Absorage

Plantago Major Seed Extract

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C O N T E N T S

Glycation reaction and Skin-aging [1 to 3)]

Glycation

Sugar abundant in the body is converted to highly reactive carbonyl compounds (RCOs) by oxidative stress. The reactive carbonyl compounds denature protein by binding the adjacent protein to result in a decrease in the protein functions. **The binding reaction between sugar and protein under such oxidative stress is called the Glycation**. The reason why the reaction is called the Glycation is that L.C. Maillard (Louis Maillard), a French chemist found in 1912 that yellow-brown pigment was produced by heating an amino acid and sugar (reducing sugar). The tasteful brown coloration of scorched rice and of the surface of the grilled wing is produced by the Millard reaction. Thus it can be said that this reaction occurs in our daily life. (Fig.2) Since the Glycation is involved in the coloration and flavoring (production of flavoring components) of soy sauce, caramel and chocolate, the researches on this field have been actively carried out.

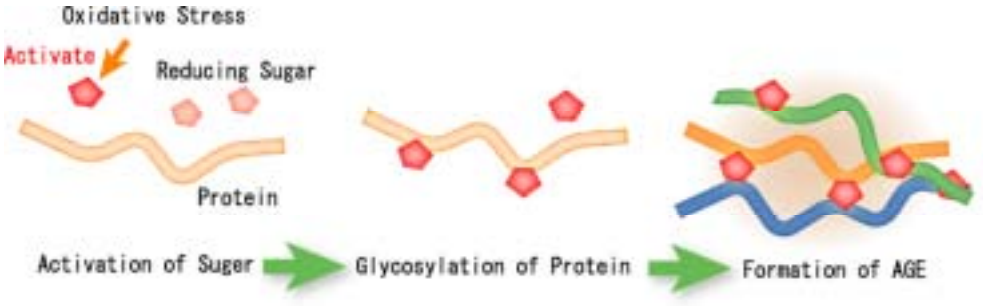


Fig.1 Glycation

Advanced Glycation Endproduct (AGE)

Fig.2 Glycation on Food

The Glycation starts from the formation of Schiff base by reaction of amino group of protein and aldehyde group of a reducing sugar. The rearrangement of double bond of Shiff base (Amadori rearrangement) forms very reactive products such as 1,2-enaminol. These compounds are generally called Amadori rearrangement products and the reactions until this step are designated as the early Glycation. The following complicated reactions such as oxidation, dehydration and condensation produce Advanced Glycation Endproduct (AGE) with peculiar physico-chemical characteristics such as brown in color, fluorescence and crosslink formation. These reactions are designated as the late Glycation. (Fig.3)

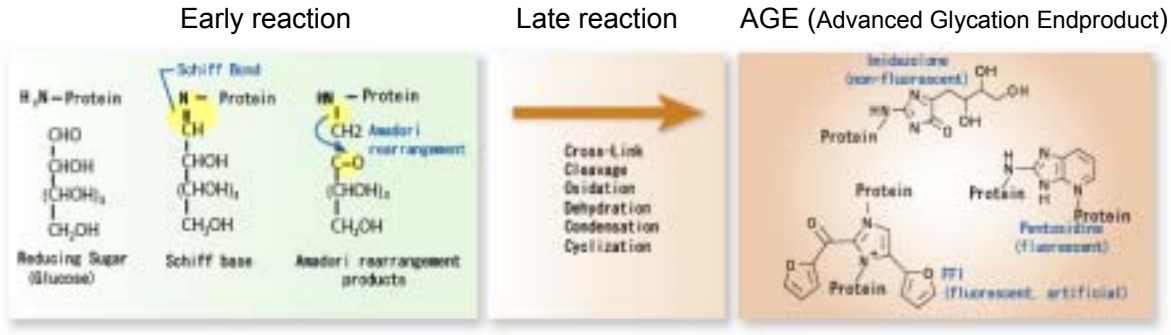


Fig. 3, Early Glycation Reaction, Late Glycation Reaction, and AGE [4)]

AGE and aging [5) 6)]

Recently, it has been reported that such AGE plays a role in the occurrence of various diseases such as diabetes, arteriosclerosis, cataract and Alzheimer's disease. It has been pointed out that AGE is involved especially in diabetic complications such as diabetic nephropathy, acute renal failure, arteriosclerosis and dialysis-related amyloidosis. It has been presumed that arteriosclerosis is produced by loss of vascular elasticity due to denaturation of vascular wall caused by the AGE formation on collagen and elastin in the vascular wall.

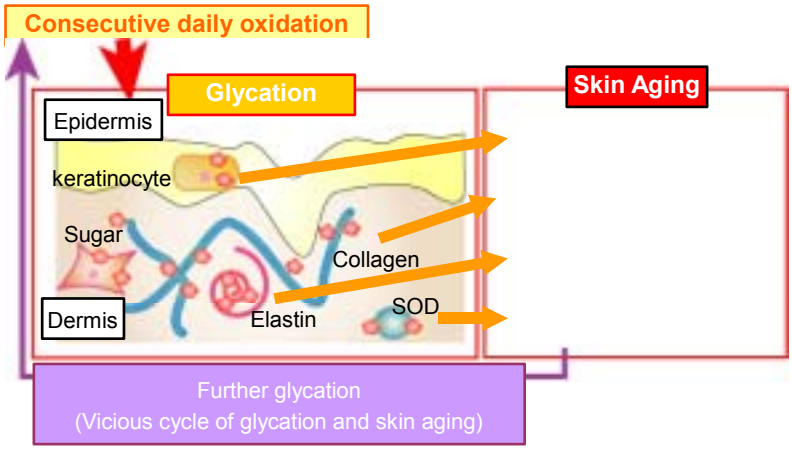


Fig.4 Skin aging and glycation

AGE and skin aging [7 to 11)]

It is considered that also in the skin, once the AGE formation on dermal collagen and elastin by the Glycation produces protein crosslinking (aging crosslinking), the skin loses its own elasticity and plasticity to lead to aging skin such as wrinkles and pouches (Fig. 4). Since the metabolic rate of dermal collagen and elastin is slow, it is considered that aging crosslinking is easily accumulated. The AGE formation on super-oxide dismutase (SOD), which plays a role in the protection of the body from oxidative stress by degrading active oxygens, decreases the SOD functions. Once the functions of anti-oxidative protein in the body such as SOD are decreased, the AGE formation in the body proceeds more and more (Fig. 4).

The AGE formation producing various disturbances in the body mechanisms is irreversible and there is no effective recovering method yet. It can be said that prevention of the AGE formation, namely the inhibition of the Glycation is now only the choice to prevent aging caused by protein glycosylation.

Anti-glycation and Absorage

Ichimaru Pharcos Co., Ltd. has explored raw materials for the prevention of skin aging by inhibiting the Glycation and found that seed extract of Plantago major L. and its component, plantagoside had a strong inhibitory action on the Glycation*. This time, we made a commercial product, "Absorage", seed extract of Plantago major L. The product name "Absorage" was derived from the following two meanings: one is the images of "the age is resetted" and "aging is alleviated" by using "Absorb" meaning "turning-off" and "Age" meaning "age and "aging" and another is the meaning that "AGE (Advanced Glycation Endproducts: the Glycation products)" is "Absorb"ed.

Note : * were released at below mentioned congress etc.

12) Aradake TADASHI et al., *Nippon Nogeikagaku Kaishi, The Rally Lecture Point Collection on 1998*, 271 (1998)
13) Chihiro SASAKI et al., *Nippon Nogeikagaku Kaishi, The Rally Lecture Point Collection on 1998*, 270 (1998)
14). Nobuyasu MATSURA et al, *The Pharmaceutical Society of Japan*, *118th The Rally Lecture Point Collection 3*, 82 (1998)
15) Hiroyuki KOJIMA et al., *Shoyakugaku Zasshi, 45th The Lecture Point Collection*, 237 (1998)
24th IFSCC Congress Osaka Japan (2006)

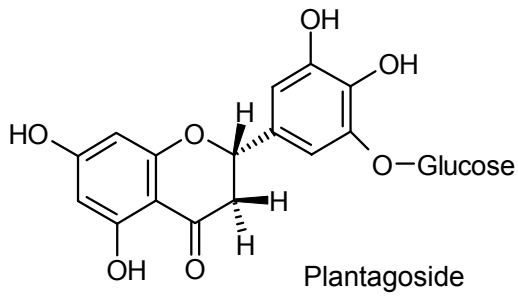
Study of Glycation was investigated by cooperation of Mr. Nobuyasu MATSURA who is Assistant Professor at Laboratory of Pharmacositology of Department of Life Science of OKAYAMA University of Science.

Origin [16 to 20)]

Absorage is extract obtained from seed of Plantago major L. (*Plantaginaceae*), genus Plantago in Plantaginaceae. In genus Plantago, about 250 species have been known and distributed all over the world. Most of species in genus Plantago are weedy plants and grow together with not so great height plants like Pona annua and Trifolium repens L. very often.

Plantago major L. is 30 to 60 cm in height and has many radical leaves. The flowering season is between April and September, and a small ear-like flower is produced on a flower stalk with 30 to 60 cm in length. Eight to dozen or so rugby ball-like seeds with 1 to 2 mm in diameter are produced in the fruit with a cap called pyxidium. Plantago major L. grows wild on wasteland and by the roadside as a naturalized plant also in Japan and cannot be easily distinguished from Plantago asiatica L. originated from Japan. It can be, however, distinguished between them, because Plantago major L. is larger than Plantago asiatica L. and because P. asiatia produces only 4 to 6 seeds in the fruit.

The academic name and English name of genus Plantago are derived from planta (the sole of a foot) in Latin. In Wales district, Plantago major L. was called "Foot mark of Christ" etc., and it was circulated that the application of crumpled leaves to foot eased the fatigue of a journey and that the placement of crumpled leaves in the socks made a long journey endurable. In Shakespeare's era, Plantago major L. was esteemed as a herb effective for wounds and fever and it was believed also in China that it made the body refresh and could prevent aging.



Plantagoside

The leaves of Planta major L. contain polysaccharides, flavonoids such as hispidulin, apigenin and luteolin, tannins, organic acids and mucus and the seeds contain linoleic acid, oleic acid and glycosides such as asperuloside and plantagluside. As the folk remedy, the seeds are used for strengthening of the body, anti-diarrhea, decline of fever, diuresis, gastritis, peptic ulcer, etc. as a tea, and the leaves have a hemostasis action and are applied externally for contusion, bone fracture, piles, ulcer and insect bite. Besides, Plantago major L. is sometimes used to decrease the irritating activity of other herbs in herb products.

Plantagoside (shown in the following figure), an active ingredient of Absorage exists in the seed of Plantago major L, which is universally distributed in the Asian area. It has been, however, said that plantagoside cannot be detected in *Plantago asiatica*, a representative plant of Semen Plantaginis in oriental drugs.

Introduction

Absorage is obtained by extracting the seeds of *Plantago major* L. (*Plantaginaceae*) with ethanol solution, and removing the solvent from the extract, and dissolving the residue in water and 1,3-butylene glycol.

Efficacy

Absorage and Plantagoside; which is active component are observed inhibition of glycation of several kinds protein, prevent and improve skin aging.

<Patent Number : JP1999-106336 A>

Efficacy for epidermis

Inhibition of Glycation in keratin

Inhibition of change of brown coloration in the skin by glycation

Efficacy for dermis

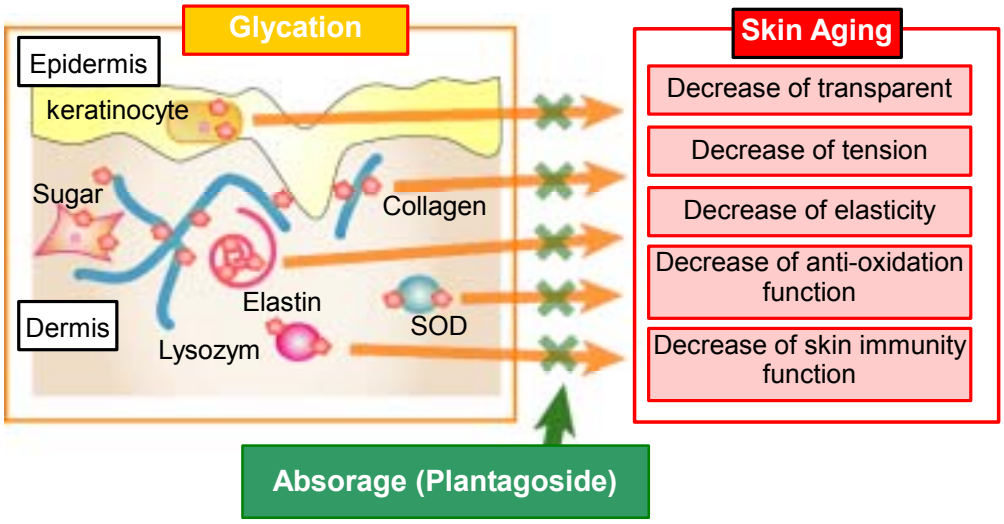
Inhibition of Glycation in collagen

Improvement of skin aging (wrinkle and elasticity) in human skin

Inhibition of Glycation in SOD

Inhibition of decrease in SOD functions (Glycation)

Inhibition of Glycation in lysozyme



Inhibition of Glycation in Keratin

It has been reported that diabetic cataract is caused by the opacification and pigmentation of transparent crystalline protein in the eye lens due to the glycation reaction.[6] It is also conceivable that the denaturation of keratin proteins in the stratum corneum and epidermis by the glycation reaction decreases skin transparency.

The inhibitory effect of plantagoside, an active ingredient of Absorage on keratin glycation was examined by measuring the amount of the fluorescent material, FFI (2-(2-furoyl)-4(5)-(2-furanyl)-1H imidazole) in the AGEs.

1.Glycation reaction at 60℃ for 40 hours

Test sample

Plantagoside, an active ingredient of Absorage was dissolved in dimethyl sulfoxide (DMSO) to make the final concentration 10 μmol/L. As the control, DMSO alone was added in place of the test substance. As the positive control, aminoguanidinesulfate (AG), a glycation inhibitor, and L-lysine were dissolved in DMSO to make the final concentration 100 μmol/L.

Test method

For the models of keratin in corneum (epidermis), wool keratin hydrolysate (final concentration: 1 mg/mL), eggshell membrane protein hydrolysate (final concentration: 1 mg/mL) and epidermal cell extracts (final concentration: 0.5 mg/mL) were used as the reaction substrates. The epidermal cell extracts were prepared as follows: the normal human epidermal cells (Kurabo) were extracted by lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris-HCl <pH 8.0>, 1 mM EDTA and 1 mM PMSF), desalted and concentrated with Sephadex gel and an ultrafiltration membrane, and dissolved again in lysis buffer. The test sample, glucose (final concentration: 200 mmol/L), substrate and 50 mmol/L phosphate buffer solution (pH 7.4) were mixed and incubated at 60°C for 40 hr. 10 μL of trichloroacetic acid were added to 100 μL of the reaction mixture, and the precipitate formed was isolated and dissolved in alkaline phosphate buffer solution. The fluorescence intensity of the solution was measured at an excitation wavelength of 360 nm and an emission wavelength of 460 nm to obtain the amount of FFI. The inhibitory rate of FFI formation in each test sample was calculated according to the following formula:

$$\text{Inhibitory rate of production of FFI \%} = \left(1 - \frac{\text{FFI amount of Sample} - \text{FFI amount of Sample Blanc}}{\text{FFI amount of Control} - \text{FFI amount of Control Blanc}}\right) \times 100$$

Result

Inhibition of Glycation of Plantagoside in Keratin (60℃ 40 hours) is shown in Fig.5 (Inhibition of production of FFI) Plantagoside is observed inhibition of glycation in Keratin. Its result is quite effected compared with AG and L-Lysine that are known as glycation inhibitor.

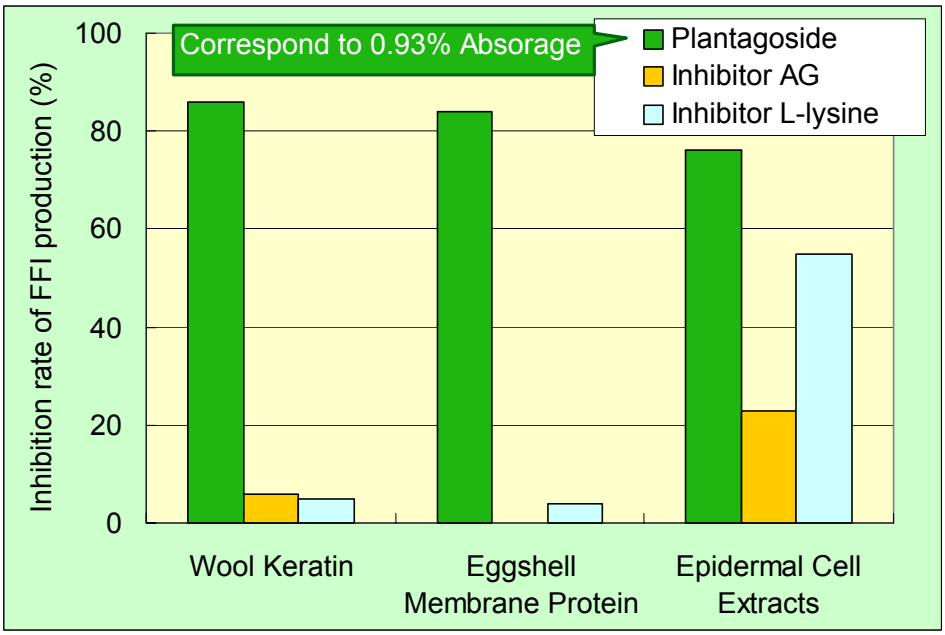


Fig.5 Inhibition of glycation in keratin at 60 ℃

2.Glycation reaction at 37℃ for one month

Test sample

Plantagoside, an active ingredient of Absorage was dissolved in dimethyl sulfoxide (DMSO) to make the final concentration 10 mmol/L. As the control, DMSO alone was added in place of the test substance. As the positive control, aminoguanidinesulfate (AG), a glycation inhibitor, and L-lysine were dissolved in DMSO to make the final concentration 100 mmol/L.

Test method

For the models of keratin in corneum (epidermis), wool keratin hydrolysate (final concentration: 1 mg/mL) and eggshell membrane protein hydrolysate (final concentration: 1 mg/mL) were used as the reaction substrates. The test sample, glucose (final concentration: 200 mmol/L), substrate and 50 mmol/L phosphate buffer solution (pH 7.4) were mixed and incubated at 37°C for one month. 10 μL of trichloroacetic acid were added to 100 μL of the reaction mixture, and the precipitate formed was isolated and dissolved in alkaline phosphate buffer solution. The fluorescence intensity of the solution was measured at an excitation wavelength of 360 nm and an emission wavelength of 460 nm to obtain the amount of FFI. The inhibitory rate of FFI formation in each test sample was calculated according to the following formula:

Inhibitory rate of production of FFI %

$$= (1 - \frac{\text{FFI amount of Sample} - \text{FFI amount of Sample Blanc}}{\text{FFI amount of Control} - \text{FFI amount of Control Blanc}}) \times 100$$

Result

Inhibition of Glycation of Plantagoside in Keratin is shown in Fig.6 (Inhibition of production of FFI) Plantagoside was observed inhibition of glycation in Keratin. Its result is quite effected compared with AG and L-Lysine that are known as glycation inhibitor.

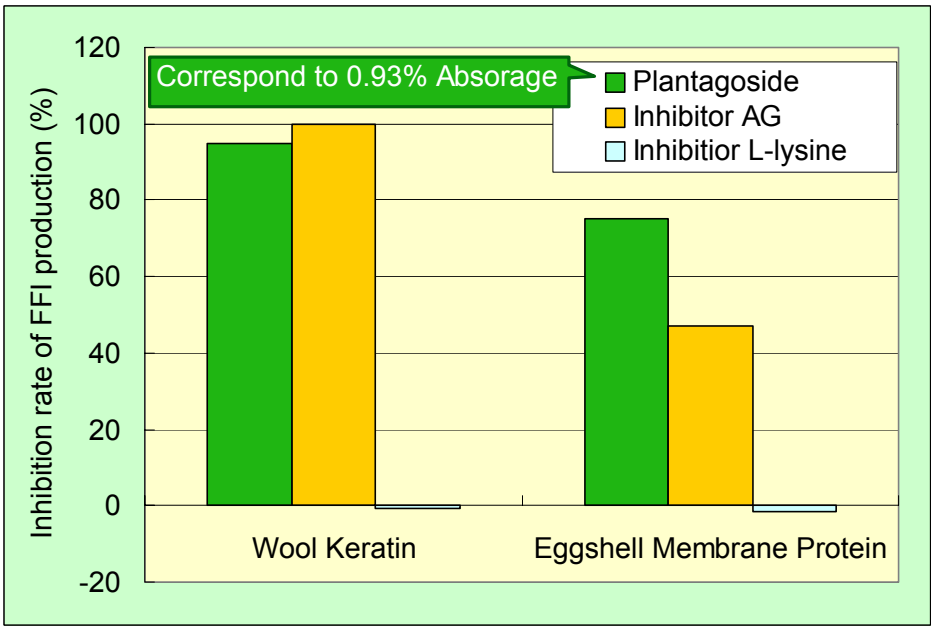


Fig.6 Inhibition of glycation in keratin at 37 ℃

Discussion

Plantagoside; which is active component of Absorage was confirmed inhibition of glycation in keratin. According to the result, Absorage is expected to prevent decrease of skin transparent by aging.

Inhibition of change of brown coloration in the skin by glycation

It is conceivable that the denaturation of keratin proteins in the stratum corneum and epidermis decreases skin transparency. On the other hand, dihydroxyacetone (DHA) has been used for self-tanning and for the treatment of vitiligo, because DHA turns the skin brown color. DHA reacts with protein in the stratum corneum to produce a brown color, which has been said to be the glycation reaction. [11)]

The inhibitory effect of Absorage on the glycation reaction in the stratum corneum was examined based on the change of brown coloration in the skin by glycation with DHA.

Test sample

Undiluted Absorage was used for test.

Test method

The healthy adult volunteers in their forties who gave informed consent were employed as the subjects. The inside of the forearm in each subject was used as the testing site. The test sample was applied only on the left arm and the right arm was not treated (control). After lightly wiping out the residual test sample on the skin with Kimwipes, 1% and 0.5% DHA solutions were applied occlusively for 3 hr on the inside of the left and right forearms to color the skin. Twenty-four hours after the removal of the application, the testing sites were examined.

Result and Discussion

It is shown the change of brown coloration in the skin by Absorage with DHA in Fig.7. Although it was actually observed the change of brown coloration in the skin with DHA on application of sample, it was inhibited the change of brown coloration in the skin with DHA on application of Absorage.

According to the result, Absorage is expected to prevent decrease of skin transparent because of inhibition of glycation in keratinocyte.

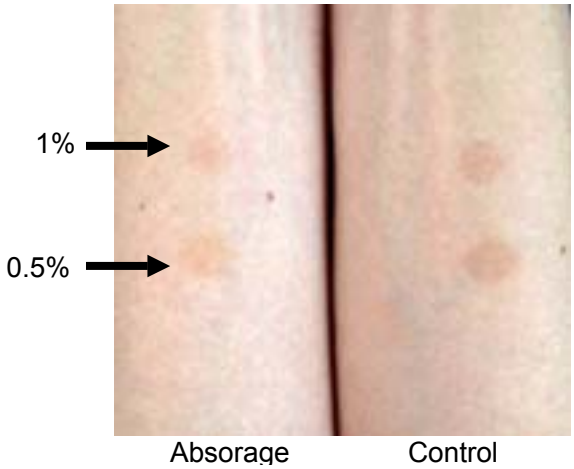


Fig. 7 Inhibition of change of brown coloration in the skin by glycation

Inhibition of Glycation in collagen

The denaturation of body protein by the Glycation caused by aging produces a decrease in the protein functions and leads to a decrease in the physiological functions all over the body. Collagen, a major protein of dermal constituents is also denatured and polymerized by the Glycation to lose flexibility and elasticity of the skin.[7 8)] This leads to the aging symptoms such as punches and wrinkles of the skin.

We investigate how to inhibit glycation of collagen and glucose by Platnagoside; which is active component by studying FFI of fluorescence AGE substance; which was produced by glycation

Test sample

Plantagoside; which is active component of Absorage and its aglycon, 5, 7, 3', 4', 5'-pentahydroxyflavanone is dissolved in DMSO and apply in the test. DSMO instead of sample is applied as control. As positive control, Aminoguanidine sulfate (AG); which is known as glycation inhibitor is dissolved and apply in the test.

Test method

10 μL of test sample solution, 50 mL of glucose (200 mmol/L), 100 μL of Type I collagen (800 μg/mL), 250 μL of 50 mM disodium hydrogen phosphate (Na2HPO4, pH 7.4) and 90 μL of purified water were mixed and incubated at 37°C for 2 weeks. After the completion of the Glycation, 10 μL of trichloroacetic acid was added to 100 μL of the reaction mixture. The precipitate thus produced was isolated and dissolved in 400 μL of alkaline phosphate buffer. The fluorescent intensity of the solution was measured using an excitation wavelength of 360 nm and an emission wavelength of 460 nm to obtain the amount of FFI. The inhibitory rate of FFI production in each test sample was calculated according to the following equation.

To exclude the effect of the fluorescent absorbance by a test sample (quenching effect), the quenching rate was obtained as described below. The control reaction solution was added to 2 μL of a test sample solution to make the final volume 100 μL. Ten microliter of trichloroacetic acid was further added to the mixture. The precipitate thus produced was isolated and dissolved in 400 μL of alkaline phosphate buffer. The fluorescent intensity of the solution was measured using an excitation wavelength of 360 nm and an emission wavelength of 460 nm and the quenching rate was obtained according to the following equation. The inhibitory rate of FFI production in each test sample was corrected by subtracting the quenching rate from the inhibitory rate of FFI production obtained previously.

Inhibitory rate of production of FFI %

$$= (1 - \frac{\text{FFI amount of Sample} - \text{FFI amount of Sample Blank}}{\text{FFI amount of Control} - \text{FFI amount of Control Blank}}) \times 100$$

Inhibitory rate of quenching %

$$= (1 - \frac{\text{Sample fluorescence amount - Sample Blank fluorescence amount}}{\text{Control fluorescence amount}}) \times 100$$

Result and Discussion

The 50% inhibition concentration (IC_{50}) values of plantagoside; which is active component of Absorage, plantagoside aglycon and AG are shown in Table 1. The IC_{50} value of plantagoside in the Glycation in collagen was 14.5 μmol/L, indicating that the degree of the inhibitory activity of plantagoside was 170 times higher than that of AG, a representative inhibitor of the Glycation. It was also cleared that plantagoside exhibited more powerful inhibitory action in the glycoside form than in the aglycon form.

According to the result, Absorage is expected to prevent the aging symptoms by inhibiting the Glycation in collagen. It was also cleared that plantagoside, an active component of Absorage was a very powerful inhibitor of the Glycation.

Table 1

Substances	50% inhibition value IC_{50} (μ mol/L)
Plantagoside	14.5 (correspond to 1.35% Absorage)
Plantagoside aglycone	42.1
Inhibitor AG	2500

Improvement of skin aging (wrinkle and elasticity) in human skin

It became clear that Absorage had an inhibitory effect on the skin collagen glycation. Since it is considered that the collagen glycation produces the deterioration of the physicochemical property of collagen to result in skin aging, it can be expected that Absorage has the preventing and improving effects on the occurrence of wrinkles and on a decrease in skin elasticity. Thus using the replica method, the improving effect of Absorage on the occurrence of wrinkles was examined. In this method, light was irradiated on the replica of the skin surface at the testing site at a certain angle and the area of shadow produced by the roughness of the skin surface due to wrinkles was calculated by the image analysis to obtain the area of wrinkles. Using a cutemeter, the effect of Absorage on skin elasticity was also examined.

1.Improvement of wrinkle

Test sample

3% Absorage lotion was applied. 50% 1,3-Butylene Glycol was applied as control.

Test method [23)]

Seven health male and female volunteers at age 30s to 40s that gave us written informed consent were enrolled in this study.

Before the treatment and 12 weeks after the treatment, a test was carried out according to the procedures for examining dermal shape by replica (SILFLO,AMIC Group).After washing face, volunteers stay in a thermo-hygrostat room (at 20℃, humidity 50%) for 20 minutes. Light was irradiated to replica from constant angle (tops and bottoms angle 25) and its shade was calculated with the use of an image data processing and picture analyzing software and area of wrinkle was the measured. By calculating ratio of the area of wrinkle per analyzing area, it is compared with control.

Result

The change of maximum wrinkle depth before application and 12 weeks later shown in Fig.8.

According to the result, degree and improvement of maximum wrinkle depth was observed in application of Absorage compared with control.

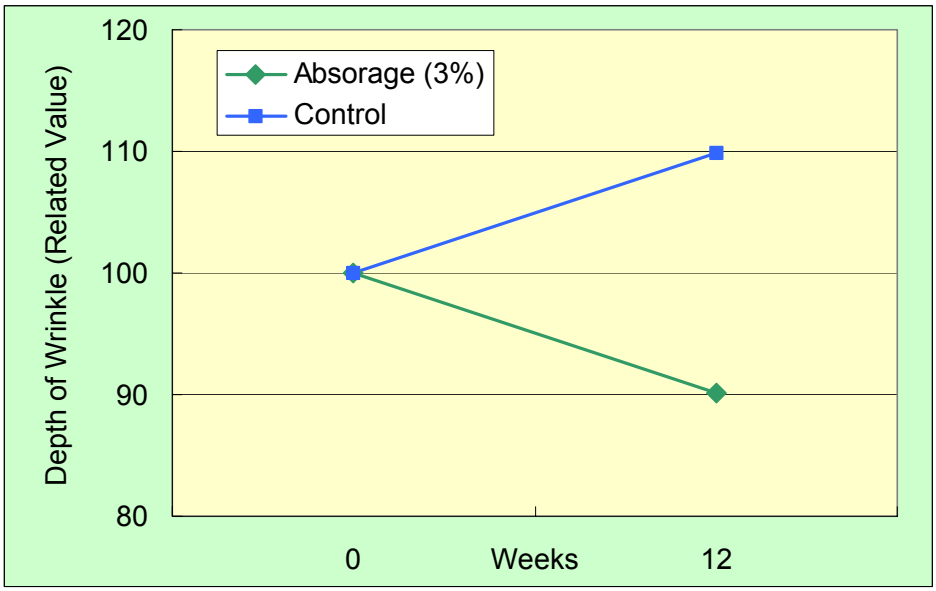


Fig.8 Improvement of wrinkle

2.Improvement of elasticity

Test method [23)]

Seven health male and female volunteers at age 30s to 40s that gave us written informed consent were enrolled in this study.

Each test sample was applied around the left and right eyes of each subject three times a day for 12 weeks. Before and at 12 weeks after the commencement of the application, the elasticity of the skin was measured by a skin viscosity and elasticity meter · Cutometer (CUTOMETER SEM474, COURAGE + KHAZAKA Electronic GmbH). The elasticity was calculated by the change of the skin condition when the skin was sucked for 5 sec by instantly reducing the pressure to 500 mb and thereafter the negative pressure was instantly released, which were done twice.

The test was performed several times each in right and left application sites, and the percentage of the change of the mean values between the value before the commencement of the application and that at 28 days after the commencement of the application was calculated to obtain the rate of the change. The measurement was performed 20 min after acclimation in an air conditioning room (room temperature: 20°C and humidity: 50%) after washing the face.

Result and Discussion

The plasticity before application and 12 weeks later is shown in Fig.9. it is shown photo of typical replica around eye area in Fig.10. Although plasticity is reported to increase by age [24 25)], plasticity is decreased on application of Absorage. (improvement of skin elasticity) It was actually observed improvement of wrinkle by photo.

According to the result, Absorage is expected to improve wrinkle and decrease of elasticity and improve skin aging.

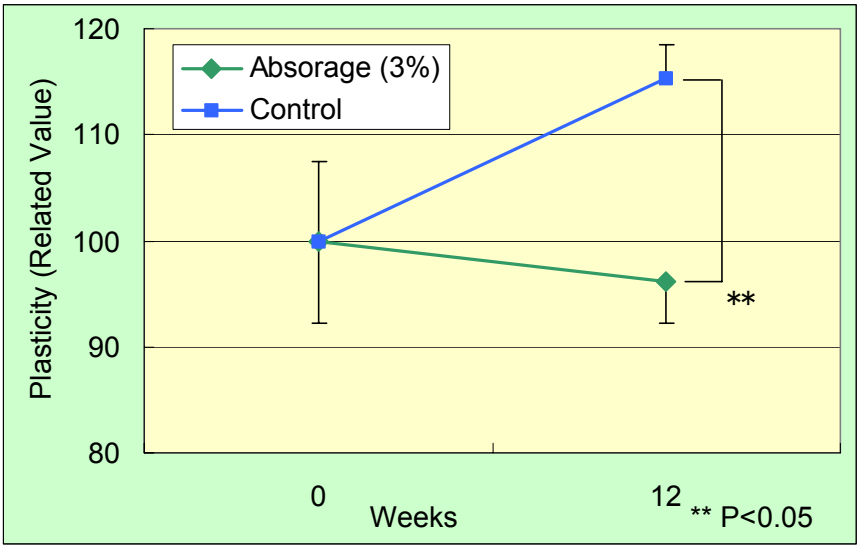


Fig.9 Improvement of elasticity

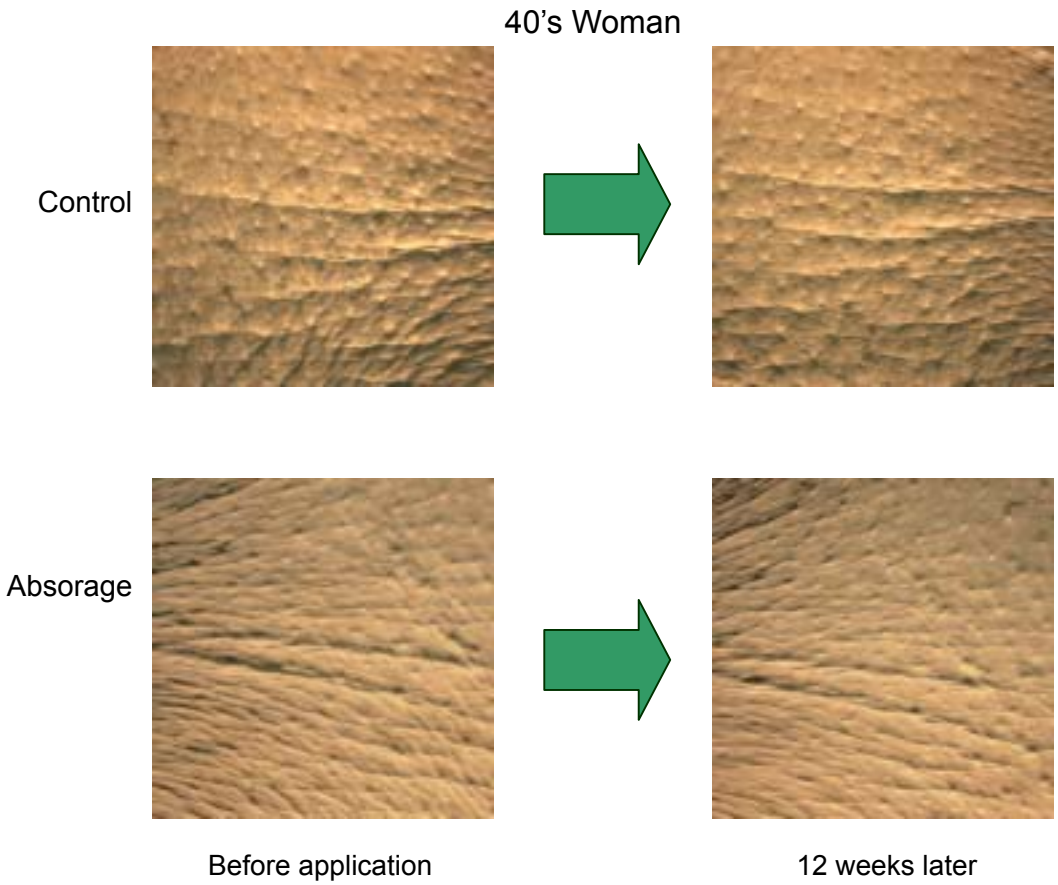


Fig. 10 Improvement of wrinkle

Inhibition of Glycation in SOD

Super-oxide dismutase (SOD) has been paid much attention from the aging prevention point of anti-aging. SOD are also denatured and inactivated by glycation to result in a decrease in physiological functions.[10)]

The degree of the inhibitory effect of plantagoside, an active component of Absorage on the Maillard reaction in SOD and BSA was examined by assaying a fluorescent AGE substance, FFI and CML produced by the glycation.

Test sample

Plantagoside, an active component of Absorage was dissolved in DMSO to make the final concentrations 12.5, 6.3, 3.1 and 1.6 μ mol/L and used for the test. The control reaction system, in which DMSO was added in place of a test sample solution, was employed. As a positive control, aminoguanidine sulfate (AG), an inhibitor of the glycation was used in the present test.

Test method

10 μ L of a test sample solution, 50 μL of glucose (200 mmol/L), 100 μL of SOD (2 mg/mL), 250 μL of 50 mM disodium hydrogen phosphate (Na_2HPO_4, pH 7.4) and 90 μL of purified water were mixed and incubated at 37°C for 2 weeks. After the completion of glycation, 10 μL of trichloroacetic acid was added to 100 μL of the reaction mixture. The precipitate thus produced was isolated and dissolved in 400 μL of alkaline phosphate buffer. The fluorescent intensity of the solution was measured using an excitation wavelength of 360 nm and an emission wavelength of 460 nm to obtain the amount of FFI. The inhibitory rate of FFI production in each test sample was calculated according to the following equation. The Inhibition rate of FFI production in each test sample was corrected by subtracting the quenching rate from the inhibitory rate of FFI production obtained previously.

The amount of CML in the above-mentioned glycation was determined by an ELISA method and the inhibition rate of CML production was calculated according to the following equation.

Inhibition rate of CML production % = (1 – Sample CML amount / Control CML amount) X 100

Result and Discussion

The inhibition effect of plantagoside on glycation in SOD (inhibitory effect on FFI and CML production) is shown in Figs. 11. The inhibition effect of plantagoside on glycation was observed in SOD.

These results indicated that plantagoside, an active component of Absorage had an inhibition effect on glycation in SOD. Therefore, it can be expected that Absorage prevents the decreases in anti-oxidative functions and physiological functions caused by aging.

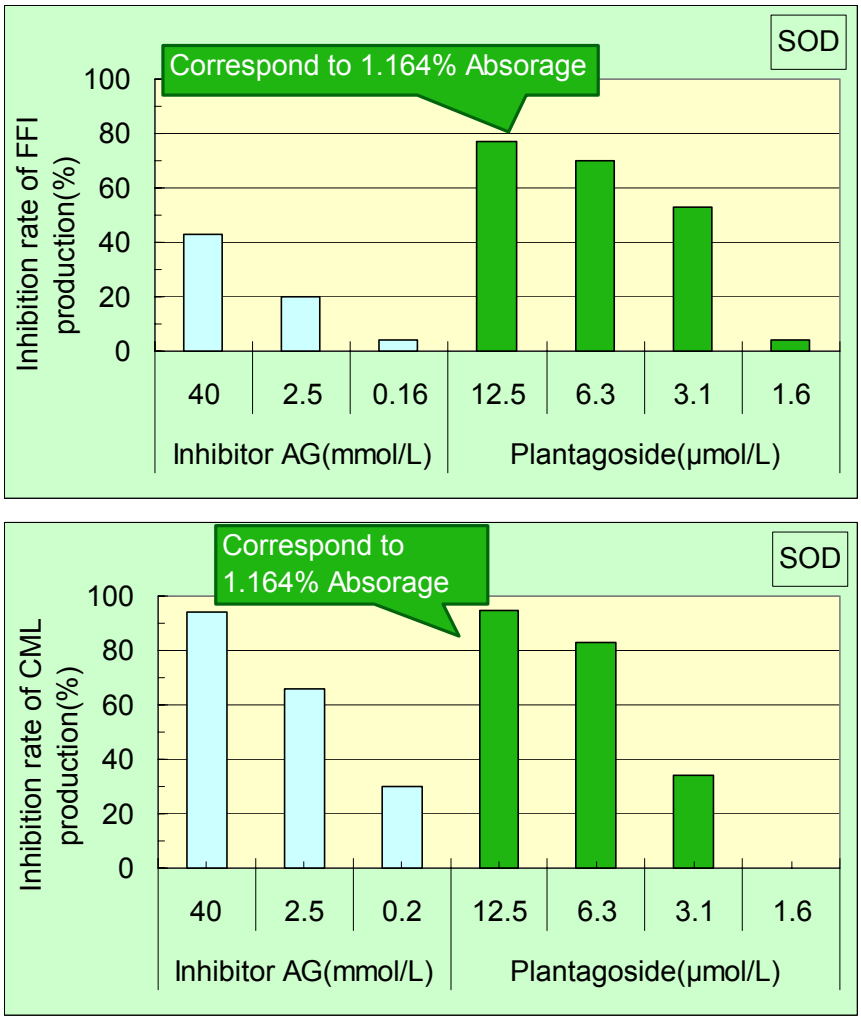


Fig.11 Inhibition of Glycation in SOD by Plantagoside; which is active component of Absorage

Inhibition of decrease in SOD functions (Glycation)

In the experiment described in the preceding section, the result that plantagoside, an active component of Absorage inhibited glycation in SOD (super-oxide dismutase) was obtained. Thus we examined whether plantagoside could actually prevent a decrease in SOD functions caused by glycation.

Test sample

Plantagoside, an active component of Absorage was dissolved in purified water to make the final concentrations 12.5, 6.3, 3.1 and 1.6 μmol/L and used for the test. The control reaction system, in which DMSO was added in place of a test sample solution, and the non-reaction system, in which glycation was substituted for glucose solution so that no glycation occurs, were employed. As a positive control, aminoguanidine sulfate (AG), an inhibitor of glycation was used in the present test.

Test method

10 μL of a test sample solution, 50 μL of glucose (200 mmol/L), 100 μL of SOD (2 mg/mL), 250 μL of disodium hydrogen phosphate (Na_2HPO_4, pH 7.4) and 90 μL of water were mixed and incubated at 37°C for 2 weeks. After the completion of glycation, SOD activity in the reaction solution was assayed by the NBT method. The NBT method is an assay method for SOD activity on the following mechanism: superoxide (0_2-) produced by the reaction of xanthine and xanthine oxidase reduces NBT (nitroblue tetrazolium) to change the color.

Result and Discussion

The inhibitory effect of plantagoside on the decrease in the SOD functions by glycation is shown in Fig. 12. SOD was denatured by glycation to result in the marked decrease in the SOD activity (super-oxide-eliminating activity). Plantagoside inhibited the decrease in the SOD activity caused by glycation. On the other hand, in the treatment of aminoguanidine known as an inhibitor of glycation, the inhibitory action on the decrease in the SOD activity as in the treatment of plantagoside was not observed.

These results and the results described in the preceding section indicated that plantagoside, an active component of Absorage prevented the decrease in the super-oxide (active oxygens)-eliminating activity of SOD by inhibiting glycation in SOD. Therefore, it can be expected that Absorage prevents a decrease in physiological functions such as anti-oxidative function caused by aging.

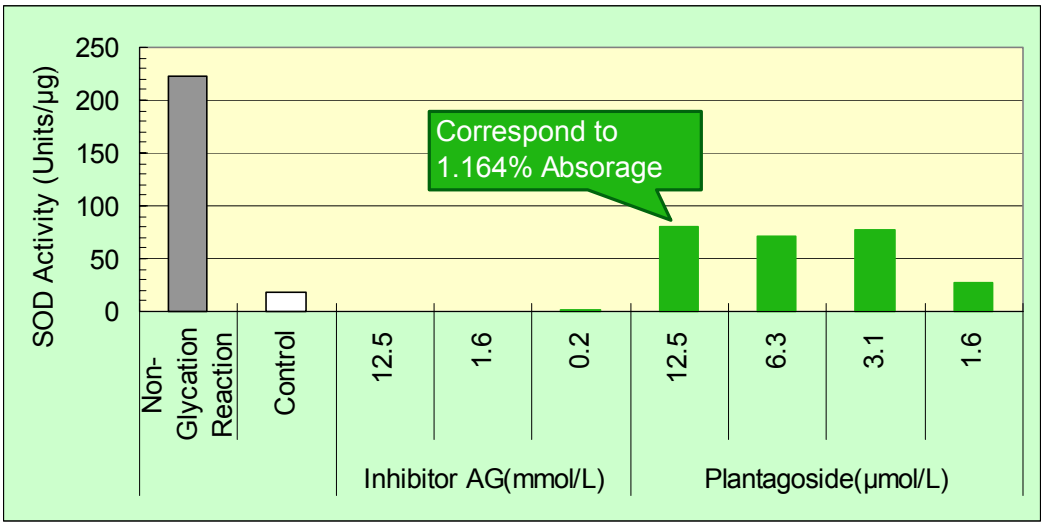


Fig.12 Inhibition of decrease in SOD functions by Plantagoside; which is active component of Absorage

Inhibition of Glycation in lysozyme

Lysozyme is contained in body fluids such as tear and mother's milk and has a bacteriolytic action against Gram positive bacteria and other such actions. Therefore, lysozyme is a very important enzyme for the body's immunity at the early stage of development. A decrease in the lysozyme functions by glycation gives a great disadvantage to body immune reactions. It is also considered that the decrease in the lysozyme functions leads to skin aging.

The degree of the inhibitory effect of plantagoside, an active component of Absorage on the Maillard reaction between lysozyme and ribose, a kind of sugar, was examined by analyzing the molecular weight distribution of the Maillard reaction products using a protein electrophoresis method (SDS-PAGE).

Test sample

Plantagoside, an active component of Absorage was used at the final concentrations of 12.5, 25, 50, 100 and 200 μmol/L. The control group, in which no test sample solution was added, was also employed. As a positive control, aminoguanidine sulfate (AG) (100 mmol/L) or sodium cyanoborohydride ($NaCNBH_3$) (100 mmol/L), either of which has been known as an inhibitor of glycation, was used for the present test.

Test method

Ribose (20 mM), lysozyme (5 mg/mL) and a test sample were mixed and reacted in phosphate buffer (pH 7.4) at 37 °C for 1 week. After the completion of the reaction, 10 μL of each reaction mixture was subjected to 17.5% T SDS-PAGE electrophoresis. After the completion of electrophoresis, the gel was stained with coomassie brilliant blue.

Result and Discussion

The electrophoresis images of the reaction products in each test sample are shown in Fig. 13. In the control reaction, bands were observed at about 14kDa*, original molecular weight of lysozyme, and at 30kDa, two times higher molecular weight, indicating that the molecular weight of lysozyme was doubled due to crosslinking by glycation. In the positive control group treated with AG or $NaCNBH_3$, an inhibitor of glycation, a band was observed only at 14kDa, suggesting that glycation was inhibited under this condition. On the other hand, in the treatment with plantagoside, the band at 30 kDa disappeared in a concentration-dependent manner, suggesting that plantagoside inhibits the Maillard reaction between lysozyme and ribose in a concentration-dependent manner.

These results indicated that plantagoside, an active component of Absorage inhibited glycation between lysozyme and ribose. Therefore, it can be expected that Absorage prevents crosslinking of protein caused by aging and a decrease in immune function caused by glycation.

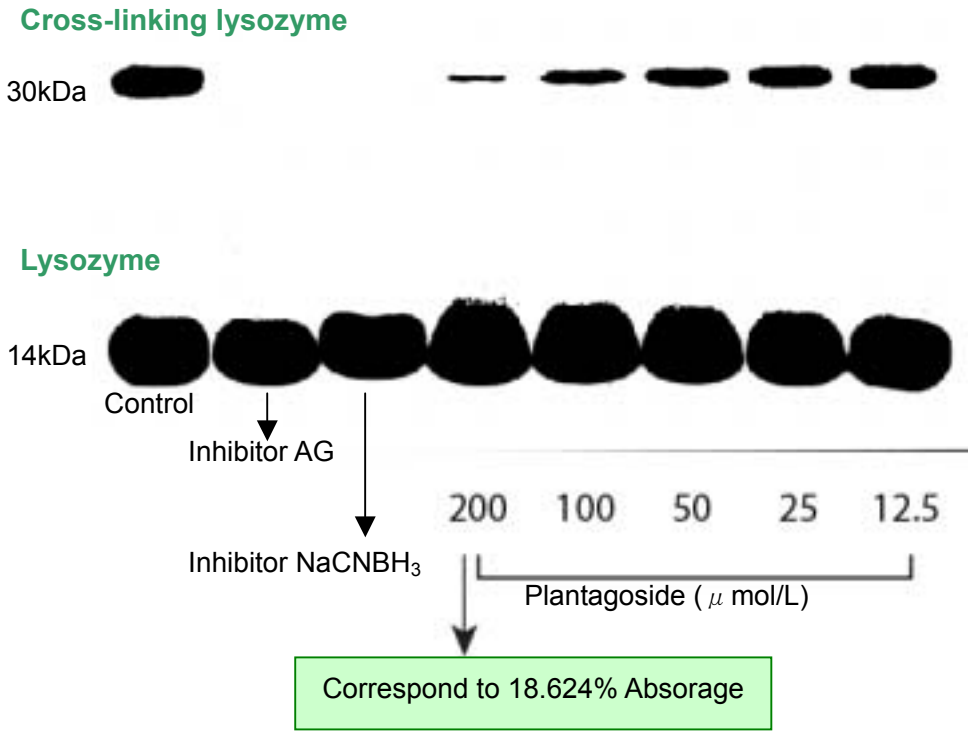


Fig.13 Inhibition of Glycation in lysozyme by Plantagoside; which is active component of Absorage

Stability

Stability of Absorage was investigated.

Long Term stability

Store Absorage in a cool dark place(4℃), room temperature, window side and at 50℃. Absorbance values at 470nm were determined.

Result and Discussion

Change of Absorbance value is shown in Fig. 14. At any condition, Absorage was very stable.

According to the result, the long term stability of Absorage is superior.

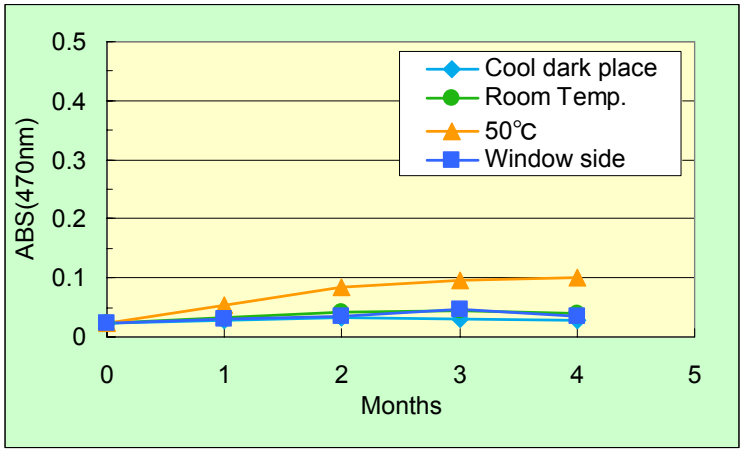


Fig. 14 Long term stability

Thermal stability

Absorage was heated in a water bath at 90℃. After cooling down, absorbance value was measured at 470 nm.

Result and discussion

Thermal stability of Absorage is shown in Fig. 15. The increase of absorbance of Absorage and precipitate were not observed. According to the result, Absorage is considered to be stable against heating.

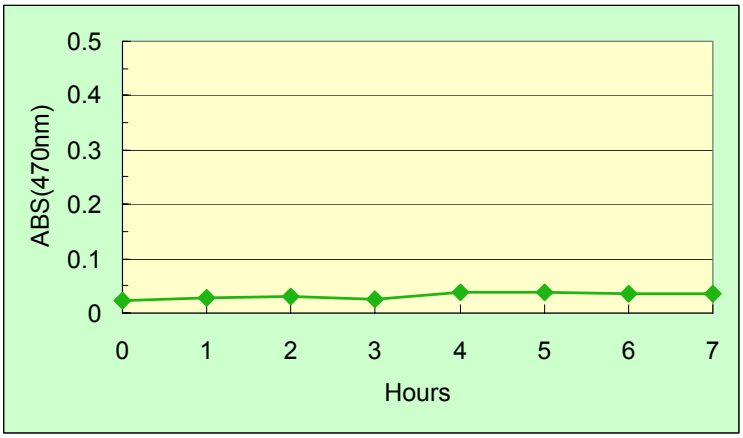


Fig.15 Thermal Stability

pH stability

pH of Absorage is adjusted from 3 to 10 by HCl and NaOH. Absorbance value (1 → 10) at 470nm were determined.

Result and Discussion

Absorbance value of Absorage is shown in Fig. 16 and visible color change is shown in Fig. 17. pH 2 to 8, it was almost stable on ABS, but over pH9 ABS was increased. And over pH 8 the visible color was darken. Precipitation was not observed at any pH range.

According to the result, Absorage is not recommended to use under alkali conditions

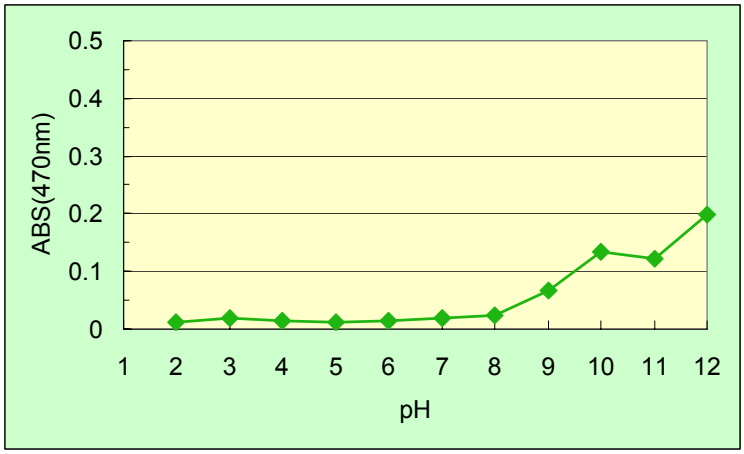


Fig.16 pH Stability

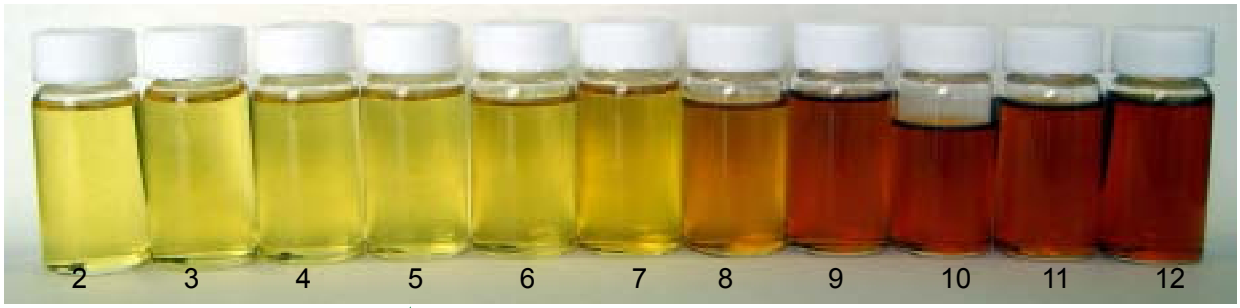


Fig.17 Color change by pH

Original pH of Absorage for this test

Compatibility

Compatibility of Absorage with other materials was evaluated.

Test method

Absorage was diluted to 5 %, and the test samples were adjusted by purified water as shown on the each table. After 24 hours, mixed solution was evaluated.

Result and Discussion

Table 2, Compatibility of 5 % Absorage with Surfactant

	%	Ingredients	Result
Cation	2.8	Stearyl Trimethyl Ammonium Chloride	○
	3.0	Cetyltrimehylammonium Chloride	○
	2.7	Lauryltrimethylammonium Chloride	○
Anion	10.0	Triethanolamine Lauryl Sulfate	○*
	25.0	Sodium Laureth Sulfate	○
	25.0	Triethanolamine Laureth Sulfate	○
	6.25	Laureth-6 Carboxylic Acid	○
	10.0	Sodium N-Cocoyl-N-methyl Taurate	○
	10.0	Potassium N-Cocoyl Glycinate	○**
	7.5	Sodium Lauroyl Methylaminopropionate	○**
	25.0	Sodium Tetradecenesulfonate	○**
Nonion	10.0	Polyethylene Glycol (50) Oleyl Ether	○
	10.0	Coconut Tatty Acid Diethanolamide	○***
	10.0	Sorbeth-60 Tetraoleate	○
	10.0	Polyoxyethylene Sorbitan Monooleate (20E.O.)	○
	10.0	Polyoxyethylene Hydrogenated Castor Oil (60E.O.)	○
Silicone	10.0	Polyoxyethylene・Methylpolysiloxane Copolymer	○
Ampholytic	3.5	Lauryl Dimethylaminoacetic Acid Betaine	○**
	4.0	Sodium N-Cocoyl-N-Carboxymethyl-N-Hydroxyethyl Ethylenediamide	○**
	2.9	Lauroyl Amide Propylhydroxysulfobetaine	○

○ : Good, △: Slight Turbidity, ×: Precipitate

*:Color changed in light pink, **:Color changed in light brown, ***:Color changed in light yellow

Table 3, Compatibility of 5 % Absorage with other ingredients

	%	Ingredients	Result
Solvent	50	Glycerin	○
	50	1,3-Butylene Glycol	○
	50	Propylene Glycol	○
	50	Isopropyl Alcohol	○
	50	Ethanol	○
Synthetic polymer	0.1	Carboxyvinyl polymer	○
	1	Polyvinyl Alcohol	○
	1	Polyvinylpyrrolidone	○
	1	Polyethylene glycol (6000)	○
Natural polymer	1	Sodium alginate	△
	1	Carboxymethyl cellulose	○
	1	Cationic cellulose	○
	0.1	Bio-Sodium Hyaluronate (HA12)	○
	1	Hydroxypropyl cellulose	○
Phospholipid	1	Lipidure-PMB	○
Vitamin-C derivative	2	Ascorbyl Glucoside	○
	2	Pacificos VAP	○

○: Good, △: Slight Turbidity, ×: Precipitate

Table 4, Compatibility of 5 % Absorage with other our products

%	Product name	INCI Name	Result
5	FM Extract LA-B	Lactobacillus / Milk Ferment Filtrate	○
5	OUGON Liquid SE	Scutellaria Baicalensis Root Extract	○
5	Caffenoage	Coffea Arabica (Coffee) Seed Extract	○
5	CHITIN Liquid (N)	Carboxymethyl Chitin	○
5	HPCH Liquid	Hydroxypropyl Chitosan	○
5	CureBerry	Vaccinium Myrtillus Leaf Extract	○
5	Clairju	Hydrolyzed Prunus Domestica	○
5	KOTHALAHIMBUTU Liquid B	Salacia Reticulata Wood Extract	○
5	SAKURA Extract B	Prunus Yedoensis Leaf Extract	○
5	MARINWORT IPC-14 SBW	Algae Extract	○
5	SILKGEN G Soluble	Hydrolyzed Silk	○
5	SILKGEN G Soluble-S	Hydrolyzed Silk	○
5	TREHALOSE 30	Trehalose	○
5	NEEM Leaf Liquid B	Melia Azadirachta Leaf Extract	○
0.1	Bio-PGA Na Powder	Sodium Polygamma-Glutamate	○
5	Bio-PGA Solution HB	Polyglutamic Acid	○
5	Bio-PGA Solution LB	Polyglutamic Acid	○
5	Bio antiage B	Pueraria Lobata Root Extract, Aloe Barbadensis Leaf Extract, and Chlorella Vulgaris Extract	○
5	Biobenefity	Cynara Scolymus Leaf Extract	○
5	PEACH Leaf Liquid B	Prunus Persica (Peach) Leaf Extract	○
5	Biocellact ALOE VERA B	Aloe Barbadensis Leaf Extract	○
5	Fermentage Chardonnary B	Lactobacillus/Grape Juice Ferment	○
5	Fermentage Pear B	Lactobacillus/Pyrus Communis (Pear) Fruit Juice Ferment	○
5	Pharconix CTP-F (BG)	Hydrolyzed Collagen	○
5	JIOU Liquid	Rehmannia Chinensis Root Extract	○
5	SOUHAKUHI Liquid (BG)	Morus Alba Root Extract	○
5	HIOUGI Liquid	Belamcanda Chinensis Root Extract	○
5	BOTANPI Liquid E	Paeonia Suffruticosa Root Extract	○
5	LEMONGRASS Liquid B	Cymbopogon Schoenanthus Extract	○
5	Phyto COLLAGE (N)	Natto Gum	○
5	Phyto HYALURON B	Hibiscus Esculentus Fruit Extract	○
5	FLAVOSTERONE SB	Glycine Soja (Soybean) Protein	○
5	PrincessCare	Geranium Robertianum Extract	○
5	Mebi-Gel 20	Butyl Acrylate/Isopropylacrylamide/PEG-18 Dimethacrylate Crosspolymer	○
5	YUZU Ceramide B	Citrus Junos Fruit Extract	○
5	LACTOSACCHARIDES B	Yogurt Filtrate	○
5	RYOKUCHA Liquid	Camellia Sinensis Leaf Extract	○
5	LUNAWHITE B	Oenothera Biennis (Evening Primrose) Seed Extract	○

○: Good, △: Slight Turbidity, ×: Precipitate

Specification

Specification	
Appearance	Light yellowish brown to reddish brown liquid having a characteristic odor
Identification Phenolic substance Sugar	 Positive Positive
Purity Heavy Metals Arsenic	 20 ppm max. 2 ppm max.
Residue on Evaporation	0.3 w/v % max
INCI Name	Butylene Glycol Water Plantago Major Seed Extract
CAS Number	84929-43-1
EINECS Number	284-526-8

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