Hair Nourishing Agent and Production Thereof By Fermentation of Vegetable Matter

The present invention relates to a hair nourishing agent and the production thereof. More particularly, the invention relates to a hair nourishing agent comprising fermented products obtained by applying, for example, a mixture of lipase and proteinase, or rice yeast on a substrate containing vegetable protein such as rice bran and vegetable lipid, and also to the production thereof.

The true mechanism of falling-out and growth of hair has yet to be discovered. Chief causes of falling-out of hair, however, are generally believed to be interruption in blood circulation, low metabolism, excessive male hormone, skin inflammation and allergies.

A hair comprises the part protruding out of skin and the part under skin. The hair bulb at the root of a hair comprises tissue called papillar cells. Numerous capillaries surround a hair bulb and papillar cells take in nutrition from blood running in the

capillaries, resulting in repeating cell divisions and growth of the hair.

However, if blood is not sufficiently supplied into the cells, the cell divisional activity slows down, resulting in blockage to growth of the hair. Accordingly, it is often claimed indispensable for the growth of hair to promote blood circulation in the skin and activate the functions of the cells.

A hair follicle which surrounds a hair possesses an enzyme called 5α-reductase, which gets converted into 5α-dihydrotestosteron by combining with male hormone, which is then transported via blood vessels to papillar cells, slowing down the activity of adenylcyclase thereof and leading to slowing down of the cell divisions. As a result, the hair follicle gets gradually astrophied, and the hair becomes downy, leading to a generation of baldness.

As mentioned above as examples, there are a number of views on the mechanism of falling-out and growth of hair, though, none of them are established ones. Presently there are a great many types of hair growth products distributed on the market, claiming they contain substances which the distributors believe are effective according to their understanding of the mechanism. For instance, for promoting blood circulation in head skin and/or activating papillar cells. However, a hair agent which is truly effective on hair has yet to appear in the market.

Under such circumstances, the inventor of the present invention has continued a long and strenuous research and finally come up with the present invention of a hair nourishing agent which works excellently on hair, comprising fermented products obtained by applying, for example, a mixture of lipase and proteinase, or rice yeast on a substrate containing vegetable protein such as rice bran and vegetable lipid.

In accordance with the present invention there is provided a hair nourishing agent comprising filtered products of fermention, obtained by applying enzymes on a substrate containing vegetable protein and vegetable lipid, wherein said enzymes are lipase and proteinase. Optionally said lipase and proteinase may be produced by rice yeast. Besides lipase and proteinase; rice yeast produce a variety of other enzymes which may also be utilised.

Said substrate may comprise anything that contains vegetable protein and vegetable lipid. The enzymes for the fermentation must possess the ability to decompose the vegetable protein and the vegetable lipid in the substrate. Other types of enzyme such as amylase may be additionally used.

The fermented products go through a filtering process and hair nourishing agents of the present invention are thus obtained. They are harmless to human body and show excellent effects in preventing

hair from falling out, as well as in growing hair.

A substrate for producing a hair nourishing agent of the present invention may be anything that contains vegetable protein and vegetable lipid. It may be, for example, grain products such as rice bran, sake lees, wheat embryo buds, fusuma bran, whole buckwheat or whole barnyard millet, bean products such as soybeans, bean-curd refuse, soybean milk, adzuki beans, soybean flour or coffee grounds, or others such as yams, taros, tea grounds, honey, sesame, peanuts, lotus sees, ashitaba, beefsteak plants, wakame seaweed or sea tangles. Rice bran, sake lees, fusuma bran, coffee grounds, wheat embryo buds, sesame, bean-cured refuse, soybeans and adzuki beans are especially preferred as they contain well-balanced vegetable protein and vegetable lipid.

Lipase to be utilised for the present invention may be spermato lipase used in the acidic range which is contained in a variety of mold, yeast, microorganisms, humors, or enzyme taken from internal organs. More particularly, pancreas lipase, liver lipase, tubercle bacteria lipase, FIB lipase and hima lipase may be used.

Proteinase to be utilised for the present invention is preferred to be acidic at the most suitable pH. In this regard, pepsin, trypsin or chymotrypsin, not possessing any particular active base except an amino acid residue of the enzyme itself

and not requiring any particular reagent for enzymatic activities, may be utilised. Most enzymes (or cathepsins) found in vegetable and animal cells such as papain or ficin, which can be activated by a reducing agent such as hydrocyanate, ascorbic acid, cysteine or glutathione, may also be used. More particularly, chymotrypsin, trypsin, pepsin, carboxy peptidase, cathepsin A, cathepsin B, cathepsin C', cathepsin III, cathepsin IV, kidney acylase I, kidney acylase II, leucine amino peptidase, aminotripeptidase, glycilglycine dipectitase, prolitase, prolinase, plasmin, thrompin, papain, ficin, streptococcus proteinase, Cl, historyticum proteinase or peptidase can be utilised.

Fermented products can be obtained by applying said enzymes on said substrate. Analyses were made upon the fermented products by HPLC and the following results were obtained. Said analyses were made using a detector (LC-9A, Shimadzu Seisakusho, Co., Ltd.) and columns (3 m in length). As an elution layer, acetonitrile solvent was used and the column temperature was kept about 30°C and the flow speed was about 10 ml/min.

The fermented products were found to contain such fatty acids as myristic acid, palmitic acid, stearic acid, arachic acid, behenic acid, lignoceric acid, dodecenic acid, tetradecenic acid, tetradecadienic acid, pentadecenic acid, hexadecenic acid, oleic acid,

linoleic acid, linolenic acid, eicosaenic acid, eicosadienic acid, arachidonic acid, eicosatrienic acid, dococenic acid, and docosahexanic acid. The fermented products were found to also contain such amino acids as leucine, isoleucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine, histidine, arginine, aspartic acid, alanine, glutamic acid, glycine, proline and serine.

As is clear from the results of the analyses, the fermented products obtained according to the present invention contain a variety of amino acids and fatty acids. Amino acids are believed to function to give nutrition to papillar cells and fatty acids are believed to function to activate and expand capillary blood vessels, to supply hair with moisture to give a shining appearance, as well as to inactivate 5α-reductase contained in hair follicles; to prevent softening of hair. The components singly or in combination are believed to promote growth of hair as well. It should be noted that the fermented products also contain amino acids which include sulphur and unsaturated fatty acids, both which are believed to possess excellent effects on the growth of hair.

The products obtained by fermentation by applying rice yeast on the substrate according to the present invention were found to contain in small quantities vitamin A, vitamin B, vitamin B_2, vitamin B_6, vitamin

B_{12}, vitamin C, vitamin E, copper, iron, calcium, phosphorus, purine, deoxyribonucleic acid, ribonuleic acid, adenine, guanine, uracil, cytosine and xanthine in addition to the components confirmed in said fermented products obtained using a mixture of lipase and proteinase, since a variety of enzymes besides lipase and proteinase, such as amylase, glutaminase, cellulase and pectinase are produced in the rice yeast, decomposing not only the vegetable protein and vegetable lipid but also other components in the substrate. It is believed that such other components also help promote hair growth by synergism with said amino acids and fatty acids.

In order to preserve the hair nourishing agents of the present invention, they may be refrigerated or boiled. There is no functional change or degradation of the effects of the hair nourishing agents, due to the refrigeration or boiling.

Also provided is a method for producing a hair nourishing agent of the present invention comprising the steps of applying proteinase and lipase on a substrate containing vegetable protein and vegetable lipid, fermenting said substrate and filtering the obtained fermented products.

The fermentation is performed under aqueous conditions. Lipase and proteinase, or rice yeast which produces additional enzyme such as amylase, is used as enzyme. The lipase and the proteinase may be

separately applied or applied together in a form of a mixture. Said mixture can contain other enzymes such as amylase in order to promote decompositional function and to decompose other components in the substrate. It is preferred to apply 0.1 W % of each enzyme in said mixture with respect to the substrate. In the case of rice yeast (as the decompositional function of said yeast is weak compared with that of lipase or proteinase), it is better to add 1 W % of rice yeast with respect to the substrate.

The fermentation temperature is preferred to be room temperature or 35°C to 45°C, and as a buffer solution, pH 6 to 11 of hydrochloric acid-collidine, primary potassium phosphate-secondary potassium phosphate, hydrochloric acid-sodium Veronal, hydrochloric acid-trisaminomethane, hydrochloric acid-borax, boric acid-sodium carborate, hydrochloric acid-aminomethyl propane diol, ammonium choloride-ammonia, glycine-sodium hydroxide, boric acid-sodium hydroxide, hydrochloric acid-sodium dimethylglycinate, sodium bicarbonate-sodium carbonate, borax-sodium hydroxide, borax-sodium carbonate, hydrochloric acid-sodium carbonate, secondary sodium phosphate-sodium hydroxide or the like, can be used. Any combination or combinations of said substances may also be used in the form of a mixture.

The reaction time for the fermentation is preferably 5 to 10 hours under agitation. The

completion of the reaction can be determined, e.g. for lipase, by giving a predetermined coloring matter as an indicator and checking the amount of the extricated fatty acids which are produced by hydrolysis of ester, or by electrical titration using alkali. When the above is not easily performed, use colorimetry for the alcohol extricated in nydrolysis. For proteinase, ninhydrine colorimetry may be used, which is colorimetry of amino acids by the bluish matter produced as ninhydrine is heated. When it is not easily performed, formol titration of S ø r e n s e n is possible. This method is a utilization of the phenomenon of divergence of the titration curve for α-amino radical or imino radical under the existence of formaldehyde. For sterilization, autoclave sterilization is preferred.

A hair nourishing agent of the present invention is obtained by adding water or alcohol to the fermented products after having filtered said fermented products. The hair nourishing agent thus obtained can be used as it is, or can be mixed in hair lotion, hair cream, hair liquid, hair tonic, pomade, shampoo or rinse.

It is possible to mix in the hair nourishing agent of the present invention some components which are often used in the conventional hair growers such as blood facilitator, metabolism promoter, anti-male hormone, anti-inflammatory, anti-allergy, moistener,

anti-bacteria, and/or refrigerant. They are, for example, tocopherol acetate, capronium chloride, carbonic acid gas, hinokitiol, pantonyl ethylether benzoinate, glyceride pentadecanate, vitamin H, ethynil estradiol, glythylricinic acid, diphenylhydramin chloride, hyaluronic acid, Minisasanishiki extract, mononitroguaiacol, Togarashi tincture, Shoukyo tincture, hydrocortisone, diphenic hydramin chloride, D-pantothenyl alcohol, salicylic acid, lactic acid, sodium lactate, menthol and the like.

The present invention will now be further described with reference to the following examples.

Example 1:

Rice bran was used as a substrate. Warm water, 1,000 ml, was added to said rice bran, 500 g, and both were mixed well. The mixture was then put in an adequate container such as a glass container or a plastic container, and rice yeast, 5 g, was added to said mixture. Sodium bicarbonate, 3 g, was also added as a buffer to control pH. The container was heated by a heating means to about 45°C and kept constant at the temperature. The mixture was left for a whole day with occasional agitations. The mixture turned pasty and said pasty liquid in the container was filtered with a cloth to obtain a semitransparent hair

nourishing liquid of about 1,000 ml.

Example 2:

Soybean grounds were used as a substrate. Water, 600 ml, was added to said soybean grounds, 200 g. Animal internal organs, 100 g, were prepared and water, 200 ml, was added to said animal internal organs, which were then ground with a glass homogenizer to prepare an homogenate. Said homogenate was centrifuged at 1,500 rpm. The supernatant fluid, 100 ml, was then taken and added to the substrate. Sodium carbonate-boric acid was also added at this time as a buffer to adjust pH to 10.0. The mixture was left at about 40°C. The enzyme activities were observed by using a kletto photoelectric colorimeter (No. 540 filter) and the end of the enzyme activity was observed after about 8 hours.

Sodium carbonate-boric acid of 0.1 M (pH 10.0), 40 ml, was added as a buffer to the fermented liquid obtained as such. Then chymotripsin was added to 0.1 M. The whole mixture was left at about 40°C. The confirmation of the completion of the fermentation was made by measuring (by the Conway's method) the amount of ammonia in 0.2 ml samples, taken from said whole mixture. After about 10 hours, generation of ammonia stopped and the fermentation was over. The fermented liquid was then filtered and washed with water. A

hair nourishing liquid was thus obtained.

Example 3:

Adzuki grounds were used as a substrate. Water, 500 ml, was added to said adzuki grounds, 200 g, and the mixture was suspended. Separately, lipase obtained from FIB mycelium was floated in distilled water of 100 ml, which was then agitated for about 2 minutes with a Knapp-March Blender and left at room temperature for about 20 minutes. The top liquid obtained after centrifuging the blend at 2,000 rpm for 30 minutes was added to said mixture of adzuki grounds and water. A Britton-Robinson buffer (phosphoric acid, acetic acid, boric acid, caustic soda) was added to adjust pH to 9.0. The mixture was kept shaken for about 8 hours at about 35°C. The fermentation process was observed by titration using 0.05 M alcoholic hydrochloric acid.

Separately, papain (available on the market), was dissolved in water and hydrogen sulfide was sent into the mixture for several hours. Then alcohol was added to said mixture to settle the papain. Water was added to the refined papain (papain 1g : water 100ml) and then a Britton-Robinson buffer was also added to adjust pH to 9.0, which was added to said fermentation liquid including said substrate. The whole fermentation liquid was left at about 40°C. The end

of the fermentation was confirmed by titration using alcohol of 0.2 ml samples taken from said whole fermentation liquid. The fermentation was over after about 12 hours. The fermented liquid was filtered and a hair nourishing liquid was thus obtained.

Example 4:

Bean-curd refuse was used as a substrate. Water, 500 ml, was added to said bean-curd refuse, 100 g, and the mixture was kept at about 50°C. Separtately, kidney lipase (available on the market) was dissolved (1 %) in distilled water. Said distilled water containing kidney lipase (5 ml) was added to said mixture.

Phosphoric acid (0.6 M, pH 7.0), 5 ml was also added as a buffer and the whole mixture was kept for about 10 hours at about 40°C with occasional agitations. The end of the fermentation was confirmed by observing the stoppage of generation of carbonic acid gas using a Warburg's manometer.

Separately, pepsine (available on the market, Cudahy, 1/10,000 USP soluble pepsin), 10 g, was dissolved in a 20 % Ethanol solution, 10 ml, which was added to said fermentation liquid.

Ammonium chloride was also added to adjust pH to 7.5. This liquid was left for about 20 hours at about 35°C, then it was filtered by means of filter cell

(5g). The pH was 7.0 at this time. The filtered liquid was left at about 37°C. The end of fermentation was confirmed by putting 0.5 ml samples taken from the filtered liquid from outside of a Conway's apparatus and mixing each sample with the potassium carbonate, 1 ml, which had been placed in said apparatus. The end of the fermentation was known by means of observing the changes in amount of volatilizing ammonia by titration. The end was confirmed after about 10 hours. The liquid thus obtained was filtered in an oridinary manner and a hair nourishing liquid was obtained.

The hair nourishing liquids obtained according to Examples 1 to 4 were diluted with ethanol and the blends were applied on men and mice once in the morning and once in the evening everyday. After 30 days of application, both cases showed an outstanding effect of hair growth. Especially in the case of the mice, where the hair-growing effect was enormous.

What is Claimed is:

1. A hair nourishing agent comprising filtered products of fermentation, obtained by applying a mixture of enzymes on a substrate comprising vegetable protein and vegetable lipid, wherein said enzymes are proteinase and lipase.

2. The hair nourishing agent according to claim 1, wherein said enzymes are enzymes produced by rice yeast.

3. A method for producing a hair nourishing agent comprising the steps of applying proteinase and lipase on a substrate comprising vegetable protein and vegetable lipid, fermenting said substrate, and filtering the obtained fermented products.

4. The method for producing a hair nourishing agent according to claim 3, wherein said proteinase and lipase are produced by rice yeast.

5. The method for producing a hair nourishing agent according to claims 3 or 4, further comprising a step of adding as a buffer to said substrate one or any combination or combinations of the

chemicals of pH6 to 11 selected from the following:

hydrochloric acid-collidine, primary potassium phosphate-secondary potassium phosphate, hydrochloric acid-sodium Veronal, hydrochloric acid-trisaminomethane, hydrochloric acid-borax, boric acid-sodium carbonate, hydrochloric acid-aminomethyl propane diol, ammonium chloride-ammonia, glycine-sodium hydroxide, boric acid-sodium hydroxide, hydrochloric acid-sodium dimethylglycinate, sodium bicarbonate-sodium carbonate, borax-sodium hydroxide, borax-sodium carbonate, hydrochloric acid-sodium carbonate, secondary potassium phosphate-sodium hydroxide and the like.

6. Use of an agent as defined in claims 1 or 2 for nourishing hair.

7. Use of an agent as defined in claims 1 or 2 for the manufacture of a medicament for nourishing hair.

8. A hair nourishing agent substantially as disclosed in any of Examples 1 to 4; or use thereof.

Hair Nourishing Agent and Production Thereof

Abstract of the Disclosure

The hair nourishing agent according to the present invention is a product of fermentation by enzyme applied on a substrate comprising vegetable protein and vegetable lipid. The hair nourishing agent obtained by filtering the fermented products is safe to use as only harmless substances compose the agent and shows excellent effects for preventing hair from falling out as well as for growing hair.