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10 **Cosmetic or dermatological preparation**
 containing proteins for skin-whitening

 The invention relates to a cosmetic or dermatological
preparation containing proteins or enzymes and which can be used
15 for whitening the skin.

 From WO98/58628, kojic acid, ascorbic acid, bearberry
extract, mulberry extract, licorice extract, arbutin and
mixtures thereof are known for skin-whitening, which agents are
used in the form of a cosmetic plaster in WO02/36090.

20 EP 909161 describes substituted diphenols in a water-in-
silicone gel composition as skin-whitening agents.

 US 5 609 875 discloses a combination of Glycyrrhiza glabra
with α - or β -hydroxy acids, keto amides or salts thereof for
skin whitening.

25 US 6 416 756 relates to enzymes which can be modified by
means of polymer molecules such as PEG, which stabilize them and
reduce their sensitivity potential.

 The object of the invention is to provide new cosmetic or
dermatological products for whitening human skin. Another object
30 is to influence the skin-tanning process.

 According to the invention, the cosmetic or dermatological
preparation comprises an effective amount of modified, copper-
binding proteins or enzymes whose prosthetic group copper has

been extracted by a prior exchange reaction, or of such modified, copper-binding proteins which have been prepared without copper by recombinant methods.

The preparation preferably contains such modified, i.e. copper-free, copper-binding proteins which are selected from the group consisting of oxidoreductase, auracyanin, azurin, phytocyanin, plastocyanin, rusticyanin and mixtures thereof in their modified, copper-free form. Particularly preferred are those from the group of the so-called blue copper proteins such as auracyanin, azurin, phytocyanin, rusticyanin, ascorbate oxidase, ceruloplasmin, laccase, nitrite reductase and mixtures thereof.

Other preferred modified, copper-binding enzymes comprise Cu,Zn superoxide dismutase, dioxygenase, monooxygenase, amine oxidase, diamine oxidase, ascorbate oxidase, ceruloplasmin, cytochrome c oxidase, galactose oxidase, lysyl oxidase, catechol oxidase, N₂O reductase, haemocyanin, tyrosinase, ubiquinone oxidase and mixtures thereof in their modified, copper-free form.

Said modified, substantially copper-free copper proteins make up 0.001 to 1% by weight, preferably 0.005 to 0.01% by weight, relative to the preparation's total weight.

It has been found that these proteins, which are generally referred to as "copper proteins" or "copper-binding proteins" and which are blue or non-blue complexes in their copper-containing form, i.e. when they contain Cu ions in their prosthetic group, can be made copper-free by means of a chelation reaction. They can also be produced by recombinant methods using a copper-free molecule, i.e. a molecule without a Cu ion. Surprisingly, such modified copper proteins are able to effectively extract copper from the enzyme tyrosinase, which is also a copper protein, thus interrupting the natural melanin synthesis in which the non-essential amino acid tyrosine is involved.

The chelation reaction, which can e.g. be used to extract copper from the copper proteins, is performed in a known manner using a cation exchanger or chelating agents such as polyoxycarboxylic acids, polyamines, ethylenediamine tetraacetic acid and the Na or K salts thereof, nitrilotriacetic acid, etc. Such reactions are known to those skilled in the art.

In contrast to other known skin-whiteners, such as e.g. hydroquinone and derivatives thereof, azelaic acid, kojic acid or arbutin, which also inhibit the melanin synthesis, there are no side effects and the modified copper proteins can be obtained in a relatively easy manner by simply chelating commercially available protein or enzyme products. The latter can be obtained from plant extracts or isolated from bacterial or yeast cultures and are subsequently treated with a chelating agent.

The preparation can, in addition, contain skin-whitening agents such as bearberry leave extract, mustard seed extract obtained from white mustard, white willow bark extract, hibiscus flower extract and mixtures thereof. Said extracts can reduce the pigmentation of the skin. For example, bearberry extract contains active agents which inhibit the production of tyrosinase. Mustard seed extract contains campherol which prevents the transfer of melanosomes into the keratinocytes. Extracts from the bark of white willows contain salicosides which increase the speed at which skin cells regenerate, thus accelerating skin-whitening effects. Hibiscus flower extracts are rich in organic acids which have a keratolytic effect and speed up the removal of pigmented dermal layers.

Further, it is advantageous to add 0.1 to 10% by weight of a ground, pure white porcelain whose particle size ranges between 0.1 and 100 μm to the preparation, whereby a particular strong whitening effect is achieved if combined with said modified, copper-binding proteins.

Alternatively or additionally, 0.1 to 15% by weight of reflective pigments can also be added, selected from the group consisting of ground glass particles, TiO_2 , ZnO , mother of pearl, pearlescent pigments and mixtures thereof.

5 Another advantageous embodiment of the invention consists in that ground white pearls whose particle size ranges between 0.1 and 100 μm are added to the copper-binding proteins. A combination of pearl proteins (conchagens) and copper-binding proteins brings about an additional, surprisingly intense
10 whitening effect and makes the skin shimmer. In addition, said conchagens enhance the anchoring of glycoproteins in the skin and counteract the destabilization of the skin collagen's fibre network, thus achieving an anti-ageing effect. Ground pearls can be added in an amount ranging between 0.1 and 8% by weight.

15 Another advantageous embodiment of the invention consists in that a root extract from *Glycyrrhiza glabra* or a complex consisting of a root extract from *Glycyrrhiza glabra*, a root extract from *Morus alba*, a root extract from *Scutellaria baicalensis*, an extract from *Saxifraga stolonifera*, a grapefruit
20 extract and disodium ethylenediamine tetraacetic acid (EDTA) is added to the copper-binding proteins. In this way, the skin-whitening effect is intensified further. *Glycyrrhiza glabra* or the aforesaid complex can be added in an amount ranging between 0.1 and 6% by weight.

25 Further, it is advantageous that suitable water- and/or oil-soluble UVA or UVB filters or both be added to the inventive compositions. Advantageous oil-soluble UVB filters include derivatives of 4-aminobenzoic acid such as 4-(dimethylamino)-benzoic acid-(2-ethylhexyl) ester; esters of cinnamic acid such
30 as 4-methoxycinnamic acid-(2-ethylhexyl) ester; benzophenone derivatives such as 2-hydroxy-4-methoxybenzophenone; derivatives of 3-benzylidene camphor such as 3-benzylidene camphor.

Preferred oil-soluble UV filters are Benzophenone-3, Butyl Methoxybenzoylmethane, Octyl Methoxycinnamate, Octyl Salicylate, 4-Methylbenzylidene Camphor, Homosalate and Octyl Dimethyl PABA.

Water-soluble UVB filters include e.g. sulphonic acid derivatives of benzophenone or of 3-benzylidene camphor, or salts such as the Na or K salt of 2-phenylbenzimidazol-5-sulphonic acid.

UVA filters include derivatives of dibenzoylmethane such as 1-phenyl-4-(4'-isopropylphenyl)propane-1,3-dione.

It is preferred that the organic UVA and/or UVB filters added make up 1 to 20% by weight, advantageously 1 to 12% by weight, particularly 1 to 3% by weight, relative to the total weight of the overall composition.

The inventive preparation further contains cosmetic auxiliaries and carriers as they are commonly used in such preparations, e.g. water, preservative, colourants, white pigments, thickeners, fragrances, alcohols, polyols, esters, electrolytes, gel-forming agents, polar and non-polar oils, polymers, copolymers, emulsifiers, waxes, stabilizers.

The inventive preparation can contain further active agents. These include e.g. free radical scavengers, moisturizers, vitamins, plant-based active agents, polymers, antioxidants, anti-inflammatory natural active agents, asymmetric lamellar aggregates loaded with oxygen according to W094/00109; products obtained by the gentle ultrasonic decomposition of yeasts or vegetable matter according to W094/13783; kaolin; as well as kaolin which has been modified with SiO₂ according to W094/17588.

The aforescribed preparation can be provided as an emulsion or a gel. Known suitable gel-forming agents include carbomer, xanthan gum, carrageenan, acacia gum, guar gum, agar-agar, alginates and tyloses, carboxymethyl cellulose, hydroxyethyl cellulose, quaternised cellulose, quaternised guar,

certain polyacrylates, polyvinyl alcohol, polyvinylpyrrolidone, montmorillonite.

The oils used for the invention can be common cosmetic oils such as mineral oil; hydrogenated polyisobutene; synthetic
5 squalane or squalane made from natural products such as Synthesqual[®], Cosbiol[®]; cosmetic esters or ethers which can be branched or unbranched, saturated or unsaturated; vegetable oils; or mixtures of two or more thereof.

Particularly suitable oils are e.g. silicone oils, mineral
10 oils, Hydrogenated Polyisobutene, Polyisoprene, Squalane, Tri-decyl Trimellitate, Trimethylpropane Triisostearate, Isodecyl Citrate, Neopentyl Glycol Diheptanoate, PPG-15 Stearyl Ether as well as vegetable oil such as calendula oil, jojoba oil, avocado oil, macadamia nut oil, castor oil, cocoa butter, coconut oil,
15 maize oil, cotton seed oil, olive oil, palm kernel oil, rapeseed oil, safflower oil, sesame seed oil, soybean oil, sunflower seed oil, wheatgerm oil, grapeseed oil, kukui nut oil, thistle oil and mixtures thereof.

Depending upon the oils used, the cosmetic characteristics of
20 the solid composition, e.g. degree of transparency, softness, hardness spreading effect, vary.

Suitable moisturizers include e.g. glycerine, butylene glycol, propylene glycol and mixtures thereof.

A number of compounds can normally be used as emollient, e.g.
25 stearyl alcohol, glyceryl monoricinoleate, glyceryl monostearate, propane-1,2-diol, butane-1,3-diol, cetyl alcohol, isopropyl isostearate, stearic acid, isobutyl palmitate, isopropyl myristate, isopropyl palmitate, oleyl alcohol, isopropyl laurate, decyl oleate, octadecan-2-ol, isocetyl alcohol, cetyl palmitate,
30 silicone oils such as dimethylpolysiloxane, polyethylene glycol, lanolin, cocoa butter, vegetable oils such as maize oil, cotton seed oil, olive oil, mineral oils, butyl myristate, palmitic acid, etc.

The invention will hereinafter be explained in more detail by means of examples. All quantities are in % by weight unless indicated otherwise.

Example 1 **Whitening and shimmering face and body lotion SPF 15**

5 **Phase A**

Water	q.s. ad 100
Glycerine	5
Timeron (mica, TiO ₂ , iron oxides)	1

Phase B

Isopropyl Palmitate	1
Tri-C12-13 Alkyl Citrate	6
C12-15 Alkyl Benzoate	1.2
Butyl Methoxydibenzoylmethane	3
Ethylhexyl Methoxycinnamate	7.5

Phase C

RPF active formulation*	0.5
Ascorbate oxidase (modified, i.e. Cu-free)	0.1
Glycyrrhiza glabra (licorice)	0.2
Ground white pearls 0.5-30 µm	0.5
Hibiscus flower extract	0.1
Perfume oil	0.05
Preservative	0.1

* containing Quebracho blanco (2%), silkworm extract (1%), dry gel (1%), phospholipids (7%) and water (89%); prepared as described in WO99/66881, Example 1.

Phases A and B are prepared separately by mixing the respective ingredients at approx. 75° C, combined with one another and homogenized. The mixture is cooled down to approx. 40° C and Phase C is stirred in, which has been prepared at said temperature by mixing the individual ingredients.

The modified enzyme is produced at approx. 30° C using EDTA.

Example 2 **Whitening and shimmering face cream SPF 20**

Phase A

Water	q.s. ad 100
EDTA	0.1
Propylene Glycol	3
Crosspolymer	0.8
Timeron (mica, TiO ₂ , iron oxides)	1.5

Phase B

Benzophenone-3	5
Isoamyl p-Methoxycinnamate	5
Butyl Methoxydibenzoylmethane	3
Ethylhexyl Methoxycinnamate	7

Phase C

RPF active formulation*	0.8
Yeast extract**	0.1
Azurin (modified, i.e. Cu-free)	0.1
Glycyrrhiza glabra (licorice)	0.5
Bearberry leave extract	0.1
White willow bark extract	0.3
Ground white pearls 0.5-30 µm	1.0
Perfume oil	0.1
Preservative	0.1

5 * containing Quebracho blanco (2%), silkworm extract (1%), dry gel (1%), phospholipids (7%) and water (89%); prepared as described in WO99/66881, Example 1.

** yeast extract according to DE 4241154C1, Example 1

10 Phases A and B are prepared separately by mixing the respective ingredients at approx. 75° C, combined with one another and homogenized. The mixture is cooled down to approx. 40° C and Phase C is stirred in, which has been prepared at said temperature by mixing the individual ingredients.

The modified azurin is produced at approx. 28° C using nitrilotriacetic acid.

15 Example 3 **Whitening and shimmering face cream SPF 20 II**

Phase A

Water	q.s. ad 100
EDTA	0.1
Propylene Glycol	3
Crosspolymer	0.8
Timeron (mica, TiO ₂ , iron oxides)	1.5

Phase B

Benzophenone-3	5
Isoamyl p-Methoxycinnamate	5
Butyl Methoxydibenzoylmethane	3
Ethylhexyl Methoxycinnamate	7

Phase C

Active formulation*	0.8
Yeast extract**	0.1
Laccase (modified, i.e. Cu-free)	0.1
Complex consisting of Glycyrrhiza glabra (licorice), Morus alba, Scutellaria baicalensis, Saxifraga stolonifera, grapefruit extract, EDTA	1.0
Ground white pearls 0.5-30 µm	1.0
Ground porcelain 0.1-20 µm	0.8
Perfume oil	0.1
Preservative	0.1

5 * containing Quebracho blanco (2%), silkworm extract (1%), dry gel (1%), phospholipids (7%) and water (89%); prepared as described in WO99/66881, Example 1.

** yeast extract according to DE 4241154C1, Example 1

Processing was done as in Example 2.

Patent claims

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1. A cosmetic or dermatological preparation containing proteins for skin whitening, which preparation comprises an effective amount of modified, i.e. copper-free, copper-binding proteins or enzymes whose prosthetic group copper has been
10 extracted by a prior exchange reaction, or which were produced without copper by recombinant methods.

2. A preparation according to claim 1, wherein said preparation contains the modified, i.e. substantially copper-free, copper-binding proteins selected from the group of the
15 blue copper-binding proteins, which group consists of auracyanin, azurin, phytocyanin, plastocyanin, rusticyanin, ascorbate oxidase, ceruloplasmin, laccase, nitrite reductase and mixtures thereof.

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3. A preparation according to claim 1, wherein the copper-binding proteins in their reduced, copper-free form are selected from the group consisting of Cu,Zn superoxide dismutase, dioxygenase, monooxygenase, amine oxidase, diamine oxidase,
25 cytochrome c oxidase, galactose oxidase, lysyl oxidase, catechol oxidase, N₂O reductase, haemocyanin, tyrosinase, ubiquinone oxidase and mixtures thereof.

4. A preparation according to claim 1, wherein the modified,
30 substantially copper-free, copper-binding proteins make up 0.001 to 1% by weight, relative to the preparation's total weight.

5. A preparation according to claim 1, wherein said preparation additionally contains skin-whitening agents selected from among bearberry leave extract, mustard seed extract obtained from white mustard, white willow bark extract, hibiscus flower extract and mixtures thereof.

6. A preparation according to claim 1, wherein said preparation contains 0.1 to 10% by weight of a ground, pure white porcelain or of ground white pearls whose particle size ranges between 0.1 and 100 μm , relative to the preparation's total weight.

7. A preparation according to claim 1, wherein said preparation contains 0.1 to 15% by weight of reflective pigments selected from the group consisting of ground glass particles, TiO_2 , ZnO , mother of pearl, pearlescent pigments and mixtures thereof.

8. A preparation according to claim 1, wherein said preparation contains 1 to 20% by weight of an organic UV filter.

9. A preparation according to claim 1, wherein said preparation contains an extract from *Glycyrrhiza glabra* in an amount ranging between 0.1 and 6% by weight, relative to the preparation's weight.

10. A preparation according to claim 1, wherein said preparation contains a complex consisting of extracts from *Glycyrrhiza glabra*, *Morus alba*, *Scutellaria baicalensis*, *Saxifraga stolonifera*, a grapefruit extract and disodium ethylenediamine tetraacetic acid, said complex making up 0.1 to 6% by weight, relative to the preparation's weight.

11. A cosmetic or dermatological preparation containing proteins for skin whitening, which preparation comprises an effective amount of modified, i.e. copper-free, copper-binding proteins or enzymes obtainable by reacting enzymes or proteins containing copper in their prosthetic group with a copper-binding chelating agent, or by ion exchange using a cation exchanger, and separation of the copper-free, modified enzymes or proteins.
- 10 12. The use of proteins or enzymes whose prosthetic group copper has been extracted by a chelation reaction or a cation exchange reaction as a skin-whitening agent in cosmetic preparations.