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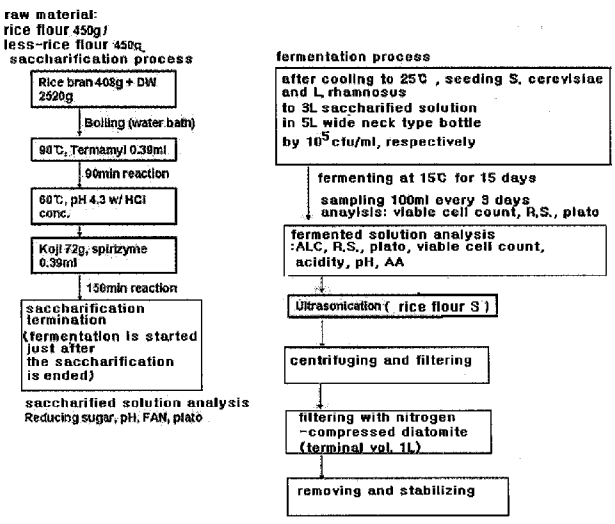
(54) Title: COMPOSITION FOR PROTECTING AND IMPROVING SKIN COMPRISING THE RICE BRAN FERMENTED BY LACTIC ACID BACTERIA



(57) Abstract: Disclosed is a composition containing the rice bran-lactic acid bacteria fermentation and used for protecting or improving skin, skin whitening, preventing or improving skin aging or wrinkle, protecting skin against the ultraviolet or alleviating inflammatory response and reinforcing skin barrier function recovery. The rice bran-lactic acid fermentation is obtained by fermenting the rice bran as it is or saccharifying and then fermenting the rice bran with lactic acid bacteria or a mixture of lactic acid bacteria and yeast. The rice bran- lactic acid bacteria fermentation of the invention impedes the melanin synthesis to exhibit the skin whitening effect, promotes the procollagen synthesis to prevent or improve the skin aging or wrinkle, prevents the skin cell damage against the ultraviolet or suppresses the inflammatory response to protect the skin from the ultraviolet or to alleviate the inflammatory response due to the ultraviolet and reinforces the skin barrier function recovery.

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【DESCRIPTION】

【Invention Title】

COMPOSITION FOR PROTECTING AND IMPROVING SKIN COMPRISING THE RICE BRAN FERMENTED BY LACTIC ACID BACTERIA

【Technical Field】

The invention relates to a composition containing the rice bran fermented by lactic acid bacteria ("rice bran-lactic acid bacteria fermentation"), as effective ingredients, and more particularly to a composition containing the rice bran-lactic acid bacteria fermentation and used for protecting or improving skin, skin whitening, preventing or improving skin aging or wrinkle, protecting skin against the ultraviolet or alleviating inflammatory response, and reinforcing skin barrier function recovery.

【Background Art】

Since the rice includes many nutrient ingredients, the Korean have used the rice as the principal food from ancient times and the functional nutrient food and cosmetic food using the rice have been widely used. The rice obtained by drying and threshing the rice plant and then removing the rice hulls is referred to as unpolished rice. The unpolished rice consists of rice bran, embryo bud and albumen, which are the fruits of the nutrient ingredients, so that the unpolished rice has been known as nutritious food. In recent years, the unpolished rice is much highlighted with respect to the well-being trend.

Among them, the rice bran plentifully contains fat, protein, essential fatty acid, essential amino acid, vitamin, dietary fiber and mineral. In particular, the vitamin contained in the rice bran includes vitamin B such as myoinositol, choline and niacin, and vitamin E (tocopherol) which is antioxidant material removing free radicals or activated oxygens causing cell aging and damage. In addition, the mineral includes phosphorus, potassium, magnesium, calcium and the like. The mineral also includes sitosterol, which is a protein having a function of lowering blood cholesterol, and gamma-

oryzanol, having effects of blood circulation promotion and skin metabolism promotion, whitening and ultraviolet blocking, which are reported in recent years.

Although the rice bran has the valuable nutrient and effective values, it is considered as by-product of the rice hulling and is simply used as rice bran oil and feed without systematic research about the effectiveness thereof.

As known already, since the rice bran fermentation plentifully contains amino acid group which is main ingredient of the natural moisturizing factor, it has an effect of making the skin glossy and moisturized. In addition, the remaining voluminous organic acid, saccharide and peptide keep the skin moisturized and refreshed.

In the mean time, when the lactic acid bacteria are taken through yogurt, they activate the enterokinesia and improve the physiological environment in the intestines, thereby improving the skin through the intestines improvement.

However, it has not been proved what effect the materials which the lactic acid bacteria make from the rice bran fermentation and the useful materials of the lactic acid bacteria cell have for the skin.

【Disclosure】

【Technical Problem】

In order to examine the effectiveness of the rice bran-lactic acid bacteria fermentation, the inventors fermented the rice bran as it was, or saccharified and then fermented the rice bran with lactic acid bacteria. Alternatively, the inventors fermented the rice bran with a mixture of yeast and lactic acid bacteria. As a result, the inventors confirmed that the rice bran-lactic acid bacteria fermentation impeded the melanin synthesis, promoted the procollagen synthesis, prevented the skin cell damage and inflammatory response due to the ultraviolet and reinforced the skin barrier function recovery.

Accordingly, an object of the invention is to provide a composition

containing the rice bran fermented by lactic acid bacteria (hereinafter, "rice bran-lactic acid bacteria fermentation") and used for protecting or improving skin, skin whitening, preventing or improving skin aging or wrinkle, protecting skin against the ultraviolet or alleviating inflammatory response and reinforcing skin barrier function recovery.

【Technical Solution】

In order to achieve the above object, there is provided a composition for skin protection or improvement containing rice bran-lactic acid bacteria fermentation in which the rice bran is fermented by lactic acid bacteria.

In addition, there is provided a composition for skin whitening containing rice bran-lactic acid bacteria fermentation in which the rice bran is fermented by lactic acid bacteria.

Additionally, there is provided a composition for preventing or improving the skin aging or wrinkle containing rice bran-lactic acid bacteria fermentation in which the rice bran is fermented by lactic acid bacteria.

Furthermore, there is provided a composition for preventing the skin from the ultraviolet or alleviating an inflammatory response due to the ultraviolet, which composition containing rice bran-lactic acid bacteria fermentation in which the rice bran is fermented by lactic acid bacteria.

In addition, there is provided a composition for reinforcing a skin barrier function recovery containing rice bran-lactic acid bacteria fermentation in which the rice bran is fermented by lactic acid bacteria.

In the compositions, the rice bran is at least one selected from a group consisting of rice flour, rice flour S and less-rice flour.

In the compositions, the rice bran-lactic acid bacteria fermentation is obtained by fermenting the rice bran with a mixture of lactic acid bacteria and yeast.

In the compositions, the lactic acid bacteria is *Lactobacillus* rhamnosus.

In the compositions, the yeast is *Saccharomyces cerevisiae.*

In the compositions, the rice bran-lactic acid bacteria fermentation is

obtained by saccharifying and then fermenting the rice bran.

In the compositions, the composition is a cosmetic, pharmaceutical or health functional food composition.

【Advantageous Effects】

The rice bran-lactic acid bacteria fermentation of the invention impedes the melanin synthesis to exhibit the skin whitening effect, promotes the procollagen synthesis to prevent or improve the skin aging or wrinkle, prevents the skin cell damage against the ultraviolet or suppresses the inflammatory response to protect the skin from the ultraviolet or to alleviate the inflammatory response due to the ultraviolet and reinforces the skin barrier function recovery. Accordingly, the composition containing the rice bran-lactic acid bacteria fermentation can be usefully used in the cosmetic, pharmaceutical or health functional food composition for protecting or improving skin, skin whitening, preventing or improving skin aging or wrinkle, protecting skin against the ultraviolet or alleviating inflammatory response and reinforcing skin barrier function recovery.

【Description of Drawings】

FIG. 1 shows a method for manufacturing rice bran-lactic acid bacteria fermentation according to an embodiment of the invention;

FIG. 2 is a graph showing an effect of the rice bran-lactic acid bacteria fermentation of the invention on the melanin synthesis impediment in the melanin tumor cell and a photograph;

FIG. 3 shows an effect of the rice bran-lactic acid bacteria fermentation of the invention on the procollagen synthesis promotion in the human fibroblast;

FIG. 4 shows an effect of the rice bran-lactic acid bacteria fermentation of the invention on the skin keratinocyte protection against the ultraviolet;

FIG. 5 is a graph showing an effect of the rice bran-lactic acid bacteria fermentation of the invention on the inflammatory response decrease in the skin keratinocyte, which is caused by the ultraviolet irradiation; and

FIG. 6 is a graph showing an effect of the rice bran-lactic acid bacteria fermentation of the invention on the skin barrier function recovery reinforcement in a glabrous rat model.

【Best Mode】

In the specification including the claims, the "rice flour" is defined as a white part which is polished by about 11% in the rice hulling.

The "rice flour S" is defined as rice bran-lactic acid bacteria fermentation containing bacteria cell ingredients which are obtained by crushing the bacteria cell in the rice bran-lactic acid bacteria fermentation with the ultrasonic.

The "less-rice flour" is defined as rice bran part which is polished by about 13~15% in the rice hulling.

In the invention, as the lactic acid bacteria for fermenting the rice bran, it may be used *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus delbruekii*, *Lactobacillus johnsonii*, *Lactobacillus rhamnosus*, *Lactobacillus sakei*, *Streptococcus thermophilus*, *Streptococcus pythium*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides* and the like, and more preferably, *Lactobacillus rhamnosus*.

In the invention, as the yeast for fermenting the rice bran, it may be used *Saccharomyces cerevisiae*, *Saccharomyces sakei*, *Xanthophyllomyces dendrorhous* and the like, and more preferably, *Saccharomyces cerevisiae*.

In the invention, the method for saccharifying the rice bran is as follows. First, the rice bran and distilled water are put and heated in a saccharification vessel of a predetermined capacity. When a temperature reaches 90℃, liquefaction enzyme (α-amylase) is introduced into the vessel and subject to a reaction for 90 minutes. Then, after the temperature is lowered to 60℃, the solution is adjusted to be pH 4.3 with heavy hydrochloric acid. Then, Koji and saccharification enzyme (glucoamylase) are put in the vessel in predetermined amounts. After that, the solution is

stirred at 60℃ and subject to a reaction for 150 minutes, thereby saccharifying.

In order to examine the skin whitening efficacy of the rice bran-lactic acid bacteria fermentation, the inventors treated the melanin tumor cells with 0.1% of the rice bran-lactic acid bacteria fermentation, cultured the cells for 3 days and measured the amount of the synthesized melanin in absorbency, thereby proving the efficacy thereof.

Furthermore, in order to examine the skin aging and wrinkle prevention and improvement efficacies of the rice bran-lactic acid bacteria fermentation, the inventors treated the fibroblast with 0.1% of the rice bran-lactic acid bacteria fermentation, cultured it for one day and quantified the procollagen (collagen precursor) secreted in the culture medium with a western blot method, thereby proving the efficacies.

Additionally, in order to examine the skin protection and inflammatory response alleviation efficacies against the ultraviolet of the rice bran-lactic acid bacteria fermentation, the inventors irradiated the skin keratinocyte with UVB of 25 mJ/cm^2, treated it with 0.1% of the rice bran-lactic acid bacteria fermentation, cultured the treated keratinocyte for one day, quantified the TNF-α secreted in the culture medium with an ELISA method and analyzed the cell survival rate with a MTT analysis method, thereby proving the efficacies.

Further, in order to examine the skin barrier function reinforcement efficacy of the the rice bran-lactic acid bacteria fermentation, the inventors measured the water-evaporation amounts of the glabrous rat having taken the rice bran-lactic acid bacteria fermentation and the glabrous rat having not taken it, thereby proving the efficacy.

The composition of the invention preferably contains the rice bran-lactic acid bacteria fermentation of the invention in an amount of 0.0001~99.9 wt% for a total weight of the composition. More preferably, the composition contains the fermentation in an amount of 0.1~60.0 wt% in consideration for the effective effect, the stability and the formulation

stability.

The composition of the invention may be a cosmetic or pharmaceutical composition.

Preferably, in addition to the rice bran-lactic acid bacteria fermentation, the cosmetic composition of the invention may further contain the other ingredients capable of providing a synergetic effect to the main effects, within a range not deteriorating the main effects of the invention.

The rice bran-lactic acid bacteria fermentation of the invention is preferably prepared into each cosmetic formulation and applied as it is. However, it may be formulated into a typical external preparation.

The cosmetic composition of the invention can be applied to the cosmetics for the above effects in various manners. The products into which the composition of the invention can be added include a variety of cosmetics such as cream, lotion and toner, cleansing, washing agent, soap, cosmetic solutions and the like.

The cosmetics into which the composition containing the rice bran-lactic acid bacteria fermentation of the invention is added may be formed into solution, emulsion, viscous mixture and the like.

In other words, the cosmetics of the invention are not particularly limited with regard to the formulations thereof, and the formulations include emulsion, cream, cosmetic water, essence, pack, gel, powder, makeup base, foundation, lotion, ointment, patch, cosmetic solution, cleansing foam, cleansing cream, cleansing water, body lotion, body cream, body oil, body essence, shampoo, rinse, body cleansing agent, soap, hair dye, aerosol and the like.

In the respective cosmetic compositions, the other ingredients except the rice bran-lactic acid bacteria fermentation may be properly selected and mixed by one skilled in the art in accordance with the formulations or purposes of the cosmetics.

The above formulations may contain a skin absorption promoting material so as to enhance the effects described above.

In addition, the cosmetics of the invention may contain an ingredient selected from a group consisting of water-soluble vitamin, oil-soluble vitamin, high-molecular peptide, high-molecular polysaccharide, sphingolipid and seeweed extracts.

The cosmetics of the invention may contain the other ingredients which are mixed in the typical cosmetics, as necessary, in addition to the essential ingredients.

The other mixed ingredients which can be added may include oil and fat ingredient, moisturizing agent, emollient agent, surfactant, organic and inorganic pigments, organic powder, ultraviolet absorbent, antiseptics, fungicide, antioxidant, plant extract, pH adjustor, alcohol, colorant, flavour, blood circulation accelerant, frigidity agent, anhydrotics, purified water and the like.

In addition, the mixed ingredients which can be added are not limited to the above ingredients and any ingredient may be mixed within a range of not deteriorating the object and effect of the invention.

The pharmaceutical composition of the invention may be administrated such as oral, non-oral, rectal, local, percutaneous, intravenous, intramuscular, intraperitoneal, and subcutaneous administrations. The percutaneous administration is most preferable.

In addition, the dosage of the active ingredient is different depending on ages, sexes and weights of the patient to be treated, specific diseases or pathological state to be treated, seriousness of the disease or pathological state, administration route, prescriber's decision and the like. The dosage is decided based on such factors by one skilled in the art. In general, the dosage is 0.001 mg/kg/day to 2000 mg/kg/day. The preferred dosage is 0.5 mg/kg/day to 2.5 mg/kg/day. Alternatively, 1.0 to 3.0 ml/day may be applied one to five times per a day for one month or more.

In the mean time, the pharmaceutical composition may be administrated in a solid, semi-solid or liquid form depending on the intended administration types. The administration types may include, but not limited

to, tablet, pill, capsule, suppository, small bag, granule, powder, cream, lotion, ointment, gel, patch, spray, sticking plaster, liquid solution, suspensions, dispersions, emulsions, syrup and the like. The active ingredient may be encapsulated in liposome, micro particles or micro capsules. However, the most preferred formulation is the formulation for the percutaneous administration, such as cream, lotion, ointment, gel, patch, spray, liquid solution, suspensions, dispersions or emulsions.

Among the formulations, the solid composition such as tablet, pill and granule may be coated for convenience sake. Typically, the composition for the intravenous administration is the solution in sterilized isotonic aqueous buffer solution and includes the local anesthetics so as to alleviate the pain in the syringed portion.

Additionally, the pharmaceutical composition may contain a small amount of a non-toxic adjuvant material, such as wetting agent, emulsifying agent, pH buffer agent and the like. The non-toxic adjuvant material may include, but not limited to, sodium acetate, sorbitan monolaurate, triethanolamine and triethanolamine oleate.

Further, the pharmaceutical composition of the invention may further include stabilizer, antioxidant, binding agent, colorant, flavouring, excipient such as antiseptic and thickening agent, carrier and diluents.

Furthermore, the invention provides a health functional food composition including the rice bran-lactic acid bacteria fermentation and a sitologically acceptable food adjuvant additive. The food to which the rice bran-lactic acid bacteria fermentation can be added may include various foods such as beverage, gum, tea, vitamin complex, health functional food and the like.

At this time, the rice bran-lactic acid bacteria fermentation in the food or beverage may be added in an amount of 0.001~99.9 wt% for the total food weight and the health beverage composition may be added in a ratio of 0.001~0.1g, more preferably 0.05~0.1g on the basis of 100 ㎖.

The health functional beverage composition of the invention necessarily

contains the rice bran-lactic acid bacteria fermentation, as essential ingredient, in the amount described above. However, the composition may contain the other ingredients without particular limitations. For example, as the typical beverages, the composition may contain the flavouring or natural carbohydrate, as additional ingredients.

In addition, the composition of the invention may further contain various nutrients, vitamin, mineral (electrolyte), synthesized flavour and natural flavour, colorant and promoters (cheese, chocolate and the like), pectic acid and salts thereof, alginic acid and salts thereof, organic acid, protective colloid thickeners, pH adjustor, stabilizer, antiseptics, glycerine, alcohol, carbonation agent used for carbonated beverage and the like. Further, the compositions of the invention may contain flesh of fruit for preparing the natural fruit juice, fruit juice beverage and vegetable beverage. These ingredients may be used individually or in combination. Although the ratio of the ingredients is not particularly important, the ingredients are generally selected within a range of 0~20 weight parts for 100 weight part of the composition of the invention.

In the mean time, since rice bran-lactic acid bacteria fermentation of the invention do not have severe toxicity and side effects, it can be used safely for a long time, for the prevention purpose.

【Mode for Invention】

Hereinafter, the invention will be more specifically described with reference to embodiments and experimental examples. However, it should be noted that the invention is not limited thereto.

[embodiment 1]

The solution, in which rice flour (white parts which are polished by about 11% in the rice hulling) 408g and *Lactobacillus plantarum* (separated from Koji) 2×10^5 cfu/mℓ were dispersed in water 2,520g, was put in a 5L wide neck type bottle and fix-cultured at 15℃ for 15 days, thereby fermented with the lactic acid bacteria. After the fermentation, the fermented solution was

centrifuged (4300 rpm, 30 minutes, SORVALL LEGEND RT), filtered with nitrogen-compressed diatomite so as to remove the solid body, and concentrated five times using an evaporator to prepare the rice flour-lactic acid bacteria fermentation.

[embodiment 2]

In this embodiment 2, the bacteria cells in the rice flour fermented solution obtained in the embodiment 1 were crushed with ultrasonic (sonics, vibra cell™) at 30℃ for 30 minutes with 50% sonic so that the solution include the bacterial cell ingredients therein. The rice flour S-lactic acid bacteria fermentation, which was the rice bran-lactic acid bacteria fermentation containing the bacteria cell ingredients obtained, was prepared.

[embodiment 3]

In the embodiment 3, the less rice flour-lactic acid bacteria fermentation was produced in the same manner as the embodiment 1, except that the less-rice flour (rice bran parts which are polished by about 13~15% in the rice hulling) was used instead of the rice flour in the embodiment 1.

[embodiment 4]

In this embodiment 4, the rice flour-lactic acid bacteria fermentation was produced in the same manner as the embodiment 1, except that *Lactobacillus rhamnosus* (KCTC5033) 2×10^5 cfu/ml was used instead of *Lactobacillus plantarum* in the embodiment 1.

[embodiment 5]

The rice bran 408g and distilled water 2,520g were put and heated in a saccharification vessel. When a temperature reached 90℃, α-amylase (Termamyl, liquefaction enzyme) 0.39 mℓ was introduced into the vessel and subject to a reaction for 90 minutes. Then, after the temperature was lowered to 60℃, the solution was adjusted to be pH 4.3 with heavy

hydrochloric acid. Then, Koji (prepared with Aspergillus oryzae) 72g and glucoamylase (spirizyme, saccharification enzyme) 0.39 mℓ were put in the vessel (having a stirrer attached thereto and capable of controlling a temperature thereof; 600 rpm, Lab-stirrer, POONGLIM. Co.), respectively. After that, the solution was stirred at 60℃ and subject to a reaction for 150 minutes, thereby saccharified.

After the saccharification was completed, *Lactobacillus rhamnosus* 2x 10^5cfu/ml was put in the obtained saccharified solution and fix-cultured at 15 ℃ for 15 days, thereby fermenting the solution. After the fermentation, the fermented solution was centrifuged (4300 rpm, 30 minutes, SORVALL LEGEND RT), filtered with nitrogen-compressed diatomite so as to remove the solid body, and concentrated five times using an evaporator to prepare the rice flour-lactic acid bacteria fermentation.

[embodiment 6]

In this embodiment 6, the rice flour was used to obtain the saccharified solution in the same manner as the embodiment 5. The obtained saccharified solution, *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* were put by 1x10^5cfu/ml, respectively, and fix-cultured at 15℃ for 15 days, thereby conducting the mixing-fermentation. After the fermentation, the fermented solution was centrifuged (4300 rpm, 30 minutes, SORVALL LEGEND RT), filtered with nitrogen-compressed diatomite so as to remove the solid body, and concentrated five times using an evaporator to prepare the rice flour-lactic acid bacteria fermentation (refer to Fig. 1).

[embodiment 7]

In this embodiment 7, the bacteria cells in the rice flour fermented solution obtained in the embodiment 6 were crushed with ultrasonic (sonics, vibra cell™) at 30℃ for 30 minutes with 50% sonic so that the solution include the bacterial cell ingredients therein. The rice flour S-lactic acid

bacteria fermentation, which was the rice bran-lactic acid bacteria fermentation containing the bacteria cell ingredients obtained, was prepared (refer to Fig. 1).

[embodiment 8]

In this embodiment 8, the less rice flour-lactic acid bacteria fermentation was prepared in the same manner as the embodiment 6, except that the less rice flour was used instead of the rice flour in the embodiment 6 (refer to Fig. 1).

[comparative example 1]

In the comparative example 1, the high polished rice-lactic acid bacteria fermentation was prepared in the same manner as the embodiment 1, except that the high polished rice was used instead of the rice flour in the embodiment 1.

[comparative example 2]

In the comparative example 2, the unpolished rice-lactic acid bacteria fermentation was prepared in the same manner as the embodiment 3, except that the unpolished rice was used instead of the less rice flour in the embodiment 3.

[comparative example 3]

In the comparative example 3, the high polished rice-lactic acid bacteria fermentation was prepared in the same manner as the embodiment 4, except that the high polished rice was used instead of the rice flour in the embodiment 4.

[comparative example 4]

In the comparative example 4, the unpolished rice-lactic acid bacteria fermentation was prepared in the same manner as the embodiment 4, except that

the unpolished rice was used instead of the rice flour in the embodiment 4.

[comparative example 5]

In the comparative example 5, the high polished rice-lactic acid bacteria fermentation was prepared in the same manner as the embodiment 6, except that the high polished rice was used instead of the rice flour in the embodiment 6.

[comparative example 6]

In the comparative example 6, the unpolished rice-lactic acid bacteria fermentation was prepared in the same manner as the embodiment 6, except that the unpolished rice was used instead of the rice flour in the embodiment 6.

[experimental example 1] melanin synthesis prohibiting effect in the melanin tumor cell

In order to examine the melanin synthesis prohibiting effect in the melanin tumor cell of the fermentations obtained in the embodiments and comparative examples, the following experiment was conducted.

First, the malignant melanin tumor cell line B16F10 (Korean Cell Line Bank; KCLB8008), which was originated from the mouse, was put in the DMEM (Dulbecco's Modified Eagle's Medium) culture medium containing penicillin (100 IU/mL), streptomycin (100 g/mL) and 10% FBS (fetal bovine serum), kept at 37℃ and cultured in the fermentation vessel including 5% carbon dioxide. The obtained melanin tumor cells were seeded to the six well plate by 1.5 x 10^5 cells/well and cultured under cell cultivation conditions for one day, and the culture medium was removed. Then, the respective fermentations obtained in the embodiments and comparative examples, by 0.1% respectively, and Kojic acid (positive control group) 100 μM was added to DMEM culture medium and cultured for 3 days. The culture medium of each treatment group was collected in a 5 mℓ test tube and the change in the colors of the culture medium was photographed with a digital camera (refer to a lower part in

Fig.2). In addition, 10% DMSO (Dimethyl sulfoxide) and 1N NaOH by 100 ㎕, respectively, were added to the cells of each treatment group, thereby dissolving the melanin. Then, the absorbency at 405 nm was measured. The result is shown in Fig. 2 (upper part in Fig. 2).

As a result, Fig. 2 shows a degree that the medium color became deep due to the melanin secreted as the melanin was synthesized. As shown, the colors of the culture media of the rice bran-lactic acid bacteria fermentations in the embodiments are lighter, compared to the non-treatment group, the Kojic acid treatment group and the comparative examples.

In addition, the absorbency results of the non-treatment group, the Kojic acid treatment group, the comparative examples and the embodiments were as follows: 0.74 for the non-treatment group, 0,6 for the Kojic acid treatment group, and 0.612, 0.604, 0.576, 0.534, 0.592 and 0.497 for the comparative examples 1 to 6, respectively. The results for the embodiments 1 to 8 are shown in the graphs of Fig. 2. Accordingly, it could be seen that the melanin production amounts in the rice bran-lactic acid bacteria fermentations of the embodiments were generally decreased, compared to the non-treatment group, the Kojic acid treatment group and the comparative examples.

Further, as shown in the graphs of Fig. 2, the melanin production amount was more decreased in the embodiment 4 wherein the rice bran was fermented with *Lactobacillus rhamnosus*, than the embodiments 1 to 3 wherein the rice bran was fermented with *Lactobacillus plantarum*, and the decrease effect on the melanin production amount was highest in the embodiments 5 to 8 wherein the rice bran was saccharified and then fermented. This decrease effect was higher than the effect in the Kojic acid treatment group. In particular, the effect was highest in the embodiment 6 wherein the rice flour was saccharified and then fermented with the mixture of *Lactobacillus rhamnosus* and yeast. And the effects was high in order of the rice flour S-lactic acid bacteria fermentation of the embodiment 7, the less rice flour-lactic acid bacteria fermentation of the embodiment 8 and the rice flour-

lactic acid bacteria fermentation of the embodiment 5 wherein the rice bran was fermented with *Lactobacillus rhamnosus* only.

[experimental example 2] procollagen synthesis promoting effect in the human fibroblast

In order to examine the procollagen synthesis promoting effect in the normal human fibroblast of the fermentations obtained in the embodiments and comparative examples, the following experiment was conducted.

First, the human fibroblast (Cambrex) was put in the DMEM (Dulbecco's Modified Eagle's Medium) culture medium containing penicillin (100 IU/mL), streptomycin (100 g/mL) and 10% FBS (fetal bovine serum), kept at 37℃ and cultured in the fermentation vessel including 5% carbon dioxide. The obtained fibroblast was seeded to the six well plate by 3 x 10^5 cells/well and cultured under cell cultivation conditions for 24 hours, and the culture medium was removed. Then, the cultivation was conducted for 24 hours in the serum-free culture medium (DMEM) containing the 0.1% fermentations obtained in the embodiments and comparative examples and vitamin C of 5, 10 and 20 μM (positive control groups). The cultured solution of each treatment group was collected and used as sample for protein electrophoresis. The culture medium collected for the protein electrophoresis and a western sample buffer was prepared in a ratio of 1:2 as the electrophoresis sample of about 30 ㎕. The prepared sample was denatured with a boiling method and was subject to the protein electrophoresis in 8% SDS-PAGE. After that, the protein moved to the PVDF membrane was made to be visible in a X-ray film by using the anti-procollagen type 1 antibody as the primary antibody.

As seen from the western blot band, the procollagen synthesis amount was small in the non-treatment group and the comparative examples 1 to 6. The results for the embodiments 1 to 8 are shown in Fig. 3. Accordingly, it could be seen that the rice bran-lactic acid bacteria fermentations of the embodiments could promote the procollagen synthesis to the considerable level, as compared to the non-treatment group and the comparative examples.

Further, as shown in the graphs of Fig. 3, the procollagen synthesis was much promoted in the embodiment 4 wherein the rice bran was fermented with *Lactobacillus rhamnosus*, than the embodiments 1 to 3 wherein the rice bran was fermented with *Lactobacillus plantarum*, and the procollagen synthesis promoting effect was much higher in the embodiments 5 to 8 wherein the rice bran was saccharified and then fermented. In particular, it can be seen that the rice flour S-lactic acid bacteria fermentations of the embodiments 6 and 7 wherein the rice flour was saccharified and then fermented with the mixture of *Lactobacillus rhamnosus* and yeast could promote the procollagen synthesis to the considerable level. This promoting effect is similar to the effect in the vitamin C-treatment groups. The less rice flour-lactic acid bacteria fermentation of the embodiment 8 and the rice flour-lactic acid bacteria fermentation of the embodiment 5 much promoted the procollagen synthesis, as compared to the embodiments 1 to 4 and the non-treatment group, although the effect was not same as the effect in the embodiments 6 and 7.

[experimental example 3] skin keratinocyte protecting effect against the ultraviolet damage

In order to examine the skin keratinocyte protecting effect against the ultraviolet damage of the fermentations obtained in the embodiments and comparative examples, the following experiment was conducted.

First, the skin keratinocyte line (HaCaT) (originated in Dr. Norbert Fusenig's laboratory) was seeded to the 35 mm^2 cell culture plate by 3 x 10^5 cells/dish and then cultured for one night. Next day, the culture medium was removed, and the dish was washed with 1X phosphate-buffered saline (PBS). Then, 700 $\mu\ell$ of 1XPBS was added to each dish by the same amount, and 25 mJ/cm^2 of UVB was irradiated to the dish. Then, the PBS was removed, and the DMEM culture medium, to which the 0.1% fermentations obtained in the embodiments and the comparative examples and 200 μM of WY-14643 (Sigma, USA)(positive

control group) were added, was added and cultured for 24 hours. Next day, the culture media were collected, and the cell survival rates were analyzed with a MTT method. The result is shown in Fig. 4.

As a result, the absorbency results of the normal group, the non-treatment group, the WY14643 treatment group, the comparative examples and the embodiments were as follows: 0.7 for the non-treatment group, 1.4 for the WY14643 treatment group, and 0.75, 0.79, 0.78, 0.95, 0.775 and 0.99 for the comparative examples 1 to 6, respectively. The results for the embodiments 1 to 8 are shown in the graphs of Fig. 4. Accordingly, it could be seen that the cell survival rates in the rice bran-lactic acid bacteria fermentations were higher than the survival rates in the non-treatment group and the comparative examples.

In addition, as shown in Fig. 4, the cell survival rate was higher in the embodiment 4 wherein the rice bran was fermented with *Lactobacillus rhamnosus*, than the embodiments 1 to 3 wherein the rice bran was fermented with *Lactobacillus plantarum*, and the cell survival rate was much higher in the embodiments 5 to 8 wherein the rice bran was saccharified and then fermented. In particular, it could be seen that the effect was highest in the embodiment 6 wherein the rice flour was saccharified and then fermented with the mixture of *Lactobacillus rhamnosus* and yeast. This effect was approximately similar to the effect in the WY14643 treatment group. And the effect was high in order of the rice flour S-lactic acid bacteria fermentation of the embodiment 7, the less rice flour-lactic acid bacteria fermentation of the embodiment 8 and the rice flour-lactic acid bacteria fermentation of the embodiment 5 wherein the rice bran was fermented with *Lactobacillus rhamnosus* only.

[experimental example 4] decrease effect of an inflammatory response caused by ultraviolet irradiation in the skin keratinocytes.

In order to examine the decrease effect of the fermentations obtained in the embodiments and the comparative examples on an inflammatory response

caused by ultraviolet irradiation in the skin keratinocytes, the following experiment was conducted.

At this time, the cell preparation and the sample treatment were conducted in the same manner as the experimental example 3. In other words, the skin keratinocyte line (HaCaT) (originated in Dr. Norbert Fusenig's laboratory) was seeded to the 35 mm^2 cell culture dish by 3 x 10^5 cells/dish and then cultured for one night. Next day, the culture medium was removed, and the dish was washed with 1X phosphate-buffered saline (PBS). Then, 700 $\mu\ell$ of 1XPBS was added to each dish by the same amount, and 25 mJ/cm^2 of UVB was irradiated to the dish. Then, the PBS was removed, and the DMEM culture medium, to which the 0.1% fermentations obtained in the embodiments and the comparative examples and 200 μM of WY-14643 (positive control group) were added, was added and cultured for 24 hours. Next day, the culture media were collected and the amounts of TNF-α, which was known as inflammatory cytokines, were measured with a ELISA (Enzyme-Linked Immunosorbent assay) method. The result is shown in Fig. 5.

As a result, the measurement results of TNF-α secretion amount of the normal group, the non-treatment group, the WY14643 treatment group, the comparative examples and the embodiments were as follows: based on the 100% for the non-treatment group, 55% for the WY14643 treatment group, and 94%, 92%, 93%, 85%, 91% and 80% for the comparative examples 1 to 6, respectively. The results for the embodiments 1 to 8 are shown in the graphs of Fig. 5 (88%, 89%, 87%, 85%, 72%, 62%, 72% and 72%, respectively). Accordingly, it could be seen that the amounts of the inflammatory cytokines TNF-α increased due to the ultraviolet irradiation were decreased in the rice bran-lactic acid bacteria fermentations of the embodiments, as compared to the non-treatment group and the comparative examples.

In addition, as shown in Fig. 5, the amount of TNF-α was much decreased in the embodiment 4 wherein the rice bran was fermented with *Lactobacillus rhamnosus*, than the embodiments 1 to 3 wherein the rice bran

was fermented with *Lactobacillus plantarum*, and the decrease effect on TNF-α amount was highest in the embodiments 5 to 8 wherein the rice bran was saccharified and then fermented. In particular, it could be seen that the effect was highest in the embodiment 6 wherein the rice flour was saccharified and then fermented with the mixture of *Lactobacillus rhamnosus* and yeast. This effect was approximately similar to the effect in the WY14643 treatment group. And the effect was high in order of the rice flour S-lactic acid bacteria fermentation of the embodiment 7, the less rice flour-lactic acid bacteria fermentation of the embodiment 8 and the rice flour-lactic acid bacteria fermentation of the embodiment 5 wherein the rice bran was fermented with *Lactobacillus rhamnosus* only.

[experimental example 5] reinforcing effect on a skin barrier function recovery in a glabrous rat model

In order to examine the reinforcing effect of the fermentations obtained in the embodiments and the comparative examples on a skin barrier function recovery in a glabrous rat model, the following experiment was conducted.

First, the water evaporation amounts at both sides of the back of 8~12 week old, induced hairless rat were measured with a TEWL device (TEWLAMETER, TM210, Germany) (in case of normal skin, about 102g/hm^2). Then, the operation which is attaching and detaching the cellophane adhesive tape to and from the same parts about 15~20 times was repeated until the water evaporation amount reached 40~50 g/hm^2. After that, the 2% fermentations of the embodiments and the comparative examples were applied just after the damage, after 6 hours and after 12 hours, respectively. Nothing was applied to the other side of the back, as a control group. As time went by (before the damage, just after the damage, after 6 hours and after 12 hours), the water evaporation amounts were measured to examine the degrees of the skin barrier function recovery. The result is shown in Fig. 6.

As a result, the measurement results of the water evaporation amount-recovery degree (%) of the non-treatment group, the comparative examples and the embodiments are as follows: after 6 hours, 51% for the non-treatment group and 51%, 60%, 52%, 61%, 54% and 64% for the comparative examples 1 to 6. The results for the embodiments 1 to 8 are shown in Fig. 6. Accordingly, it could be seen that the recovery degrees of the skin barrier, which were confirmed by the water evaporation amount, were higher in the case where the rice bran-lactic acid bacteria fermentations were treated, as compared to the non-treatment group and the comparative examples.

In addition, as shown in Fig. 6, the recovery degree of the skin barrier was much higher in the embodiment 4 wherein the rice bran was fermented with *Lactobacillus rhamnosus*, than the embodiments 1 to 3 wherein the rice bran was fermented with *Lactobacillus plantarum*, and the recovery degree of the skin barrier was highest in the embodiments 5 to 8 wherein the rice bran was saccharified and then fermented. In particular, it could be seen that the effect was highest in the embodiment 6 wherein the rice flour was saccharified and then fermented with the mixture of *Lactobacillus rhamnosus* and yeast. This effect was approximately similar to the effect in the WY14643 treatment group. And the effect was higher in order of the rice flour S-lactic acid bacteria fermentation of the embodiment 7, the less rice flour-lactic acid bacteria fermentation of the embodiment 8 and the rice flour-lactic acid bacteria fermentation of the embodiment 5 wherein the rice bran was fermented with *Lactobacillus rhamnosus* only.

Hereinafter, although it will be described formulation examples of the above composition, it should be noted that they are provided to specifically illustrate the invention, not to limit it.

Formulation example 1: soap preparation

rice bran-lactic acid bacteria fermentation of embodiment 6

20.00 (wt%)

oil and fat	proper quantity
sodium hydroxide	proper quantity
sodium chloride	proper quantity
perfume	small quantity

Soap which has the total weight 100 including purified water was prepared in accordance with the mixing ratio.

Formulation example 2: lotion preparation

rice bran-lactic acid bacteria fermentation of embodiment 6

	30.00 (wt%)
L-ascorbic acid-2-magnesium phosphate salt	1.00
water-soluble collagen (1% aqueous solution)	1.00
sodium citrate	0.10
citric acid	0.05
licorice extract	0.20
1,3-butyleneglycol	3.00
oil and fat	2.00
serine	1.00

Lotion which has the total weight 100 including purified water was prepared in accordance with the mixing ratio (%).

Formulation example 3: cream preparation

rice bran-lactic acid bacteria fermentation of embodiment 6

	10.00 (wt%)
polyethyleneglycol monostearate	2.00
self-emulsified type monostearic acid glycerine	5.00
cetyl alcohol	4.00
squalene	6.00
tri-2-ethyl hexanoic acid glycerol	6.00
sphingoglycolipid	1.00
1,3-butyleneglycol	7.00

vitamin C	1.00

Creams which has the total weight 100 including purified water was prepared in accordance with the mixing ratio (%).

Formulation example 4: pack preparation

rice bran-lactic acid bacteria fermentation of embodiment 6	50.00 (wt%)
polyvinylalcohol	13.00
L-ascorbic acid-2-magnesium phosphate salt	1.00
lauroylhydroxyproline	1.00
water-soluble collagen (1% aqueous solution)	2.00
1,3-butyleneglycol	3.00
ethanol	5.00
serine	1.00

Pack which has the total weight 100 including purified water was prepared in accordance with the mixing ratio (%).

Formulation example 5: cosmetic solution preparation

rice bran-lactic acid bacteria fermentation of embodiment 6	20.00 (wt%)
hydroxyethylenecellulose (2% aqueous solution)	12.00
xanthan gum (2% aqueous solution)	2.00
1,3-butyleneglycol	6.00
rich glycerine	4.00
sodium hyaluronic acid (1% aqueous solution)	5.00
oil and fat	2.00
serine	1.00
vitamin C	1.00

Cosmetic solution which has the total weight 100 including purified water was prepared in accordance with the mixing ratio (%).

Formulation example 6: powders preparation

rice bran-lactic acid bacteria fermentation of embodiment 6	100 mg
lactose	100 mg
talc	10 mg
oil and fat	5 mg

The above ingredients were mixed and filled in the air-tight bag, thereby preparing the powders.

Formulation example 7: tablet preparation

rice bran-lactic acid bacteria fermentation of embodiment 6	50 mg
corn starch	100 mg
lactose	100 mg
magnesium stearate	2 mg
vitamin C	50 mg

The above ingredients were mixed and tabletted in accordance with a typical tablet preparing method, thereby preparing tablets.

Formulation example 8: capsule preparation

rice bran-lactic acid bacteria fermentation of embodiment 7	50 mg
corn starch	100 mg
lactose	100 mg
magnesium stearate	2 mg
vitamin C	50 mg
serine	50 mg

The above ingredients were mixed and filled in a gelatin capsule in accordance with a typical capsule preparing method, thereby preparing capsules.

Formulation example 9: injection preparation

rice bran-lactic acid bacteria fermentation of embodiment 8	50 mg
sterilized distilled water for injection	proper quantity
pH adjustor	proper quantity

In accordance with a typical injection preparation method, the injection was prepared in accordance with the above ingredient amounts per ampoule (2mℓ).

Formulation example 10: liquid drug preparation

rice bran-lactic acid bacteria fermentation of embodiment 6	100 mg
isomerized sugar	10 g
mannitol	5 g
vitamin C	50 mg
serine	50 mg
oil and fat	proper quantity
purified water	proper quantity

In accordance with a typical liquid drug preparation method, the respective ingredients were added and dissolved in the purified water, and the lemon perfume was added in a proper quantity. Then, the above ingredients were mixed and then added with purified water. The overall was adjusted to be 100 mℓ with purified water and then filled and sterilized in a brown bottle, thereby preparing the liquid drug.

Formulation example 11: health food preparation

rice bran-lactic acid bacteria fermentation of embodiment 6	1000 mg
vitamin mixture	
vitamin A acetate	70 μg
vitamin E	1.0 mg

vitamin B_1	0.13 mg
vitamin B_2	0.15 mg
vitamin B_6	0.5 mg
vitamin B_{12}	0.2 μg
vitamin C	10 mg
biotin	10 μg
nicotinic acid amide	1.7 mg
folic acid	50 μg
calcium pantothenate	0.5 mg
inorganic matter mixture	
ferrous sulfate	1.75 mg
zinc oxide	0.82 mg
magnesium carbonate	25.3 mg
potassium phosphate monobasic	15 mg
potassium phosphate dibasic	55 mg
potassium citrate	90 mg
calcium carbonate	100 mg
magnesium chloride	24.8 mg

The above vitamin and mineral mixtures were mixed, based on the preferred embodiment for the ingredients suitable for the health food. The mixing ratio may be arbitrarily modified. In accordance with a typical health food preparing method, the above ingredients may be mixed to prepare the granules which may be used for the health food composition preparation according to the typical method.

Formulation example 12: health beverage preparation

rice bran-lactic acid bacteria fermentation of embodiment 6	1000 mg
citric acid	1000 mg

oligosaccharide	100 g
Japanese apricot concentrate	2 g
taurine	1 g
purified water	to 900 mℓ

In accordance with a typical health beverage preparing method, the above ingredients are mixed, stirred and heated at 85℃ for about 1 hour. The obtained solution is filtered, filled and seal-sterilized in a 2ℓ sterilized vessel and kept cold. Then, it is used for preparing health beverage composition.

The above composition ratio was made in accordance with a preferred embodiment suitable for the taste beverage. However, the mixing ratio may be arbitrarily modified in accordance with the regional and national tastes such as demand classes, demand countries, uses thereof and the like.

【Industrial Applicability】

The rice bran-lactic acid bacteria fermentation of the invention impedes the melanin synthesis to exhibit the skin whitening effect, promotes the procollagen synthesis to prevent or improve the skin aging or wrinkle, prevents the skin cell damage against the ultraviolet or suppresses the inflammatory response to protect the skin from the ultraviolet or to alleviate the inflammatory response due to the ultraviolet and reinforces the skin barrier function recovery. Accordingly, the composition containing the rice bran-lactic acid bacteria fermentation can be usefully used in the cosmetic, pharmaceutical or health functional food composition for protecting or improving skin, skin whitening, preventing or improving skin aging or wrinkle, protecting skin against the ultraviolet or alleviating inflammatory response and reinforcing skin barrier function recovery.

【CLAIMS】

【Claim 1】

A composition for skin protection or improvement containing rice bran-lactic acid bacteria fermentation in which the rice bran is fermented by lactic acid bacteria.

【Claim 2】

A composition for skin whitening containing rice bran-lactic acid bacteria fermentation in which the rice bran is fermented by lactic acid bacteria.

【Claim 3】

A composition for preventing or improving the skin aging or wrinkle containing rice bran-lactic acid bacteria fermentation in which the rice bran is fermented by lactic acid bacteria.

【Claim 4】

A composition for preventing the skin from the ultraviolet or alleviating an inflammatory response due to the ultraviolet, which composition containing rice bran-lactic acid bacteria fermentation in which the rice bran is fermented by lactic acid bacteria.

【Claim 5】

A composition for reinforcing a skin barrier function recovery containing rice bran-lactic acid bacteria fermentation in which the rice bran is fermented by lactic acid bacteria.

【Claim 6】

The composition according to any one of claims 1 to 5, wherein the rice bran is at least one selected from a group consisting of rice flour, rice flour S and less-rice flour.

【Claim 7】

The composition according to claim 6, wherein the lactic acid bacteria is *Lactobacillus rhamnosus*.

【Claim 8】

The composition according to claim 6, wherein the rice bran-lactic acid

bacteria fermentation is obtained by fermenting the rice bran with a mixture of lactic acid bacteria and yeast.

【Claim 9】

The composition according to claim 8, wherein the lactic acid bacteria is *Lactobacillus rhamnosus*.

【Claim 10】

The composition according to claim 8, wherein the yeast is *Saccharomyces cerevisiae*.

【Claim 11】

The composition according to claim 6, wherein the rice bran-lactic acid bacteria fermentation is obtained by saccharifying and then fermenting the rice bran.

【Claim 12】

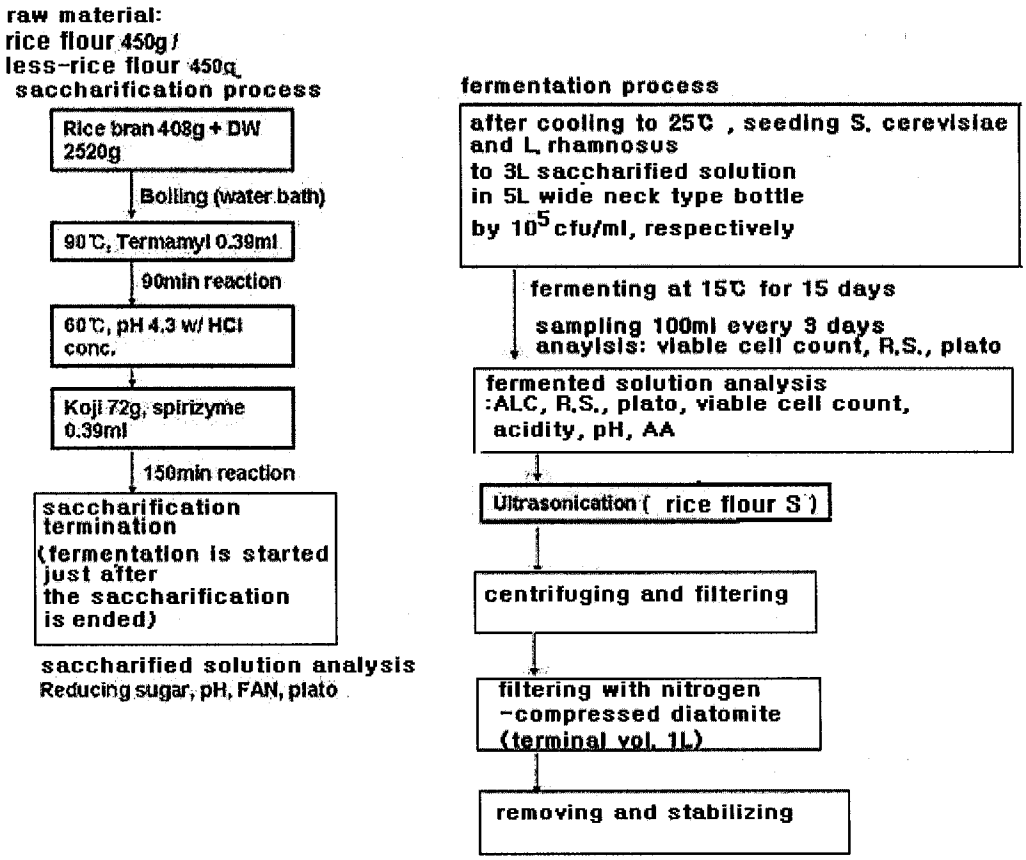
The composition according to claim 8, wherein the rice bran-lactic acid bacteria fermentation is obtained by saccharifying and then fermenting the rice bran.

【Claim 13】

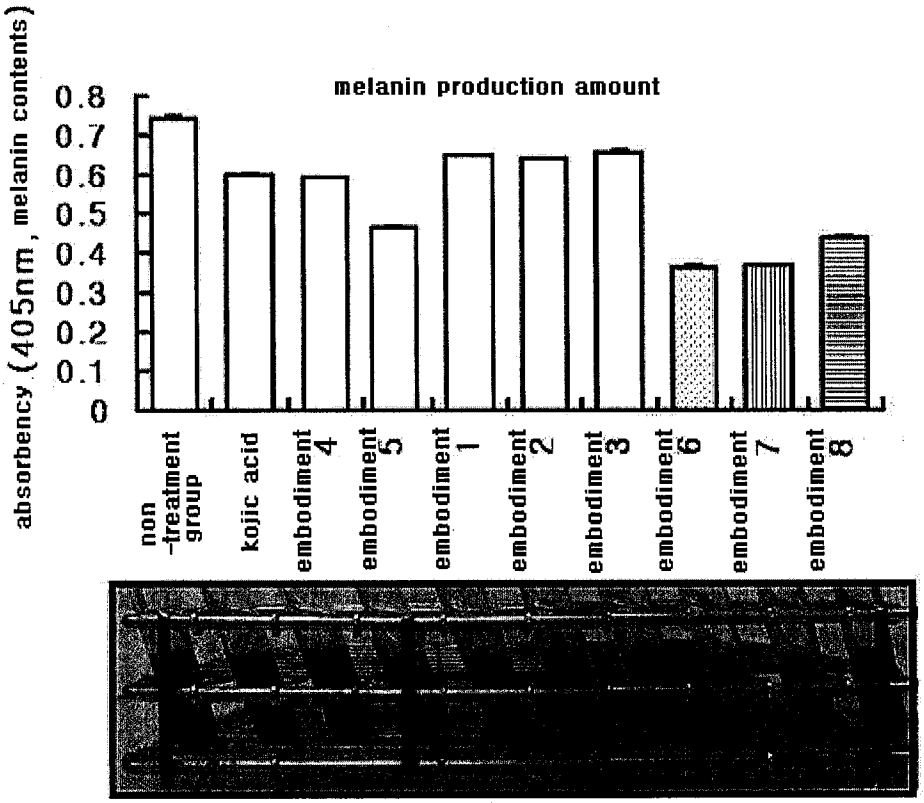
The composition according to any one of claims 1 to 5, wherein the composition is a cosmetic, pharmaceutical or health functional food composition.

【DRAWINGS】

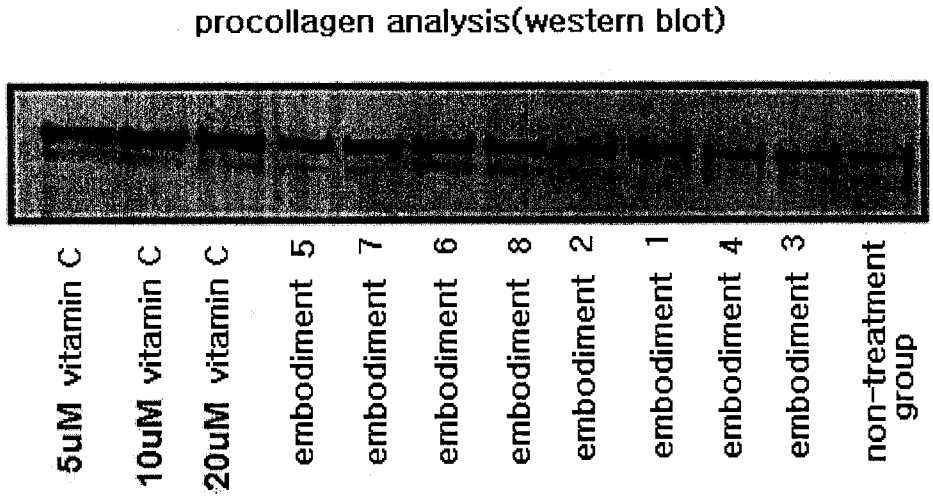
【Figure 1】



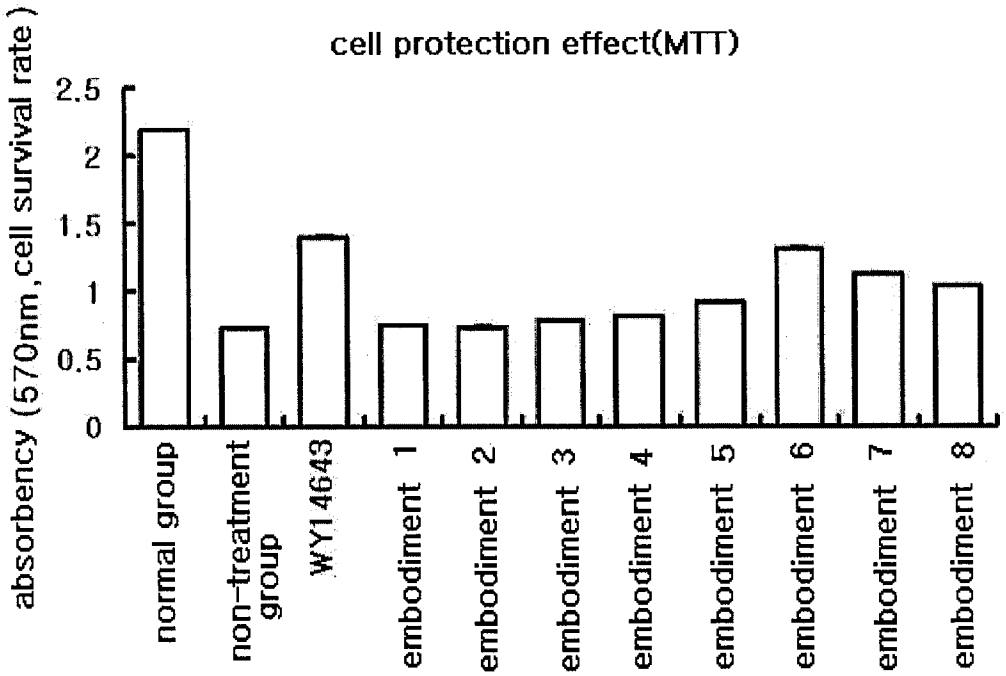
【Figure 2】



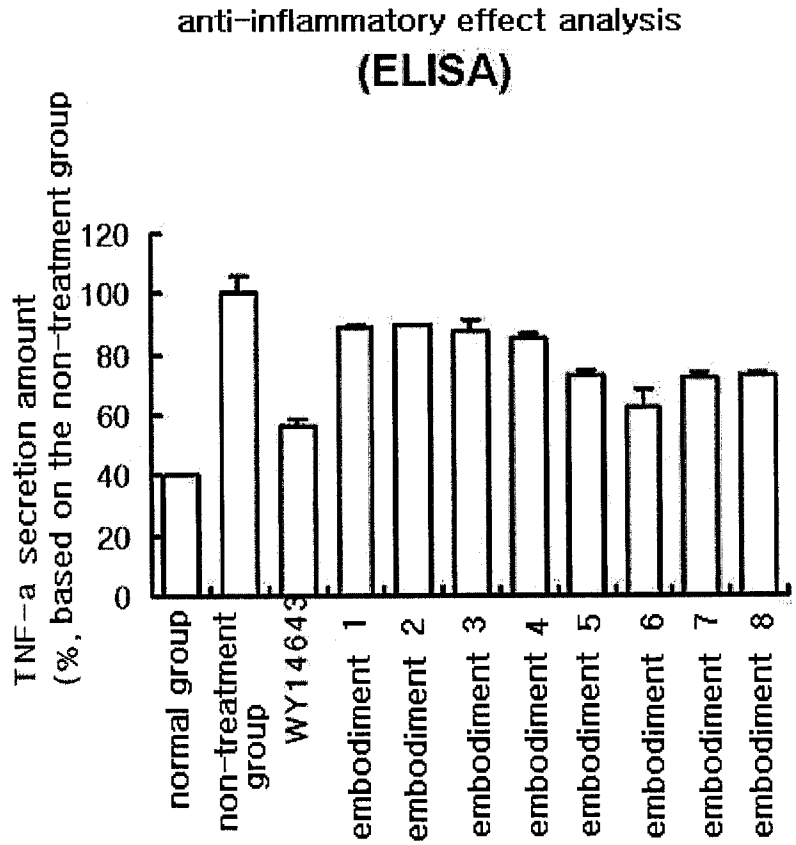
【Figure 3】



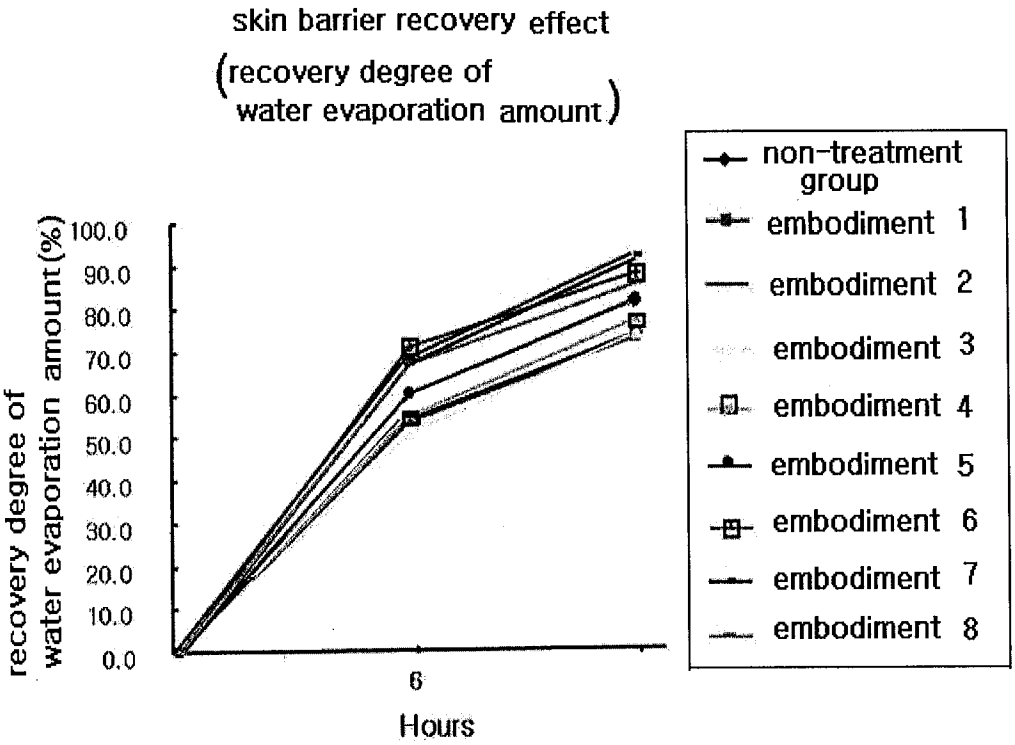
【Figure 4】



【Figure 5】



【Figure 6】


skin barrier recovery effect
(recovery degree of water evaporation amount)
recovery degree of water evaporation amount (%)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
6
Hours
non-treatment group
embodiment 1
embodiment 2
embodiment 3
embodiment 4
embodiment 5
embodiment 6
embodiment 7
embodiment 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/005678

A. CLASSIFICATION OF SUBJECT MATTER

A61K 8/97(2006.01)i, A61Q 1/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8: A61K 8/97, 7/00, 7/48, 35/74, 35/78, 38/00, A23L 1/105, A61Q 1/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

eKIPASS(KIPO internal), JPO, USPTO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	JP 3610278 B2(TKC TAKAHASHI COMPUTER KAIKEI) 12 January, 2005 see abstract, paragraph no. 0008 - 0017, claim 1 - 4	1, 2, 6, 13 3-5, 7-12
Y	JP 14037739 A(MIKIMOTO COSMETICS Ltd. and TECHNOBUL Ltd.) 6 February, 2002 see abstract, paragraph no. 0001, 0002, 0009 and 0047 - 0050	3-5, 7-12
A	JP 14037742 A(MIKIMOTO COSMETICS Ltd. and TECHNOBUL Ltd.) 6 February, 2002 see abstract, paragraph no. 0001, 0008, 0009 and 0010	1-13
A	US 20030031659 A1(SEAN FARMER) 13 February, 2002 see abstract, paragraph no. 0061, 0068 and 0138	1-13

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
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"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 APRIL 2007 (05.04.2007)	**05 APRIL 2007 (05.04.2007)**

Name and mailing address of the ISA/KR
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Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
PARK, Yeong Gwan
Telephone No. 82-42-481-8407

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2006/005678

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