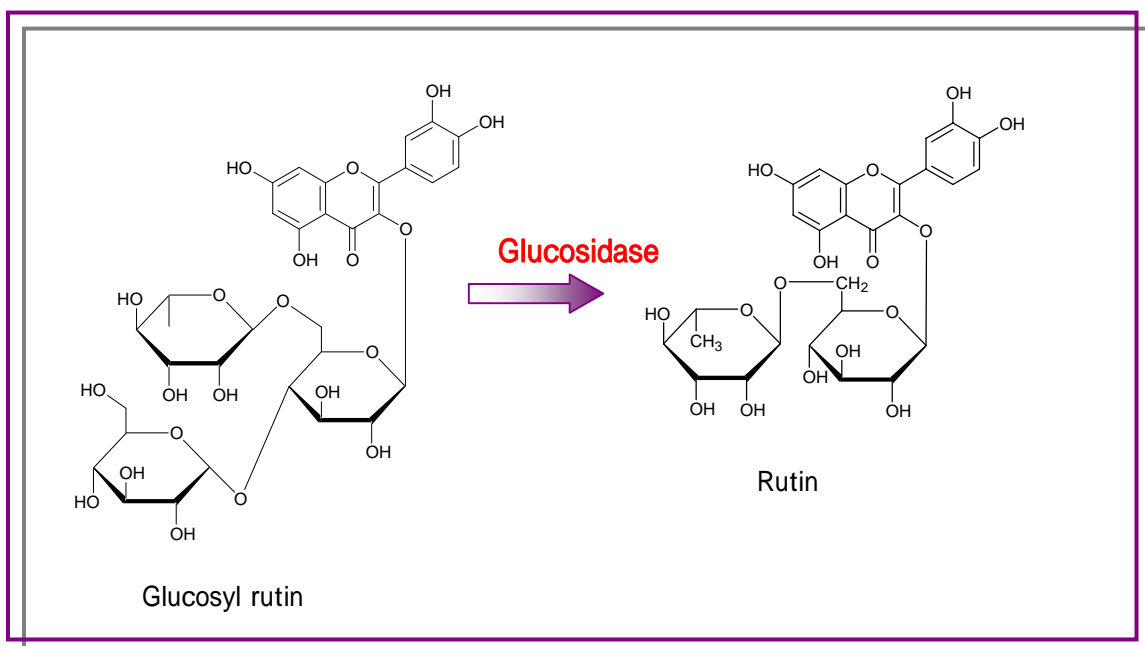
## ■ Prevention of discoloration

The addition of glucosyl rutin into dye solutions inhibited their discoloration.



## ■ Release of rutin from glucosyl rutin by enzyme present in our body

Glucosyl rutin is hydrolyzed by alpha-glucosidase, an enzyme in our small intestine and skin.



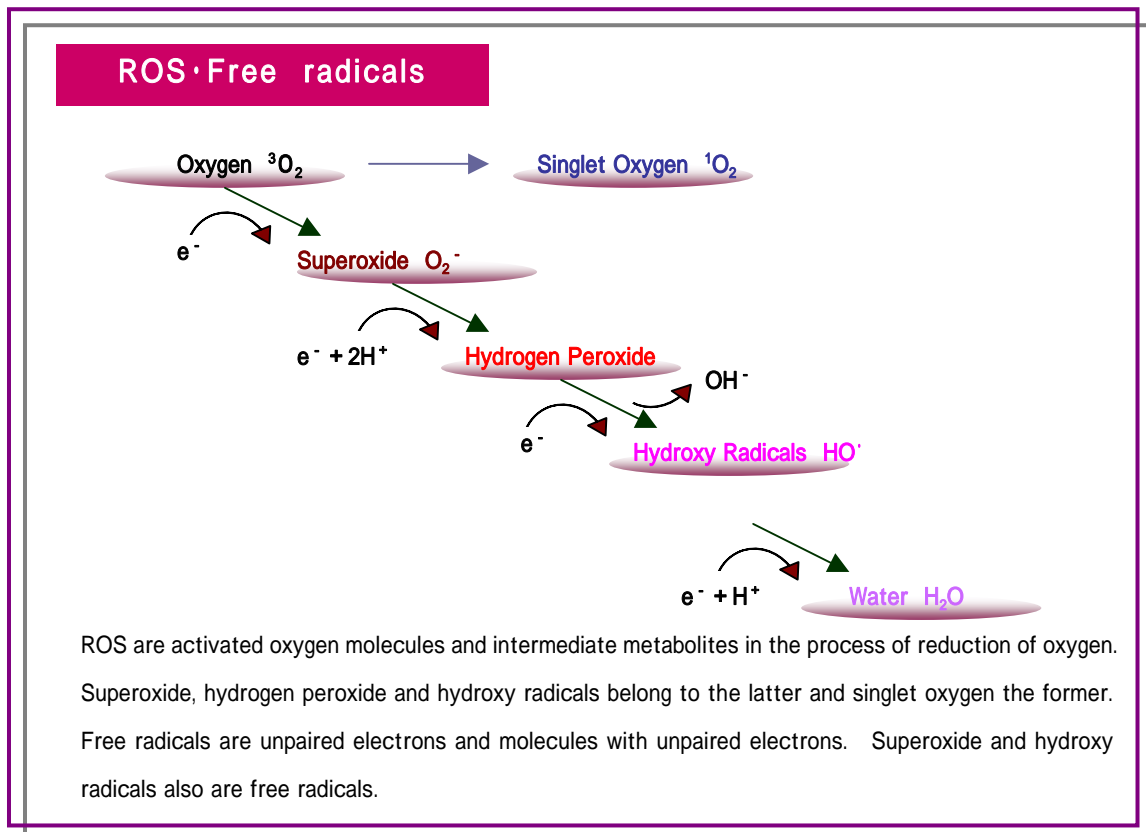
## Functions of glucosyl rutin

### ■ Aging and reactive oxygen species

Oxygen is required for normal cell function. However it is a double-edged sword. Almost all living organisms use oxygen for energy generation. In the process of energy production, a small percentage of oxygen is reduced and known to become some intermediate metabolites called reactive oxygen species (ROS). The ROS are highly reactive substances and considered to lead to many diseases such as brain infarction, cataract, myocardial infarction, rheumatoid, inflammation, tumor and stomach ulcer.

Skin contacts oxygen in air and receives UV light in sunlight. UV radiation and the ROS (photo-aging) it generates in our bodies are one of the external causes of skin aging. Quantitative and qualitative changes of extracellular matrices such as collagen fibers and elastin fibers in the dermis induce the formation of wrinkles, one of the signs of skin aging.

Reduced collagen amounts and thin collagen fiber bundles are observed in photo-aging. In addition, ROS, one of the causes of photo-aging, is reported to promote linkage of collagen fibers. Signs of photo-aging are blotches, wrinkles and sages. Following the growing consciousness on skin problems, the development of new cosmetic ingredients such as whitening and anti-aging compounds is highly expected.

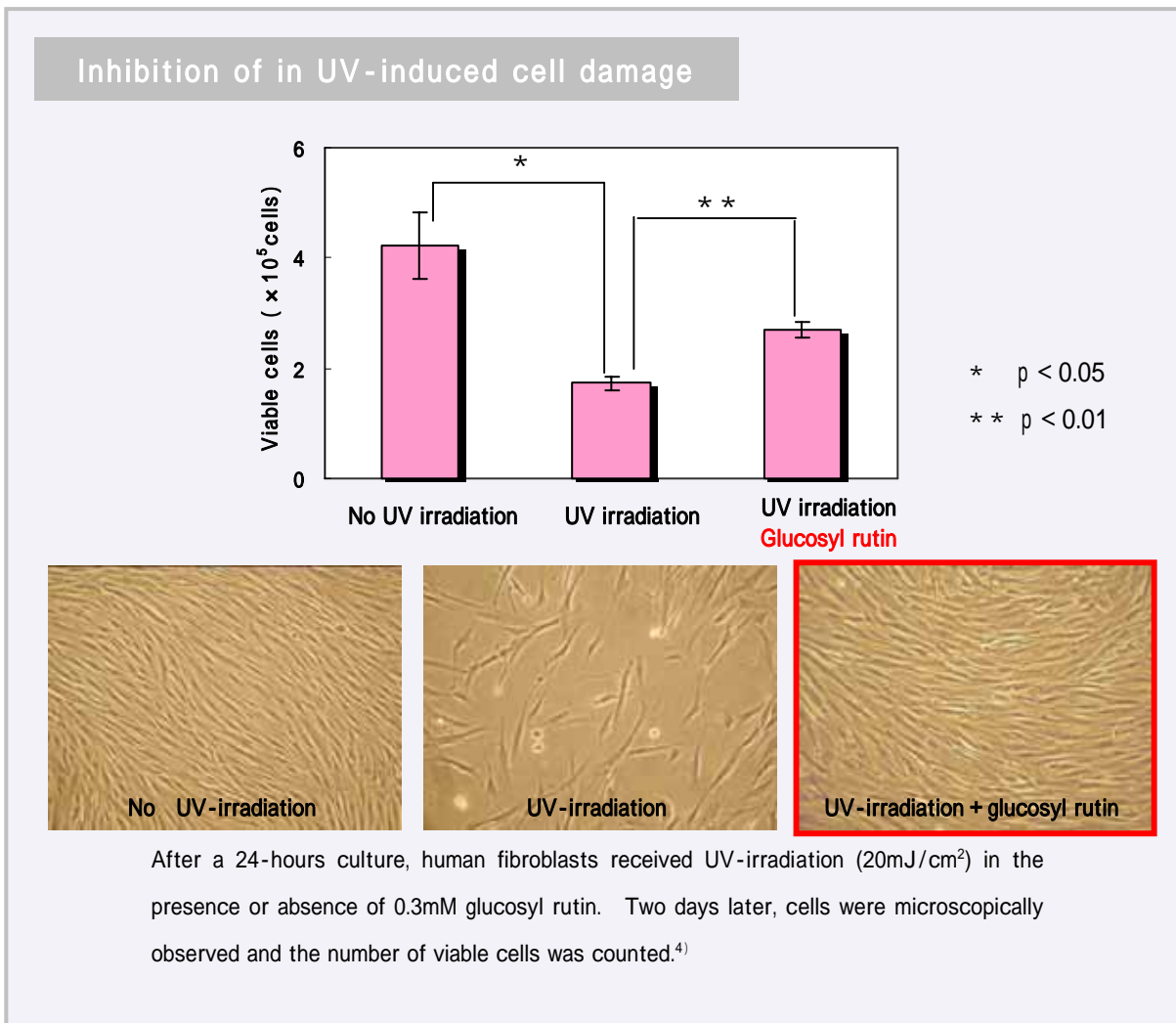


## ■ Protection of cells against damages induced by UV-irradiation

Significant progress is being achieved in research on the effects of UV light on the genetic material within the cells. UV-induced genetic mutation or activation is found to be linked to photo-aging, wrinkles and sages among others. Additionally, many findings support that UV-induced ROS plays a key role in photo-aging.

Immunostain of collagen in skin unveiled marked reduction of the collagen amounts resulting from photo-aging compared with that from natural aging. The reduced collagen is thought to be the main cause of the formation of wrinkles and spots. Fibroblasts synthesize and degenerate collagen fibers appropriately. Some researchers indicated the possibility of fiber degeneration by UV light, causing photo-aging.

Glucosyl rutin was found to protect fibroblasts from UV-induced damages. UV-irradiation reduced the number of viable fibroblasts but the presence of glucosyl rutin in culture increased it.



## ■ Inhibition of singlet oxygen-induced linkages of collagen fibers

Signs of skin aging are reduced elasticity and flexibility and thus wrinkles formation attributable to collagen fiber linkage. In vitro studies revealed the involvement of singlet oxygen in collagen fiber linkage.

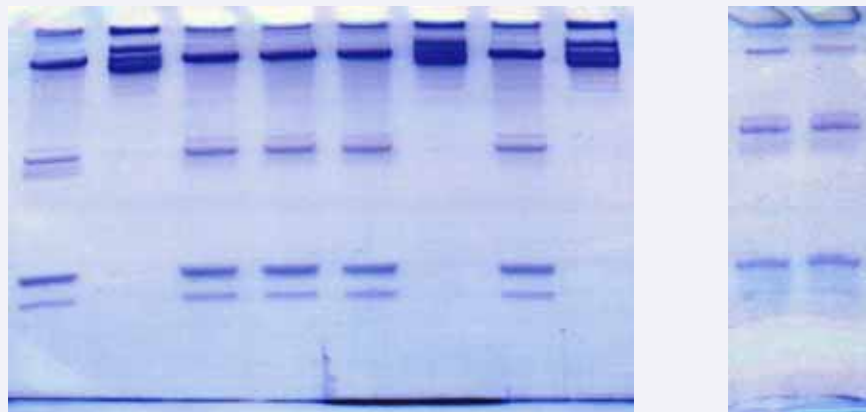
Glucosyl rutin and beta-carotene have the highest scavenging reaction constant of singlet oxygen. As a result, glucosyl rutin was found to inhibit collagen fiber linkage induced by singlet oxygen in vitro studies.

More stable than the relatively photosensitive beta-carotene, glucosyl rutin is expected to be widely used as an anti-aging agent preventing wrinkles formation.

Comparison of singlet oxygen<sup>5)</sup>

Reagent	Scavenging rate constant [M <sup>-1</sup> s <sup>-1</sup> ]
<b>Glucosyl rutin</b>	<b>3 × 10<sup>9</sup></b>
-carotene	9 × 10 <sup>9</sup>
Histidine	2.7 × 10 <sup>9</sup>
- tocopherol	5 × 10 <sup>8</sup>
NaN <sub>3</sub>	3.8 × 10 <sup>8</sup>
DABCO	1.5 × 10 <sup>7</sup>
Tyrosine	2.7 × 10 <sup>7</sup>
Squarene	4 × 10 <sup>6</sup>
Oleic acid	1.3 × 10 <sup>5</sup>
Cholesterol	1.8 × 10 <sup>4</sup>

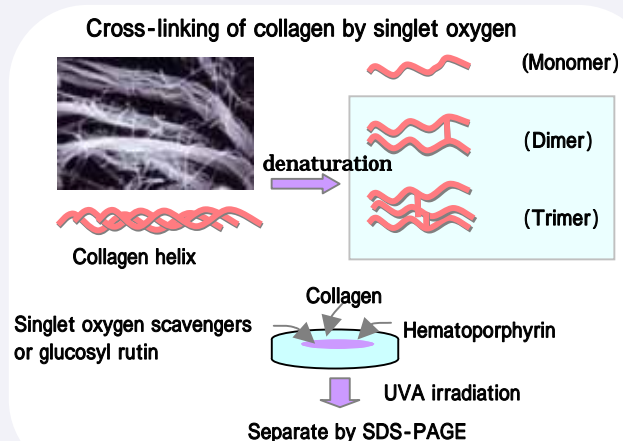
### Inhibition of collagen fiber linkage



Time (min)      0    20      0    20      0    20      0    20      0    20

Quenchers      Control      NaN<sub>3</sub>      SOD      Mannitol      **Glucosyl rutin**

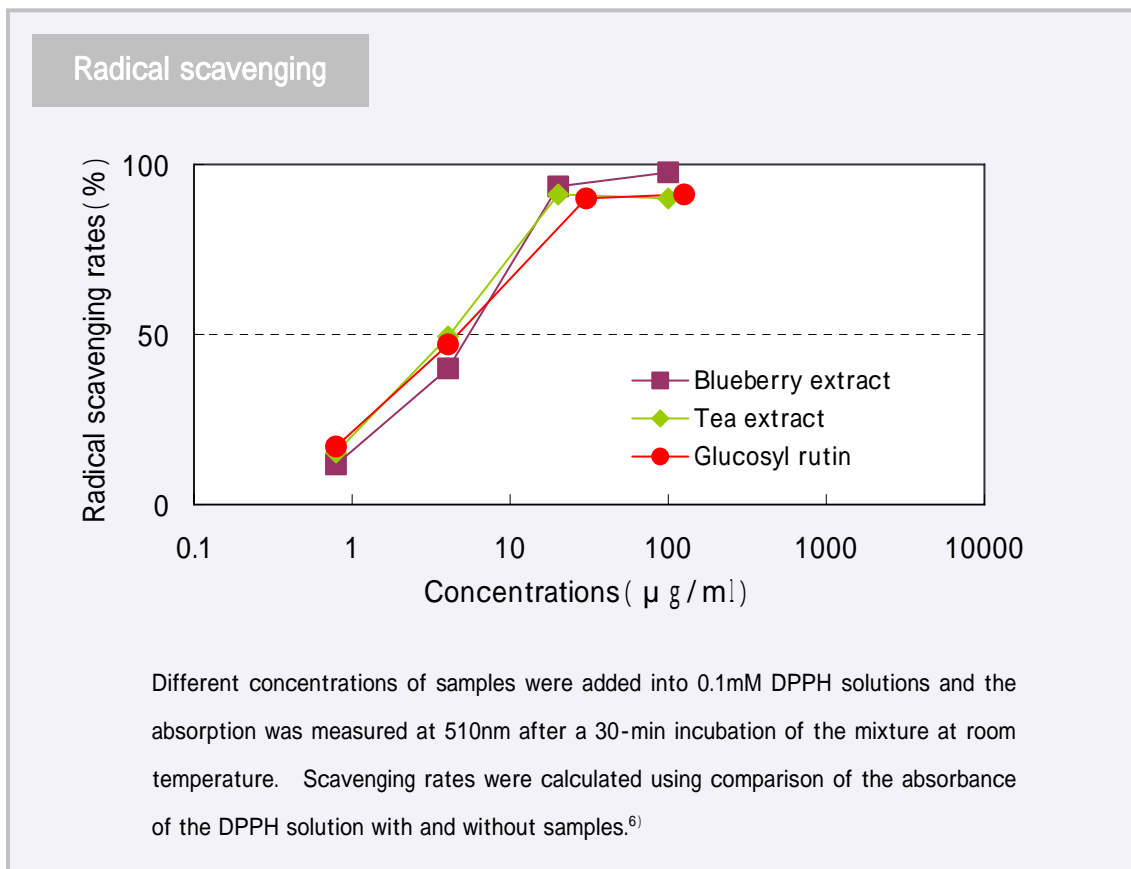
Solutions of collagen in the presence of singlet oxygen scavengers and hematoporphyrin were UV-irradiated and the quantitative change of single chain ( ), double chain ( ) and triple chain ( ) of collagen fibers were examined by SDS-PAGE.<sup>5)</sup>



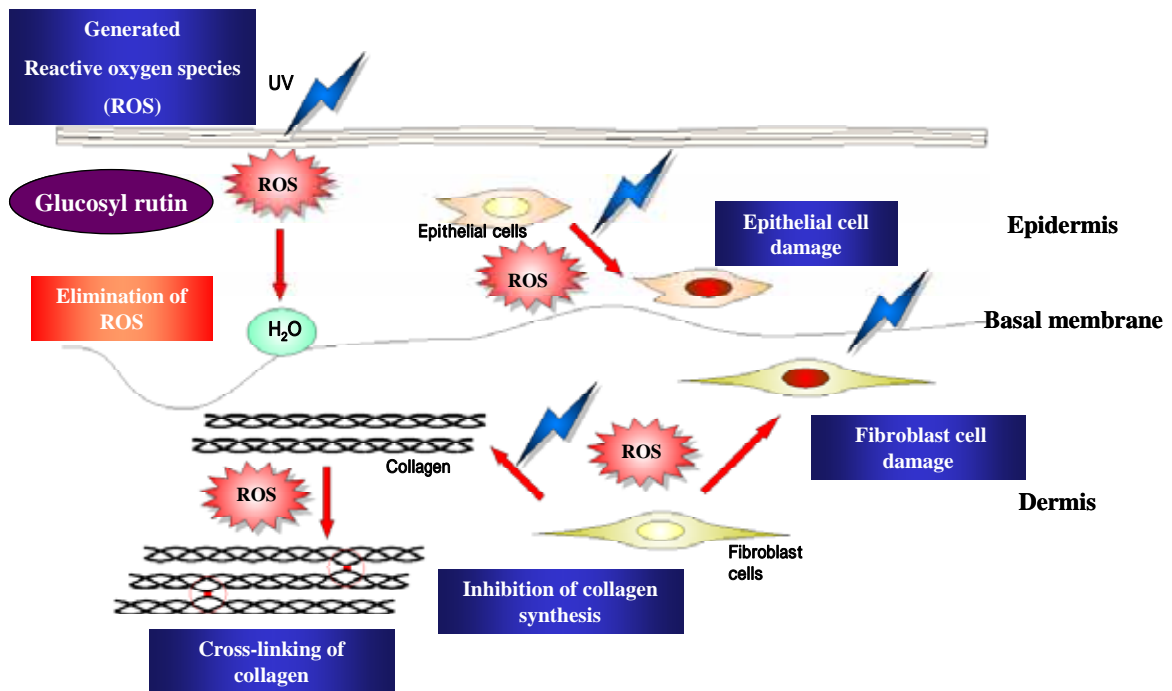
### ■ High anti-oxidants and radical scavenging

Several anti-oxidants play key roles in the reduction of ROS-induced oxidative stress. Generally anti-oxidants have two important protective mechanisms: inhibition of the initial formation of ROS and free radicals, and scavenging of intermediates during the constitutive process of their formation.

Glucosyl rutin was found to have such a strong radical scavenging activity as seen in tea extract (catechin) and blue berry extract (anthocianidin).



## ■ Skincare by ROS scavenging



One of the several physiological attributes of glucosyl rutin is its ROS-scavenging activity. UV-induced ROS/free radicals lead to skin damages including cytotoxicity and skin aging through linkage of collagen fibers produced by fibroblasts. Glucosyl rutin is reported to remove UV-induced singlet oxygen and thus inhibit the collagen fiber linkage, a potent cause for wrinkle. In addition, glucosyl rutin protects fibroblasts from UV-induced damages.

On the whole, glucosyl rutin has intrinsic skin protection attributes, preventing UV-induced damages and photo-aging.

## Safety

Given orally, glucosyl rutin is an industrial product. Glucosyl rutin given orally is catalyzed to release natural products, rutin and glucose in our body, suggesting its safety as food.

## Specifications of glucosyl rutin

Commercial name: G Rutin (for cosmetics)

The purity of glucosyl rutin is 95% or more (95-115 %).

Variables	Specifications	Test methods
Appearance	Yellow or orangish yellow powder with a slightly peculiar odor.	JSCI*
Identification test ( 1 ) Quercetin  ( 2 ) Quercetin  ( 3 ) Sugar ( 4 ) IR spectrum	The solution turns brown  The solution turns orange  The solution turns orange Absorption peak at wavenumber 3400 ~ 3200cm <sup>-1</sup> , 2930cm <sup>-1</sup> , 1660cm <sup>-1</sup> , 1610cm <sup>-1</sup> ,1510cm <sup>-1</sup> ,1450cm <sup>-1</sup> ,1360cm <sup>-1</sup> , 1300cm <sup>-1</sup> , 1200cm <sup>-1</sup> and 1100 ~ 1000cm <sup>-1</sup>	Color reaction with ferric chloride TS  Color reaction with magnesium-hydrochloric acid Phenol-sulfuric acid method KBr disk method
Purity test ( 1 ) Color of the solution ( 2 ) Heavy metals, as Pb ( 3 ) Arsenic, as As <sub>2</sub> O <sub>3</sub>	Clear and light yellow/ yellow Not more than 20 ppm Not more than 2 ppm	JSCI JSCI
Loss on drying	Not more than 10.0 %	JSCI
Residue on ignition	Not more than 2.0 %	JSCI
Quantification (purity)	95 ~ 115 %	Spectrophotometry

JSCI\*: The Japanese Standards of Cosmetic Ingredients

## Packages

Net 10 kg in a carton box (1 kg × 10 laminated multilayer bags)

## Regulation

Indication name: glucosyl rutin

INCI name: GLUCOSYLRUTIN, ISOQUERCITRIN

Glucosyl rutin is available as an additive for quasi-drugs in Japan.

## References

- 1) Yumoto T. et al. New Food Industry, 43(4), 57-63 (2001)
- 2) Takaya I. Syokuhin Kougyou, 33(10), 48-54 (1990)
- 3) Provided by Toyo Seito Co. Ltd
- 4) Provided by Hayashibara Biochemical Laboratories, Inc.
- 5) Ryu A. and Arakane K. Fragrance Journal, 26(4), 59-64 (1998)  
Ryu A. et al. Chem. Pharm. Bull. 45(8), 1243-1247 (1997)
- 6) Mitsuzumi H. and Nagao Y. Fragrance Journal, 30(5), 65-72 (2002)

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