

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

21 September 2023 (21.09.2023)



(10) International Publication Number

WO 2023/175531 A1

(51) International Patent Classification:

A61K 8/64 (2006.01) A61Q 17/00 (2006.01)

A61K 8/9789 (2017.01) A61Q 17/04 (2006.01)

A61Q 5/00 (2006.01) A61Q 19/08 (2006.01)

(21) International Application Number:

PCT/IB2023/052524

(22) International Filing Date:

15 March 2023 (15.03.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

22382248.7 16 March 2022 (16.03.2022) EP

(71) Applicant: LUBRIZOL ADVANCED MATERIALS, INC. [US/US]; 9911 Brecksville Road, Cleveland, Ohio 44141 3247 (US).

(72) Inventors: SUBIRÓS, Ramon; Calle Isaac Peral, 17, Poligono Industrial Cami Ral, Gava, 08850 Barcelona (ES). SOLEY ASTALS, Albert; Calle Isaac Peral, 17, Poligono Industrial Cami Ral, Gava, 08850 Barcelona (ES). ALMINANA DOMÈNECH, Núria; C/Concepció Arenal 215, Baixos, 08030 Barcelona (ES). BONELL, Cristina; Calle Isaac Peral, 17, Poligono Industrial Cami Ral, Gava, 08850 Barcelona (ES). GARCIA HERNÁNDEZ, Consuelo; Calle Isaac Peral, 17, Poligono Industrial Cami Ral, Gava, 08850 Barcelona (ES). YOUSSEF, Catrin; Calle Isaac Peral, 17, Poligono Industrial Cami Ral, Gava, 08850 Barcelona (ES). MÉNDEZ, Sandra; C/Menéndez y Pelayo 28, bajo 1ª, 08917 Badalona (ES). IBARZ BLANCH, Néstor; Avda. Los Hondos 8, 6ªC, Fraga, 22520 Huesca (ES). MUGUERZA MARQUÍNEZ, Begoña; C/ Marina alta, 6, Esc.14, pta.5, 46015 Valencia (ES). BRAVO VÁZQUEZ, Francisca Isabel; C/Fernando de Baxeres, 49, Santa Marta de los Barros, 06150 Badajoz (ES).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report (Art. 21(3))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: PROTEIN HYDROLYSATES OF AZUKI BEANS AND COSMETIC USES THEREOF

(57) Abstract: Protein hydrolysate obtained from azuki beans and its use, in particular, in the cosmetic treatment and/or care of the skin and/or hair. The protein hydrolysate comprises at least one compound chosen from: (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1); (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2); (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4). A method for preparing the protein hydrolysate is also disclosed.



WO 2023/175531 A1

**TITLE****PROTEIN HYDROLYSATES OF AZUKI BEANS AND COSMETIC USES THEREOF****FIELD OF THE INVENTION**

**[001]** The invention relates to protein hydrolysates obtained from azuki beans and their use, in particular, in the cosmetic treatment and/or care of the skin and/or hair. The protein hydrolysates are useful in protecting the skin and/or hair against adverse effects of the exposome, including adverse effects arising from air pollution and/or sunlight. Further, the protein hydrolysates are useful in protecting hair against chemical and heat stress, for example, chemical and heat stress arising from personal care regimes. Methods of preparing the protein hydrolysates are also disclosed.

**BACKGROUND OF THE INVENTION**

**[002]** The totality of extrinsic factors to which an individual is exposed over a lifetime is referred to as the exposome. The appearance of skin, the appearance of hair, and aging are influenced by the combined action of intrinsic and extrinsic factors. Intrinsic factors that negatively impact skin and hair include, for example, chronological aging, a person's genetic makeup, and other biological changes that occur from within the skin. For decades, external damaging factors on the skin and hair have been ignored. However, there is growing evidence that the exposome can have an adverse effect on skin and hair appearance [Vierkötter A1, et al. Airborne particle exposure and extrinsic skin aging. J Invest Dermatol. 2010; and Paarado C et al "Environmental Stressors on Skin Aging. Mechanistic insights", Front Pharmacol. 2019; 10:759, published 9 Jul. 2019, doi:10.3389/fphar.2019.00759].

**[003]** Examples of extrinsic factors associated with the exposome include exposure to ultraviolet (UV) rays emanating from the sun, high energy visible light (violet-blue) emitted by devices such as TV/computer/smartphone screens, as well as harmful chemical agents found in airborne pollution such as smog and cigarette smoke.

**[004]** Ultraviolet (UV) radiation is one of the main external factors that has a detrimental effect on the appearance of skin and hair. Extrinsic aging has been regarded to be mainly the result of sun radiation exposure, also known as photoaging.

**[005]** Following in-depth study of the effects of pollution on health, awareness about the deleterious effects of pollution on the appearance of skin and hair has increased [Vierkötter A, et al. Airborne particle exposure and extrinsic skin aging. J Invest Dermatol. 2010]. Additionally, it is known that the adverse effects of various pollutants

on the skin and hair can be amplified due to their interaction with UV radiation [Drakaki E, et al. Air pollution and the skin. Front. Environ. Sci., 2014]. For example, it is well known that the main role of the skin is to form a barrier between the host and the environment. The skin must protect an organism from chemicals, ultraviolet light, mechanical damage, and pathogenic microorganisms. Also, the skin must provide an efficient permeability barrier that prevents the loss of water and electrolytes. [Feingold K, Elias P. The important role of lipids in the epidermis and their role in the formation and maintenance of the cutaneous barrier. Biochim Biophys Acta. 2014] Air pollutants can have major effects on the function of the skin. Alterations that disturb the skin barrier function, in either stratum corneum lipid metabolism or protein components of the corneocytes, adversely affect skin appearance. Air pollutants may induce severe interference of normal functions of lipids, DNA and/or proteins of the human skin via oxidative damage [Eleni Drakaki, Clio Dessinioti and Christina V. Antoniou. Air pollution and the skin. Front. Environ. Sci., 2014].

**[006]** Furthermore, continued anthropogenic emissions of greenhouse gases (mainly carbon dioxide) have resulted in an increase of the Earth's temperature by more than 1°C since 1880, with the majority of warming occurring in the last 45 years [National Aeronautics and Space Administration. *NASA ozone watch. 2020b [cited 2020 April 4]. Available from: [https://ozonewatch.gsfc.nasa.gov/facts/history\\_SH.html](https://ozonewatch.gsfc.nasa.gov/facts/history_SH.html)]. Therefore, although solar radiation and pollutants are two important components of the exposome, other environmental components such as greenhouse gases and temperature (that affects all biochemical reactions) are also highly relevant.*

**[007]** The above-mentioned extrinsic factors can lead to high production of reactive oxidative species (ROS). In the skin, the production of ROS can affect the skin's defenses by quickly depleting the enzymatic (glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase) and nonenzymatic (vitamin E, vitamin C, and glutathione) antioxidant capacity [Valacchi G, et al. Cutaneous responses to environmental stressors. Ann N Y Acad Sci. 2012]. In hair, oxidative stress can also have significant negative effects. Proteins are the principal components of hair shafts (65% to 95%) and protein carbonylation, which is an irreversible oxidative protein modification that can significantly damage hair. The protection of hair proteins from carbonylation due to pollutants is desirable for hair and scalp protection against urban stress. [Baraibar, Martin. Urban pollution induces irreversible carbonylation of hair proteins. Conference: IFSCC, 2018].

**[008]** In addition, skin and, in particular, hair are also subjected to other stressors from daily personal care routines. Diverse causes of extrinsic damage to the hair shaft can be roughly divided into physical and chemical causes. The human hair shaft consists of the cortex with a central axial medulla and an external cuticular layer. Chemical causes of hair damage include bleaching and hair dyeing, while physical causes include thermal treatments (such as treatments with straightening irons), friction from hair accessories and washing [Lee Y, et al. Comparison of hair shaft damage after chemical treatment in Asian, White European, and African hair. *Int J Dermatol.* 2014].

**[009]** The effects of external factors on skin and hair can be distinguished from those associated with intrinsic factors. Skin that is adversely affected by the exposome may show more symptoms of aging such as coarse wrinkles. Hair adversely affected by the exposome can look damaged and be less manageable.

**[0010]** From a preventive point of view, counteracting exposome factors (i.e. extrinsic factors) is of particular interest because extrinsic factors can be modified more easily than intrinsic factors.

**[0011]** It is desirable for the skin and/or the hair to appear aesthetically pleasing. There is a great need to find active ingredients that can protect the skin, scalp and hair from the aggressions of the exposome, e.g. the aggressions of the environment to which they may be exposed to on a daily basis, including stressors due to daily personal care routines. In particular, there is a great need to find such active ingredients that can provide such protection to the aesthetic benefit of the skin and/or the hair.

**[0012]** US20210177733 discloses a composition and method for enhancing the skin's ability to defend itself against free radical aggression caused by exposome-induced oxidative stress. The compositions comprise leaf extract of *Aristolelia chilensis*; leaf extract of *Buddleja globosa*; and leaf extract of *Ugni molinae*.

**[0013]** Zoryalys® from Greentech is a natural active ingredient "derived from several molecules: Shogaol & Gingerol (grows in sunny areas), Magnolol & Honokiol (lives in polluted areas) and Pracaxi (biomimetic lipid)" (<https://www.greentech.fr/en/zoryalys-2/>). ZORYALYS® is described as protecting the hair against protein oxidation and being active from the surface to the heart of the hair fiber allowing a full protection of hair against exposome aggressions. This active is described as maintaining hair mechanical properties and improving hair shine for a healthy and beautiful hair, by maintaining keratin integrity.

**[0014]** Peptides released from extruded adzuki bean protein through simulated gastrointestinal digestion that exhibit anti-inflammatory activity are disclosed in Zhensing

Shi et al, "Peptides released from extruded adzuki bean protein through simulated gastrointestinal digestion exhibit anti-inflammatory activity", J. Agric. Food Chem. 2021, 69, 7028-7036.

5 [0015] There remains a need for alternative plant-based active compositions that can protect the skin and/or hair against exposome-induced oxidative stress. There remains a need for alternative plant-based active compositions that can protect/maintain/improve the aesthetic properties of the skin and/or hair when exposed to the exposome.

10 [0016] The present invention sets out to meet some or all of the above-identified needs and to solve some or all of the above-identified problems.

### SUMMARY OF THE INVENTION

[0017] In a first aspect, the invention relates to a protein hydrolysate of azuki beans comprising at least one compound (i.e. 1, 2, 3 or 4 compounds) chosen from:

- 15 (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);  
(ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);  
(iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4).

The protein hydrolysate may additionally comprise the compound:

- 20 (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5).

[0018] It has been found that the protein hydrolysate of the invention is useful in alleviating or preventing adverse effects of the exposome on the skin and/or hair. Adverse effects to the skin and/or hair include those arising from air pollution and/or sunlight. Adverse effects to the hair also include those due to chemical treatment such as dyeing and physical treatment such as from styling aids, e.g. hair dryers or straightening irons. Adverse effects to hair can manifest themselves in the form of hair that: is damaged; is less manageable; has more split ends; has reduced fiber alignment; has increased frizz; and/or is less hydrated. Adverse effects to skin can manifest themselves in the form of a reduction in barrier function; a dull appearance of the skin; a loss of brightness, luminosity and/or glossiness of the skin; a loss in homogeneity in the texture of the skin; an increase in roughness of the skin; the appearance of dark spots; and/or erythema (skin redness).

25  
30

[0019] In another aspect, the invention relates to a compound chosen from:

- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);  
(ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);

- (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);
- (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);
- (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5), and

5 stereoisomers, and cosmetically acceptable salts thereof. Surprisingly, it has been found that these compounds exhibit excellent antioxidant activity. Without wishing to be bound by theory, it is believed that this excellent antioxidant activity of these compounds contribute to the ability of the protein hydrolysates to perform as described herein.

**[0020]** In one aspect, the invention relates to combination of the protein hydrolysate according to the first aspect of the invention and a filtrate obtained by the fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc*. It has been found that this combination results in a synergistic effect with respect to antioxidant activity. The invention also extends to the combination of a compound chosen from:

- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
- (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
- 15 (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);
- (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);
- (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5); and

stereoisomers and cosmetically acceptable salts thereof and a filtrate obtained by the fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc*.

20 **[0021]** In one aspect, the invention relates a process for preparing a protein hydrolysate of azuki beans, wherein the hydrolysate is obtained by:

- a) providing a suspension of ground azuki beans in water;
- b) treating the suspension with alkali so as to solubilize proteins in the ground azuki beans;
- 25 c) enzymatically treating the suspension obtained in b) with a serine endoprotease;
- d) deactivating the serine endoprotease in the suspension obtained in c) to form a crude hydrolysate;
- e) recovering a supernatant from the the crude hydrolysate by solid/liquid separation; and
- 30 f) removing components from the supernatant having a molecular weight of greater than 3 KDa.

**[0022]** The process is useful in preparing protein hydrolysates according to the first aspect of the invention. The invention extends to protein hydrolysates obtained by the process of preparing a protein hydrolysate of azuki beans described herein.

**[0023]** In one aspect the invention relates to a personal care or cosmetic composition comprising at least one cosmetically acceptable excipient or ingredient and a protein hydrolysate of azuki beans according to the first aspect of the invention, or a compound chosen from:

- 5 (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);  
(ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);  
(iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);  
(v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5), and  
10 stereoisomers, and cosmetically acceptable salts thereof, or a combination of said compound and a filtrate obtained by the fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc*.

**[0024]** In one aspect, the invention relates to the use of a protein hydrolysate of azuki beans according to the first aspect of the invention, or a compound chosen from:

- 15 (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);  
(ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);  
(iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);  
(v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5), and  
20 stereoisomers, and cosmetically acceptable salts thereof, or a combination of said compound and a filtrate obtained by the fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc* for the treatment and/or care of the skin and/or hair. In particular, the treatment and/or care of the skin and/or hair is cosmetic and non-therapeutic.

25 **[0025]** In one aspect, the invention relates to a method of treatment and/or care of the skin and/or hair comprising administering a protein hydrolysate of azuki beans according to the first aspect of the invention, or a compound chosen from:

- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);  
(ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);  
30 (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);  
(v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5), and  
stereoisomers, and cosmetically acceptable salts thereof, or a combination of said compound and a filtrate obtained by the fermentation of *Raphanus sativus* root by the  
35 microorganism *Leuconostoc* to a subject. The treatment and/or care includes: the

alleviation or prevention of the adverse effects of the exposome on the skin and/or hair; the alleviation or prevention of the adverse effects of air pollution on the skin and/or hair; the alleviation or prevention of the adverse effects of sunlight on the skin and/or hair; the alleviation or prevention of the adverse effects of chemical treatment of the hair; the  
5 alleviation or prevention of the adverse effects of heat stress on the hair; and/or the maintenance and/or improvement of the physical barrier function of the skin.

#### **DETAILED DESCRIPTION OF THE INVENTION**

**[0026]** The invention is based on the finding of surprising properties of a protein hydrolysate of azuki beans, which properties lend the protein hydrolysate to cosmetic,  
10 non-therapeutic applications on the skin and/or hair.

#### **Definitions**

**[0027]** In the context of this invention “skin” is understood to be the layers which comprise it, from the uppermost layer or stratum corneum to the lowermost layer or hypodermis, both inclusive. These layers are composed of different types of cells such  
15 as keratinocytes, fibroblasts, melanocytes and/or adipocytes among others. In the context of this invention, the term “skin” includes the scalp. The term “skin” includes the skin of mammals, for example, the skin of humans, and includes skin comprising hair.

**[0028]** In the context of this invention “hair” includes the hair of the scalp, the skin, the eyelashes, the eyebrows, moustache region and/or beard region of a subject.

**[0029]** The term “treatment” as used in the context of this specification when accompanied by the qualification “cosmetic”, it means that the treatment is non-therapeutic and has the aim of improving the aesthetic appearance of the skin and/or the hair and includes improving the properties of the skin and/or hair such as, but not restricted to, the level of hydration, elasticity, firmness, shine, tone or texture, which  
20 properties affect the cosmetic appearance of the skin and/or hair. The term “care” in the context of this specification refers to the maintenance of the properties of the skin and/or hair. The properties of the skin and/or hair are subject to improvement and maintenance through cosmetic treatment and/or care both in healthy subjects as well as those who present diseases and/or disorders of the skin and/or hair, such as and not  
25 restricted to, ulcers and lesions on the skin, psoriasis, dermatitis, acne or rosacea, alopecia, among others.

**[0030]** The term “prevention”, as used for this invention, refers to the ability of the active of the invention to prevent, delay or hinder the appearance or development of a symptom or manifestation of an adverse effect on the skin and/or hair. The term  
30

“alleviation”, as used for this invention refers to the ability of the active of the invention to reduce or lessen a symptom or manifestation of an adverse effect on the skin and/or hair.

**[0031]** The term “about” as used herein, e.g. when referring to a measurable value (such as an amount or weight of a particular component or temperature), refers to variations of  $\pm 20\%$ ,  $\pm 10\%$ ,  $\pm 5\%$ ,  $\pm 1\%$ ,  $\pm 0.5\%$ , or, particularly,  $\pm 0.1\%$  of the specified amount.

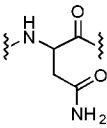
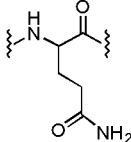
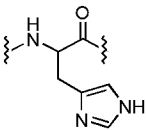
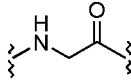
**[0032]** As used herein, the term “comprising”, which is inclusive or open-ended and does not exclude additional unrecited elements or method steps, is intended to encompass as alternative embodiments, the phrases “consisting essentially of” and “consisting of” where “consisting of” excludes any element or step not specified and “consisting essentially of” permits the inclusion of additional unrecited elements or steps that do not materially affect the essential or basic and novel characteristics of the composition or method under consideration.

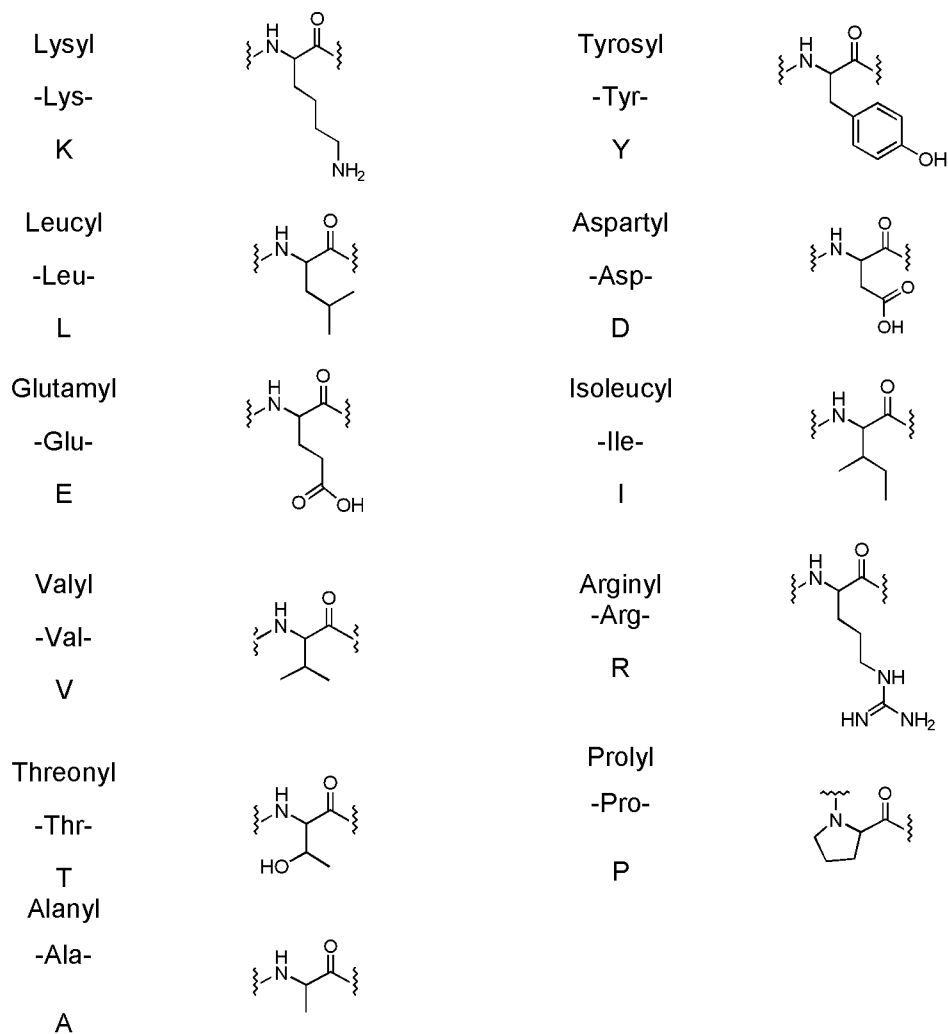
**[0033]** In this description the abbreviations used for amino acids follow the rules of IUPAC-IUB Commission of Biochemical Nomenclature specified in *Eur. J. Biochem.*, (1984), 138, 9-37. Thus, for example, Gly represents  $\text{NH}_2\text{-CH}_2\text{-COOH}$ , Gly- represents  $\text{NH}_2\text{-CH}_2\text{-CO-}$ , -Gly represents  $\text{-NH-CH}_2\text{-COOH}$  and -Gly- represents  $\text{-NH-CH}_2\text{-CO-}$ . Therefore, the hyphen, which represents the peptide bond, eliminates the OH in the 1-carboxyl group of the amino acid (represented here in the conventional non-ionized form) when situated to the right of the symbol, and eliminates the H of the 2-amino group of the amino acid when situated to the left of the symbol; both modifications can be applied to the same symbol (see the table below).

---

**Structures of the amino acid residues and their nomenclature in one and three-letter code**

---

Name	Residue	Symbol	Residue
Asparagyl -Asn- N		Glutaminyl -Gln- Q	
Histidyl -His- H		Glycyl -Gly- G	



### Protein hydrolysate of azuki beans and process for preparing same

**[0034]** In a first aspect, the invention relates to a protein hydrolysate of azuki beans.

**[0035]** The azuki bean is also known as *Vigna angularis*, and is a legume that was originally grown in China and high-altitude terrains near Tibet, and is consumed worldwide because it is a rich source of proteins (percentage > 20%). *Vigna angularis* is also known as adzuki bean (Japanese: 小豆 (アズキ), azuki, アヅキ, adzuki), azuki bean, or red mung bean.

**[0036]** A protein hydrolysate is a mixture of fragments obtained from the hydrolytic degradation of proteins and comprises, e.g., free amino acids and peptides of different

molecular weights and compositions. The protein hydrolysate of the invention can be obtained from the enzymatic hydrolysis of proteins of azuki beans.

**[0037]** The protein hydrolysate comprises at least one compound (i.e. 1, 2, 3 or 4 compounds) chosen from:

- 5 (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);  
(ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);  
(iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4).

**[0038]** Thus, the protein hydrolysate can comprise:

- 10 (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1); and  
(ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2).

**[0039]** The protein hydrolysate can comprise:

- (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);

15 **[0040]** The protein hydrolysate can comprise:

- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);  
(iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4).

**[0041]** The protein hydrolysate can comprise:

- 20 (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);  
(iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4).

**[0042]** The protein hydrolysate can comprise:

- 25 (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);  
(ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);  
(iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4).

**[0043]** The protein hydrolysate, in particular the protein hydrolysate having any of the combinations of compounds labelled (i), (ii), (iii) or (iv) given above, may additionally  
30 comprise the compound:

- (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5).

**[0044]** Each of the compounds labelled (i) to (v) above has been found to have excellent antioxidant activity. Without wishing to be bound by theory, it is believed that this activity contributes to the advantageous skin/hair treatment and/or care properties  
35 exhibited by the protein hydrolysate of the invention.

**[0045]** When present in the protein hydrolysate, each of these compounds labelled (i) to (v) above is present in the protein hydrolysate in an amount of from 0.001 to 0.15 ppm or 0.005 to 0.08 ppm. When present in the protein hydrolysate, the compound labelled (i) above can be present in the protein hydrolysate in an amount of from 0.001 to 0.1 ppm, 0.005 to 0.05 ppm, or 0.01 to 0.03 ppm. When present in the protein hydrolysate, the compound labelled (ii) above can be present in the protein hydrolysate in an amount of from 0.001 to 0.1 ppm, 0.005 to 0.05 ppm, or 0.015 to 0.04 ppm. When present in the protein hydrolysate, the compound labelled (iii) above can be present in the protein hydrolysate in an amount of from 0.001 to 0.1 ppm, 0.005 to 0.05 ppm, or 0.01 to 0.03 ppm. When present in the protein hydrolysate, the compound labelled (iv) above can be present in the protein hydrolysate in an amount of from 0.005 to 0.15 ppm, 0.01 to 0.1 ppm, or 0.03 to 0.08 ppm. When present in the protein hydrolysate, the compound labelled (v) above can be present in the protein hydrolysate in an amount of from 0.001 to 0.15 ppm, 0.001 to 0.01 ppm, or 0.003 to 0.008 ppm.

**[0046]** The protein hydrolysate can have a molecular weight of less than or equal to 3 kDa. Thus the protein hydrolysate can be absent of any components having a molecular weight of greater than 3 kDa, i.e. it can contain no components having a molecular weight of greater than 3 kDa. This can be achieved by filtering the hydrolysate.

**[0047]** The protein hydrolysate can be obtained by treating a suspension of ground azuki beans in water with alkali to solubilize proteins in the ground azuki beans, treating the resultant suspension with a serine endoprotease, deactivating the serine endoprotease in the suspension to form a crude hydrolysate, and purifying the crude hydrolysate. In this case, the enzymatic hydrolysis of the proteins in the ground azuki beans is carried out in a suspension (a suspension of ground azuki beans in water in which proteins in the auki beans have been solubilized), not on any form of isolated protein. Purifying can comprise removing any components having a molecular weight of greater than 3kDa from the crude hydrolysate. The protein hydrolysate of the invention is the purified product of this process. The process may not comprise steps of isolating proteins from the ground azuki beans and subjecting the isolated proteins to enzymatic hydrolysis. The process may not comprise subjecting proteins isolated from azuki beans to enzymatic hydrolysis and, as a result the protein hydrolysate will comprise components other than fragments of proteins, such as polyphenols and other organic compounds.

**[0048]** In particular, the protein hydrolysate can comprise polyphenols in an amount of at least 0.05 mg of polyphenols per milliliter of protein hydrolysate, particularly at least

0.15 mg of polyphenols per milliliter of protein hydrolysate. In particular the amount of polyphenols can be in the range of 0.05 to 0.30 mg/mL, more particularly 0.15 to 0.25 mg/mL. The amount of polyphenols may be measured by the Folin-Ciocalteu method and can particularly expressed as mg of gallic acid equivalents per mL of protein hydrolysate. The Folin-Ciocalteu method is a well known method for a person skilled in the analysis of compositions derived for botanicals and is extensively described in the literature (e.g. Lamuela-Raventós, "Folin–Ciocalteu method for the measurement of total phenolic content and antioxidant capacity" 2018, *Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications*). Alternatively, the protein hydrolysate can comprise polyphenols in an amount of at least 2 mg of polyphenols per g of protein hydrolysate (in dry weight), particularly at least 6 mg/g. In particular the amount of polyphenols can be in the range 2 to 12 mg/g, more particularly 6 to 9 mg/g. By polyphenols is meant naturally occurring organic compounds characterized by the presence of multiple phenol units. Polyphenols include for example flavonoids, tannic acid, and ellagitannin.

**[0049]** The protein hydrolysate can have a dry weight of 2 to 5 mg; a protein content of 19 to 35 w/w %; a DPPH activity of 74 to 80 %; and a polyphenols content of 0.05 to 0.30 mg/mL or 2 to 12 mg/g.

**[0050]** The protein hydrolysate can be prepared according to the following steps: a) to d) to obtain a crude hydrolysate, followed by purification of the crude hydrolysate.

**[0051]** Step a) involves providing a suspension of ground azuki beans in water. The azuki beans are raw azuki beans. The ground azuki beans can be obtained by grinding azuki beans into a flour. The flour can be sifted to remove larger fragments. Typically, the ground azuki beans have a particle size of less than 250 micrometers.

**[0052]** Step b) involves treating the suspension of ground azuki beans in water with alkali so as to solubilize proteins of the ground azuki beans in the water. The purpose of this step is to increase the content of protein solubilized in the suspension. Typically, this step involves adjusting the pH of the suspension to a pH of from about 6.5 to about 7.5, for example, to a pH of 7. The pH of the suspension affects the amount and type of proteins in the suspension. This can be achieved by adding an alkali such as NaOH to the suspension of ground azuki beans in water. Typically step b) is carried out at room temperature, i.e., at  $22 \pm 2^\circ\text{C}$ . After the alkali has been added to the suspension of ground azuki beans, the suspension is maintained at room temperature of a period of time, typically 1 hour, prior to step c).

**[0053]** Step c) involves enzymatically treating the suspension obtained in step b) with a serine endoprotease (also known as a serine endopeptidase). The serine endoprotease can be a subtilisin A. Subtilisin A (classification EC 3.4. 21.62, peptidase family S8) is also known as Subtilisin Carlsberg, Subtilopeptidase A, Alcalase®, ALK-enzyme, bacillopeptidase A, Bacillus subtilis alkaline proteinase biopraxe, biopraxe AL, colistinase, genenase1, Esperase®, maxatase, protease XXVII, thermoase, superase, subtilisin DY, subtilopeptidase, SP 266, Savinase®, katusase, protease VIII, protin A 3L, orientase 10B, or protease S. Subtilisin A is commonly obtained from Bacillus licheniformis. Step c) is carried out at a temperature of from 20 to 30°C, and can be carried out at a temperature of from 23 to 27°C or at a temperature of about 25°C. Step c) can be carried out for a period of about 30 to about 120, about 45 to about 90, or about 60 minutes, for example. Typically, the enzyme activity per g of protein in the ground azuki beans is from 0.1 to 0.5 AU/g. AU represents Anson Units. An Anson Unit is a unit of enzyme concentration which is defined as the amount of enzyme that can digest urea-denatured hemoglobin at the same initial rate as one milliequivalent of tyrosine at standard conditions (25C and pH 7.50). Typically the percentage of protein in the ground azuki beans is from 15 to 25 % w/w. Typically, the concentration of ground azuki beans in water is 20 -100 g (ground azuki beans)/Kg (water).

**[0054]** Step d) involves deactivating the enzyme to give a crude isolate. During step c) the pH of the suspension (undergoing enzymatic hydrolysis) is monitored. A drop of pH is associated with the release of peptide fragments and subsequent build up of acidic carboxylic acids. Typically, during step c) the suspension undergoing enzymatic hydrolysis suffers slight acidification, resulting in a drop of 0.2 to 0.3 pH units, for example. After the enzymatic hydrolysis step c), the enzyme is deactivated by increasing the temperature of the suspension obtained in c) to at least 70°C, or to from 70 to 90 °C, or to from 75 to 85°C. The suspension obtained in step c) can be obtained at this temperature for a period of time, preferably for at least about 20 minutes. The product of step d) is crude hydrosylate.

**[0055]** The crude hydrosylate product of step d) is then subjected to purification to remove any components having a molecular weight of greater than 3kDa. This can be achieved by solid/liquid separation (step e)) and ultrafiltraton (step f)). Step e) is a purification step and involves removing solid material from the crude hydrolysate by solid/liquid separation. Suitable separation techniques are known in the art and include, for example, filtration, sedimentation, decantation and/or centrifugation. Filtration can be carried out using filters having a pore size of lower than 1000 µm, or lower than 20 µm,

or lower than 10  $\mu\text{m}$ , or lower than 1  $\mu\text{m}$ , or lower than 0.1  $\mu\text{m}$ . Filtration may be performed in successive filtration operations, for instance using filters of decreasing pore size. Step f) is also a purification step and involves ultrafiltration to remove fragments from the supernatant obtained in step e) that have a molecular weight of greater than 3 kDa. Ultrafiltration is a technique that is well known in the art and involves the use of a membrane to separate molecules with different molecular weight, in this case with a cut off threshold of 3 kDa.

5 [0056] The invention extends to a protein hydrolysate obtained by the above described process.

10 [0057] The invention extends to a compound chosen from:

- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
- (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
- (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);
- (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4); and
- 15 (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5).

Each of the compounds labelled (i) to (v) above has been found to have excellent antioxidant activity. Without wishing to be bound by theory, it is believed that this activity contributes to the advantageous skin/hair treatment and/or care properties exhibited by the protein hydrolysate of the invention.

20 [0058] The invention also extends to a combination of the protein hydrolysate according to the first aspect of the invention and a filtrate obtained by the fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc*.

[0059] The invention extends to the combination of a compound chosen from:

- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
- 25 (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
- (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);
- (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);
- (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5); and

30 stereoisomers and cosmetically acceptable salts thereof and a filtrate obtained by the fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc*.

[0060] This filtrate obtained by the fermentation of *Raphanus sativus* (radish) root by the microorganism *Leuconostoc* contains antimicrobial peptides and acts as a preservative. One example of commercially filtrate obtained by the fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc* is Leucidal® (INCI Name: Leuconostoc/Radish Root Ferment Filtrate) from Active Micro Technologies LCC. The

35

most preferable species of *Leuconostoc* is *Leuconostoc kimchi*. Leucidal® is typically employed as a preservative. It has been found that the antioxidant activity of the protein hydrolysate of the invention is unexpectedly boosted in the presence of this filtrate.

### **Compositions**

5 **[0061]** The invention includes a composition which may be a personal care composition or a cosmetic composition, and which comprises the protein hydrolysate as described herein, together with at least one cosmetically acceptable excipient or adjuvant. The invention extends to a composition which may be a personal care  
10 composition or a cosmetic composition and which comprises: a combination of the protein hydrolysate as described herein and a filtrate obtained by the fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc*; together with at least one cosmetically acceptable excipient or adjuvant. The invention extends to a composition which may be a personal care composition or a cosmetic composition, comprising a compound chosen from:

15 (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);  
(ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);  
(iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);  
(v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5), and  
20 stereoisomers, and cosmetically acceptable salts thereof, together with at least one cosmetically acceptable excipient or adjuvant. The invention extends to a composition which may be a personal care composition or a cosmetic composition and which comprises: a combination of a compound chosen from:

(i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);  
25 (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);  
(iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);  
(iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);  
(v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5), and  
stereoisomers, and cosmetically acceptable salts thereof, and a filtrate obtained by the  
30 fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc*; together with at least one cosmetically acceptable excipient or adjuvant.

**[0062]** These compositions can be prepared by conventional means known to persons skilled in the art [*“Harry’s Cosmeticology”, Seventh edition, (1982), Wilkinson J.B., Moore R.J., ed. Longman House, Essex, GB*].

**[0063]** The compositions contain a cosmetically effective amount of the protein hydrolysate, or compound chosen from:

- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
- (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
- 5 (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);
- (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);
- (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5), and

stereoisomers, and cosmetically acceptable salts thereof, which should be administered, as well as their dosage, will depend on numerous factors, including age, state of the patient, the nature or severity of the condition, disorder or disease to be treated and/or  
10 cared for, the route and frequency of administration and of the particular nature of the compounds to be used.

**[0064]** The terms "cosmetically effective amount" is understood to mean a non-toxic but sufficient amount of extract of the invention to provide the desired effect. The protein hydrolysate can be present in an amount of at least 0.001 wt %, particularly from about  
15 0.001 to 10 wt %, preferably from about 0.004 to 5 wt%, based on the total weight of the personal care or cosmetic composition.

**[0065]** The compositions can be compositions for topical application which optionally include cosmetically acceptable excipients necessary for formulating the desired administration form. Topical compositions are compositions suitable for the  
20 topical application to mammalian keratinous tissue such as skin or hair.

**[0066]** Compositions for topical or transdermal application can be produced in any solid, liquid or semisolid formulation, such as and not restricted to, creams, multiple emulsions such as and not restricted to, oil and/or silicone in water emulsions, water-in-oil and/or silicone emulsions, water/oil/water or water/silicone/water type emulsions and  
25 oil/water/oil or silicone/water/silicone type emulsions, anhydrous compositions, aqueous dispersions, oils, milks, balsams, foams, lotions, gels, cream gels, hydroalcoholic solutions, hydroglycolic solutions, hydrogels, liniments, sera, soaps, shampoos, conditioners, serums, polysaccharide films, ointments, mousses, pomades, powders,  
30 bars, pencils and sprays or aerosols (sprays), including leave-on and rinse-off formulations. These topical or transdermal application formulations can be incorporated using techniques known by the person skilled in the art into different types of solid accessories for example and not restricted to, bandages, gauzes, t-shirts, socks, tights, underwear, girdles, gloves, diapers, sanitary napkins, dressings, bedspreads, wipes,  
35 adhesive patches, non-adhesive patches, occlusive patches, microelectric patches or

face masks, or they can be incorporated into different make-up products such as make-up foundation, such as fluid foundations and compact foundations, make-up removal lotions, make-up removal milks, under-eye concealers, eye shadows, lipsticks, lip protectors, lip gloss and powders among others.

5 **[0067]** The compositions of the invention can be in a form chosen from creams, multiple emulsions, solutions, liquid crystals, anhydrous compositions, aqueous dispersions, oils, milks, balsams, foams, lotions, gels, cream gels, hydroalcoholic solutions, hydroglycolic solutions, hydrogels, liniments, soaps, shampoos, conditioners, serums, polysaccharide films, ointments, mousses, pomades, powders, bars, pencils,  
10 sprays or aerosols.

**[0068]** The compositions of the invention may include agents which increase the percutaneous absorption of the compounds of the invention, for example and not restricted to, dimethylsulfoxide, dimethylacetamide, dimethylformamide, surfactants, azone (1-dodecylazacycloheptane-2-one), alcohol, urea, ethoxydiglycol, acetone,  
15 propylene glycol or polyethylene glycol, among others.

**[0069]** In particular, the compositions are for topical application and are hair care compositions such as conditioners, treatments, hair tonics, styling gels, mousses, shampoos, hair sprays, pomades, setting lotions, coloring and permanent waving compositions. Of particular interest for the purpose of the present invention are tonics,  
20 conditioners, treatments, and styling gels which may be in the form of a gel, a lotion, a tincture, a spray, a mousse, a cleansing composition or a foam and which may be applied according to individual needs, e.g., once daily as a lotion, tincture, mousse or spray; or once or twice weekly as a conditioner or treatment. Particularly, suitable compositions in the context of this invention are lotions, a shampoo, a serum or eyelashes mascara.  
25 Particularly, the cosmetic composition is a serum. The term "serum" is well known by the person skilled in the art and refers to compositions that are clear, gel-based or liquid. Serum has a fluid texture, and is more concentrated in active agents than a standard care product.

**[0070]** Further, the compositions may include other active ingredients such as anti-wrinkle agents, botox-like agents and/or anti-aging agents; firming agents, skin elasticity agents and/or restructuring agents; moisturizing agents; anti-photoaging agents, and/or blue-light protector agents; DNA protecting agents, DNA repair agents, and/or stem cell protecting agents; free radical scavengers and/or anti-glycation agents, detoxifying agents, antioxidant and/or anti-pollution agents; anti-perspirant agents; melanin  
35 synthesis stimulating or inhibiting agents; whitening or depigmenting agents;

propigmenting agents; self-tanning agents; lipolytic agents or agents stimulating lipolysis, adipogenic agents, etc. Additional examples can be found in *CTFA International Cosmetic Ingredient Dictionary & Handbook, 12th Edition (2008)*.

**[0071]** The anti-wrinkle agent, botox-like agent and/or anti-aging agent can be

5 Oxygestkin® [INCI: *Tropaeolum majus* Flower/Leaf/Stem Extract] or Eclaline™. [INCI: Hydrolyzed Lupine Protein Octenylsuccinate] from SILAB; Matrixyl® [INCI: Palmitoyl Pentapeptide-4], Matrixyl® 3000® [INCI: Palmitoyl Tetrapeptide-7, Palmitoyl Oligopeptide], Matrixyl® Synthe'6 [INCI: Glycerin, Water, Hydroxypropyl Cyclodextrin, Palmitoyl Tripeptide-38], Matrixyl® Morphomics™ [INCI: Pentylene Glycol, Caprylyl

10 Glycol], Essenskin™ [INCI: calcium hydroxymethionine], Renovage [INCI: Teprenone], Dermaxyl® [INCI: Palmitoyl Oligopeptide], Calmosensine [INCI: Butylene Glycol, Acetyl Dipeptide-1 Cetyl Ester], Volulip [INCI: Cetearyl Ethylhexanoate, Sorbitan Isostearate, Portulaca Pilosa Extract, Sucrose Cocoate, Palmitoyl Tripeptide-38], Subliskin [INCI: Sinorhizobium Meliloti Ferment, Cetyl Hydroxyethyl Cellulose, Lecithin], Biopeptide CL

15 [INCI: Palmitoyl Oligopeptide], Biopeptide EL [INCI: Palmitoyl Oligopeptide], Rigin [INCI: Palmitoyl Tetrapeptide-3], Biobustyl [INCI: Glyceryl Polymethacrylate, Rahnella/Soy Protein Ferment, Palmitoyl Oligopeptide], Dynalift [INCI: Sodium Polystyrene Sulfonate, Sorghum Bicolor Stalk Juice, Glycerin], Idealift [INCI: Acetyl Dipeptide-1 Cetyl Ester], Siegesbeckia [INCI: Siegesbeckia Orientales Extract], Ovaliss [INCI: Coco-glucoside,

20 Caprylyl Glycol, Alcohol, Glauicine], Juvinity™ [INCI: Geranylgeranylisopropanol], Prolevis [INCI: Hydrolyzed Vegetable Protein], Idealift™ [INCI: Hydroxyethylcellulose, Acetyl Dipeptide-1 cetyl ester], Beautifeye™ [INCI: Albizia Julibrissin Bark Extract, Darutoside], Chromocare™ [INCI: Sigesbeckia Orientalis Extract, Rabdosia Rubescens Extract] or Resistem™ [INCI proposed: Globularia Cordifolia Ferment] marketed by

25 Sederma/Croda. Vialox® [INCI: Pentapeptide-3], Syn®-Ake® [INCI: Dipeptide Diaminobutyroyl Benzylamide Diacetate], Syn®-Coll [INCI: Palmitoyl Tripeptide-5], Phytaluronate [INCI: Ceratonia Siliqua (Carob) Gum], Preregen® [INCI: Glycine soja (Soybean) Protein, Oxido Reductases], Pepha-Nutrix [INCI: Natural Nutrition Factors], Pepha-Tight [INCI: Algae Extract, Pullulan], Pentacare-NA [INCI: Hydrolyzed Wheat

30 Gluten, Ceratonia Siliqua Gum], Syn®-Tacks [INCI: Glycerin, Palmitoyl Dipeptide-5 Diaminobutyloyl Hydroxythreonine, Palmitoyl Dipeptide-6 Diaminohydroxybutyrate], BeauActive MTP [INCI: Hydrolyzed milk protein], Syn®-TC [INCI: Tetradecyl Aminobutyroylvalylaminobutyric Urea Trifluoroacetat, Palmitoyl Tripeptide-5, Palmitoyl

35 Dipeptide-5 Diaminobutyroyl Hydroxythreonine], Syn®-Hycan [INCI: Tetradecyl Aminobutyroylvalylaminobutyric Urea Trifluoroacetate], Syn®-Glycan [INCI: Tetradecyl

Aminobutyroylvalyl-aminobutyric Urea Trifluoroacetate], Regu-Age [INCI: Hydrolyzed Rice Bran Protein, Oxido Reductases, Glycine Soja Protein], Pepha-Timp [INCI: Human oligopeptide-20], Pepha-Age [INCI: Dunaliella Salina Extract], Colhibin [INCI: Hydrolyzed Rice Protein], Elhibin [INCI: Glycine Soja Protein, Disodium cocoamphodiacetate] or All-Q™ Plus [INCI: Ubiquinone, Tocopheryl Acetate] marketed by Pentapharm/DSM; Myoxinol™ [INCI: Hydrolyzed Hibiscus esculentus Extract], Myoxinol™ LS 9736 [INCI: Hydrolyzed Hibiscus esculentus Extract, Dextrin], Syniorage™ [INCI: Acetyl Tetrapeptide-11], Dermican™ [INCI: Acetyl Tetrapeptide-9], DN-AGE® LS [INCI: Cassia alata leaf Extract], Hyalufix GL [INCI: Alpinia Galanga Leaf Extract], Neurobiox [INCI: Achillea Millefolium Extract,], Deliner [INCI: Zea Mays (Corn) Kernel Extract], Lys'lastine V [INCI: Peucedanum Graveolens (Dill) Extract], Extracellium [INCI: Hydrolyzed Potato Protein], Proteasyl TP LS 8657 [INCI: Pisum Sativum Extract], Flavagrum PEG [INCI: PEG-6 Isostearate, Hesperetin Laurate], Micromerol [INCI: Pyrus Malus Fruit Extract], Extracellium [INCI: Hydrolyzed Potato Protein], Marine Filling Spheres [INCI: Pentaerythrityl Tetraistearate, Silica Dimethyl Silylate, Sodium Chondroitin Sulfate, Atelocollagen], Triactigen [INCI: Mannitol, Cyclodextrin, Yeast Extract, Disodium Succinate], Eterniskin [INCI: Grifola Frondosa Fruiting Body Extract, Maltodextrin], Ascotide [INCI: Ascorbyl Phosphate Succinoyl Pentapeptide-12], Hyalurosmooth [INCI: Cassia Angustifolia Seed Polysaccharide], Indinyl CA [INCI: Cassia Angustifolia Seed Polysaccharide], Arganyl [INCI: Argania Spinosa Leaf Extract], Sphingoceryl Veg [INCI: Phyto-ceramides], Vit-A-Like [INCI: Vigna Acontifolia Seed Extract], Peptiskin [INCI: Arginine/Lysine polypeptide], Prodejine [INCI: Mannitol, Cyclodextrin, Yeast Extract, Disodium Succinate], Aqu'activ [INCI: Behenyl Alcohol, Glyceryl Oleate, Cocamide MIPA, Calcium Citrate], Elestan [INCI: Glycerin, Manilkara Leaf Extract], Hibiscin HP [INCI: Hibiscus Esculentus Seed Extract], Collalift®18 [INCI: Khaya Senegalensis Bark], Collrepair™ DG [INCI: Hexylene Glycol, Niacin] or Litchiderm [INCI: Litchi Chinensis Pericarp Extract] marketed by Laboratoires Sérobiologiques/Cognis/BASF; Argireline® [INCI: Acetyl Hexapeptide-8], SNAP-7 [INCI: Acetyl Heptapeptide-4], SNAP-8 [INCI: Acetyl Octapeptide-3], Leuphasyl® [INCI: Pentapeptide-18], Inyline® [INCI: Acetyl Hexapeptide-30], Aldenine® [INCI: Hydrolyzed Wheat Protein, Hydrolyzed Soy Protein, Tripeptide-1], Preventhelia® [INCI: Diaminopropionoyl Tripeptide-33], Decorinyl® [INCI: Tripeptide-10 Citrulline], Decorinol® [INCI: Tripeptide-9 Citrulline], Trylagen® [INCI: Pseudoalteromonas Ferment Extract, Hydrolyzed Wheat Protein, Hydrolyzed Soy Protein, Tripeptide-10 Citrulline, Tripeptide-1], Eyeseryl® [INCI: Acetyl Tetrapeptide-5], Peptide AC29 [INCI: Acetyl Tripeptide-30 Citrulline], Relistase® [INCI:

Acetylglycyltryptophyl Diphenylglycine], Thermostressine® [INCI: Acetyl Tetrapeptide-22], Lipochroman™ [INCI: Dimethylmethoxy Chromanol], Chromabright® [INCI: Dimethylmethoxy Chromanyl Palmitate], Antarcticine® [INCI: Pseudoalteromonas Ferment Extract], dGlyage® [INCI: Lysine HCl, Lecithin, Tripeptide-9 Citrulline],  
5 Vilastene™ [INCI: Lysine HCl, Lecithin, Tripeptide-10 Citrulline], Hyadisine® [INCI: Pseudoalteromonas Ferment Extract], Hyanify™ [INCI: Saccharide Isomerate], Diffuporine® [INCI: Acetyl Hexapeptide-37], Silusyne® [INCI: Soybean (Glycine Soja) Oil, Sorbitan Sesquioleate, Isohexadecane, Sodium Hyaluronate, Lauryldimonium Hydroxypropyl Hydrolyzed Soy Protein, Acetyl Hexapeptide-39], Adifyline® [INCI: Acetyl  
10 Hexapeptide-38], Delisens™ [INCI: Acetyl Hexapeptide-46], Telangyn™ [INCI: Acetyl Tetrapeptide-40], Reproage™ peptide [INCI: Acetyl Hexapeptide-8], Cellynkage™ marine ingredient [INCI: Saccharide Isomerate], Eyedeline™ marine ingredient [INCI: Plankton Extract], Uplevity™ [INCI: Acetyl Tetrapeptide-2], Seacode™ marine ingredient [INCI: Pseudoalteromonas Ferment Extract] or Serilesine® peptide solution [INCI:  
15 Hexapeptide-10]; Argireline® Amplified [INCI: Acetyl Hexapeptide-8], Dawnergy™ [INCI: Nonapeptide-1] marketed by Lipotec/Lubrizon; Sirtalice™ [INCI: Bacillus Ferment], Epitensive™ [INCI: Nicotiana Benthamiana Hexapeptide-40 SH-Oligopeptide-1], Scelleye™ [INCI: Nicotiana Benthamiana SH-Oligopeptide-2], Seadermium [INCI: Aqua, Glycerin, Bacillus Ferment], Pauseile [INCI: Aqua, Glycerin, Bacillus Ferment] or  
20 Neoclair pro [INCI: Aqua, Glycerin, Caprylyl Glycol, Acetyl Tetrapeptide-2] marketed by Lipotrue; Collaxyl® IS [INCI: Hexapeptide-9], Laminixyl IS™ [INCI: Heptapeptide], Orsirtine™ GL [INCI: Oryza sativa (Rice) Extract], D'Orientine™ IS [INCI: Phoenix dactylifera (Date) Seed Extract], Phytoquintescine™ [INCI: Einkorn (Triticum monococcum) Extract], Quintescine™ IS [INCI: Dipeptide-4], Peptide Vinci 01 [INCI: Penta-decapeptide-1], Peptide Vinci 02™ [INCI: Hexapeptide-3], Aquarize IS™ [INCI: Hydrolyzed Rice Extract], Lanablue [INCI: Algae extract], Ederline™ [INCI: Pyrus Malus (Apple) Seed Extract], Dynachondrine™ ISR [INCI:Hydrolyzed Soy Protein], Prolixir S20™ [INCI: Dimer Tripeptide-43], Phytocohesine™ PSP [INCI: Sodium Beta-Sitosteryl Sulfate, Beta-Sitosterol], Perenityl™ IS [INCI: Pyrus Communis (Pear) Seed Extract],  
30 Caspaline 14™ [INCI:Hexapeptide-42], Peptide Q10™ [INCI:Pentapeptide-34 Trifluoroacetate], Survixyl IS™ [INCI: Pentapeptide-31], ChronOgen™ [INCI: Tetrapeptide-26], Elixiance [INCI: Schinus Molle Extract], Harmoniance™ [INCI: Nelumbo Nucifera Flower Extract], Serenityl [INCI: Marsdenia Condurango Bark Extract], Natriance Wrinkle-less [INCI: Hydrolyzed Corn Protein], Phytoneomatrix [INCI:  
35 Hydrolyzed Soybean Extract], Prolixir ICE [INCI: Hydrolyzed Rice Protein], PhytoRNx

Baobab™ [INCI: Hydrolyzed Adansonia Digitata Extract], Natriance Renovate Extract [INCI: Hydrolyzed Linseed Extract], Natriance Self-Hydrate Extract [INCI: Pisum Sativum Extract], Actopontine YST [INCI: Hydrolyzed Yeast Protein] or Telosense™ [proposed INCI: Hydrolized Soy Protein, Hydrolized Yeast Protein] marketed by Vincience/ISP/Ashland; BONT-L-Peptide [INCI: Palmitoyl Hexapeptide-19], TIMP Peptide [INCI: Acetylhexapeptide-20], ECM Moduline [INCI: Palmitoyl Tripeptide-28], Renaissance [INCI: Hydrolyzed Wheat Protein, Palmitoyl Decapeptide-21, Decapeptide-22, Oligopeptide-78, Zinc Palmitoyl Nonapeptide-14] or X50 Antiaging [INCI: Lactic Acid/glycolic Acid Copolymer, Polyvinyl Alcohol, Copper Palmitoyl Heptapeptide-14, Heptapeptide-15 Palmitate] marketed by Infinitec Activos; EquiStat [INCI: Pyrus malus Fruit Extract, Glycine soja Seed Extract], Juvenesce [INCI: Ethoxydiglicol and Caprylic Triglycerid, Retinol, Ursolic Acid, Phytanadione, Ilomastat], Ursolisome [INCI: Lecithin, Ursolic Acid, Atelocollagen, Xanthan Gum, Sodium chondroitin sulfate], Basaline [INCI: Hydrolyzed Malt Extract], Phytokine [INCI: Hydrolyzed Soy Protein], marketed by Coletica/Engelhard/BASF; Ameliiox [INCI: Carnosine, Tocopherol, Silybum marianum Fruit Extract] or PhytoCellTec Malus Domestica [INCI: Malus domestica Fruit Cell Culture], Lipobelle Soyaglicane [INCI: Soy Isoflavones], RoyalEpigen P5 [INCI: Butyrospermum Parkii BUTter, Hydrogenated Lecithin, Maltodextrin, Pentapeptide-48, Phenethyl Alcohol, Ethylhexylglycerin, Glycerin, Aqua] or DermCom [INCI: Crocus Chrysanthus Bulb Extract, Acacia Senegal Gum, Aqua/Water] marketed by Mibelle Biochemistr; ActiMatrix [INCI: Peptide based mushroom Extract], Peptamide 6 [INCI: Hexapeptide-11] marketed by Active Organics/Arch; and combinations thereof.

**[0072]** The firming agent, skin elasticity agent and/or restructuring agent can be selected from the group consisting of Argassential [INCI: C10-16 Alkyl Glucoside, Dicaprylyl Ether, Glycerin] or Replexium BC [INCI: Dimethyl Isosorbide, Polysorbate 20, Aqua, Acetyl Tetrapeptide-11, Acetyl Tetrapeptide-9] marketed by BASF; Prolevis [INCI: Hydrolyzed Vegetable Protein] or Poretect [INCI: Caprylic/capric Triglyceride, Sorbitan Trioleate, Apium Graveolens Seed Extract, Linum Usitatissimum Seed Extract] marketed by Sederma/Croda; Actifirm Ultra Advanced botanical ingredient [INCI: Centella Asiatica Extract, Rosmarinus Officinalis Leaf Extract, Dipropylene Glycol, Alcohol, Echinacea Angustifolia Leaf Extract] or Actifcol Advanced botanical ingredient [INCI: Aqua, Glycerin, Sodium Citrate, Lentinus Edodes Extract, Potassium Sorbate, Sodium Benzoate, Phytic Acid] marketed by Lipotec/Lubrizol; Densorphin™ [INCI: Vitex Agnus Castus Extract, Aqua, Maltodextrin] or PhytoCellTec™ nunatak® [INCI: Isomalt, Aqua,

Saponaria Pumila Callus Culture Extract, Lecithin] marketed by Mibelle; and combinations thereof.

**[0073]** The moisturizing agent can be selected from the group consisting of qua Shuttle [INCI: Sorbitol, Laminaria Digitata Extract, Diatomaceous Earth] marketed by Infinitec; Aqua-Osmoline™ [INCI: Ceratonia Siliqua (Carob) Seed Extract] marketed by Vincience/ISP/Ashland; Hydralphatine™ Asia [INCI: Hydrogenated Starch Hydrolysate, Panthenol, Bambusa Vulgaris Shoot Extract, Nelumbo Nucifera Flower Extract, Nymphaea Alba Root Extract] or Hydraporine™ [INCI: Betaine, Hydrogenated Lecithin, Honey, Pectin] marketed by Lucas Meyer Cosmetics/Unipex; PatchH2O™ [INCI: Trehalose, Urea, Serine, Glyceryl Polyacrylate, Algin, Sodium Hyaluronate, Pullulan], Aqu'activ™ [INCI: Behenyl Alcohol, Glyceryl Oleate, Cocamide MIPA], Irwinol® [INCI: Octyldodecanol, Irvingia Gabonensis Kernel Butter, Hydrogenated Coco-Glycerides], Lipodermol® [INCI: Octyldodecanol, Arachidyl Propionate, Tocopheryl Acetate, Retinyl Palmitate, Ethyl Linoleate, Ethyl Linolenate] or Seanamin® SU [INCI: Sorbitol, Algae Extract, Chondrus Crispus (Carrageenan), Fucus Vesiculosus Extract, Algin] marketed by L. Serobiologiques/Cognis/BASF; Snow Algae Powder [INCI: Coenochloris Signiensis Extract] marketed by Mibelle; Hyasol BT [INCI: Sodium Hyaluronate], Syn-Up™ [INCI: Benzylsulfonyl D-Seryl Homophenylalanine Amidinobenzamide Acetate] or Pentavitin® [INCI: Saccharide Isomerate] marketed by Pentapharm/DSM; Aqualance™ [INCI: Erythritol, Homarine HCl], Hydraprotectol™ [INCI: Glyceryl Polymethacrylate, Aleuritic Acid, Yeast Extract (Faex), Glycoprotein], Moist 24™ [INCI: Imperata Cylindrica Root Extract], Optim Hyal™ [INCI: Hydrolyzed Yeast Extract, Cetyl Hydroxyethylcellulose, Polyglucuronic Acid], Osmocide® 4 [INCI: Glycerin, Acrylates/C10-30 Alkyl Acrylate Crosspolymer] or Revidrate™ [INCI: Ethylhexyl Palmitate, Sorbitan Oleate, Sorbitan Laureate, Myristyl Malate Phosphonic Acid] marketed by Sederma/Croda; Xpertmoist® molecular film [INCI: Glycerin, Pseudoalteromonas Ferment Extract, Xanthan Gum, Proline, Alanine, Serine, Ethylhexylglycerin, Caprylyl Glycol] or Actizyme GL advanced botanical ingredient [INCI: Glycerin, Mucor miehei extract, Aqua, Sodium Citrate, Potassium Sorbate, Sodium Benzoate, Phytic Acid] marketed by Lipotec/Lubrizon; and combinations thereof.

**[0074]** The anti-photoaging agent, and/or blue-light protector agent can be selected from the group consisting of Algaktiv Genofix CPD [INCI: Plankton Extract, Aqua, Lecithin] marketed by Greenaltech; Blumilight™ Biofunctional [INCI proposed: Water/Aqua (and) Butylene Glycol (and) Theobroma Cacao (Cocoa) Seed Extract] marketed by Ashland; Lys'Sun [INCI: Hamamelis Virginiana Leaf Extract, Aqua,

Pentylene Glycol, Caprylyl Glycol, Xanthan Gum] marketed by BASF; Vitachelox [INCI: Vitis Vinifera Seed Extract, Camellia Sinensis Leaf Extract, Quercus Robur Wood Extract] marketed by Indena; L-VCG [INCI: Ascorbyl Glucoside] marketed by Freshine Bio-technology; Lumicease blue ingredient [INCI: Glycerin, Aqua, Hydrolyzed Pea Protein, Glucose, Sodium Chloride] marketed by Lipotec/Lubrizol; Lightwaves Defense [JS+M] [INCI: Jasminum Sambac Leaf Cell Extract] marketed by Naolys; Blue Oleoactif [INCI: Glycine Soja Oil, Polyglyceryl-3 Diisostearate, Oryza Sativa Germ Extract, Oryza Sativa Extract] marketed by Oleos-Hallstar; Majestem [INCI: Glycerin, Leontopodium Alpinum Callus Culture Extract, Xanthan Gum] or Senestem [INCI: Glycerin, Plantago Lanceolata Leaf Extract, Xanthan Gum] marketed by Sederma; Blueshield [INCI: Glycerin, Capsicum Annuum Fruit Extract, Xanthan Gum] marketed by Solabia; and combinations thereof.

**[0075]** The DNA protecting agent, DNA repair agent, and/or stem cell protecting agent can be selected from the group consisting of; GP4G SP [INCI: Aqua, Glycerin, Aretmia Extract], Heliostatine [INCI: Aqua, Glycerin, Pisum Sativum Extract], Orsirtine [INCI: Aqua, Glycerin, Oryza Sativa Extract], Chronogen [INCI: Aqua, Butylene Glycol, Tetrapeptide (INCI proposed)], Survixyl IS [INCI: Water, Butylene Glycol, Pentapeptide-31] and Chrondicare [INCI: Aqua, Butylene Glycol Pentapeptide-28] marketed by Vincience/ISP/Ashland; Lanacityn® [INCI: Glycerin, Aqua, Alteromonas ferment extract, Chysanthellum indicum extract] or Melinoil [INCI: Isopropyl Palmitate, Lecithin, Aqua, Acetyl Hexapeptide-1] marketed by Atrium Innovations/Lucas Meyer Cosmetics; Repair Complex [INCI: Bifida Ferment Lysate] marketed by CLR; Phycojuvenine [INCI: Laminaria Digitata] marketed by Codif; Unirepair T-43 [INCI: Butylene Glycol, Acetyl Tyrosine, Proline, Hydrolyzed Vegetable Protein, Adenosine Triphosphate] marketed by Induchem; Dragosine [INCI: Carnosine] marketed by Symrise; DN-Age [INCI: Cassia Alata Leaf Extract] marketed by Laboratories Serobiologiques/Cognis/BASF; Helioguard [INCI: Porphyra Umbilicalis encapsulated into liposomes], PhytoCellTec Malus Domestica [INCI: PhytoCellTec Malus Domestica] or PhytoCellTec Argan [INCI: Argania Spinosa Sprout Cell Extract, Isomalt, Lecithin, Sodium Benzoate, Aqua] marketed by Mibelle Biochemistry; Pepha-Protect [INCI: Water Melon Extract] marketed by Pentapharm/DSM; Celligent [INCI: Helianthus Annuus Seed Oil, Ethyl Ferulate, Polyglyceryl-5 Trioleate, Rosmarinus Officinalis Leaf Extract, Aqua, Disodium Uridine Phosphate] or Defensil [INCI: Octyl Dodecanol, Echium Plantagineum Seed Oil, Cardiospermum Halicacabum Extract, Helianthus Annuus Seed Oil Unsaponifiables] marketed by Rahn; Venuceane [INCI: Thermus Thermophilus Ferment, Glycerin], UV-

Soft [INCI: Yeast Extract], Renovage [INCI: Caprylic/Capric Triglyceride, Teprenone], Juvinity [INCI: Caprylic/Capric Triglyceride, Geranylgeranylpropanol (proposed)], Phytessence Holyherb [INCI: Butylene Glycol, Eriodictyon Californicum (Holyherb) Flower/Leaf/Stem Extract] or Resistem [INCI: Glycerin, Globularia Cordifolia Ferment] marketed by Sederma/Croda; Infraguard [INCI: Caesalpinia Spinosa Fruit Pod Extract, Propylene Glycol, Aqua, Helianthus Annuus Sprout Extract, Sodium Benzoate, Phenoxyethanol] marketed by Mibelle; Heliomoduline [INCI: Low molecular weight peptides from cottonseed] or Stem-C-Guard [Hydrolyzed Pea] marketed by Silab; and combinations thereof.

10 **[0076]** The reactive carbonyl species scavenger, free radical scavengers and/or anti-glycation agent, detoxifying agent, antioxidant and/or anti-pollution agent can be selected, for example and not restricted to, from the group formed by carnosine and its derivatives; GHK [INCI: Tripeptide-1] and its salts and/or derivatives or Quintescine IS [INCI: Dipeptide-4] marketed by Vincience/ISP/Ashland; Preregen [INCI: Glycine Soja (Soybean) Protein, Oxido Reductases], Edelweiss GC [INCI: Leontopodium Alpinum Extract], Lipogard [INCI: Squalane, Ubiquinone], Nectapure [INCI: Buddleja Davidii Extract, Thymus Vulgaris Extract], Alpaflor Nectapure [INCI: Buddleja Davidii Extract, Thymus Vulgaris Extract, Glycerin, Water] or Dismutin-BT [INCI: Highly purified SOD from a natural yeast strain of *Saccharomyces cerevisiae*] marketed by Pentapharm/DSM; Preventhelia® [INCI: Diaminopropionoyl Tripeptide-33], Aldenine® [INCI: Hydrolized Wheat Protein, Hydrolized Soy Protein, Tripeptide 1], Lipochroman™ [INCI: Dimethylmethoxy Chromanol], Thermostressine® [INCI: Acetyl Tetrapeptide-22] Pollushield™ functional ingredient [INCI: Diisopropyl Adipate, Lecithin, Acrylic Acid/Acrylamidomethyl Propane Sulfonic Acid Copolymer, Dimethylmethoxy Chromanol, Xanthan Gum] or Bodyfensine® [INCI: Acetyl Dipeptide-3 Aminohexanoate] marketed by Lipotec/Lubrizon; unactyl [INCI: Mannitol, Pisum Sativum Extract, Histidine HCl, Arginine, Cyclodextrin, Dextrin, Yeast Extract, Acetyl Trysoine, Pyridoxine HCl, Khaya Senegalensis Bark Extract, Nicotinamide, Adenine Dinucleotide, Disodium Succinate, Aspartic Acid], Imidinyl [INCI: Tamarindus Indica Seed Polysaccharide], Phytrogene [INCI: Butylene Glycol, Malva Sylvestris (Mallow) Extract, Xanthan Gum] or Purisoft [INCI: Moringa Pterogysperma Seed Extract] marketed by Laboratoires Sérobiologiques/Cognis/BASF; AquaCacteen [INCI: Glycerin, Opuntia Ficus Indica Stem Extract, Phenoxyethanol, Aqua], Trimoist (KMF) [INCI: Sodium Stearoyl Lactylate, Cetyl alcohol, Olus Vegetable oil, Tocopheryl acetate, Glycerin, Glycine soja sterol, Sodium lactate, Sodium barboxymethyl betaglucan, Carnosine, Lactic Acid], MelanoBronze

[INCI: Vitex Agnus Castus Extract (Monk's pepper berries extract (phyto-endorphins)), Acetyl Tyrosine], CM-Glucan [INCI: Sodium Carboxymethyl Betaglucan, Phenoxyethanol, SunActin [INCI: Helianthus Annuus (Sunflower) Sprout Extract, Tocopherols, Glycerin, Lecithin, Phenoxyethanol, Aqua], GSP-T skin [INCI: Glycerin, Alcohol, Aqua, PEG-40 Hydrogenated Castor Oil, Vitis Vinifera (Grape) Seed Extract] or Detoxophane [INCI: Lepidium Sativum Sprout Extract, Lecithin, Phenoxyethanol, Glycerin, Water] marketed by Mibelle Biochemistry; Bacocalmine [INCI: PEG-8, Bacopa Monniera Extract, Water (Aqua), Hydroxyethylcellulose], Kombuchka [INCI: Saccharomyces/Xylinum Black Tea Ferment, Glycerin, Hydroxyethyl cellulose], Citystem [INCI: Glycerin, Marrubium Vulgare Extract] or Prodzia [INCI: Albizia Julibrissin Extract, Glycerin] marketed by Sederma/Croda; Extramel C [INCI: Hydroxypropyltrimonium Maltodextrin Crosspolymer, Cucumis Melo (Melon) Fruit Extract] marketed by Seppic; Defensine [INCI: Triticum Vulgare Germ Extract], Apolluskin® [INCI: Taraxacum officinale (Dandelion) Extract], Detoxyl® [INCI: Water, Butylene Glycol, Butyrospermum parkii (Shea Butter) Seedcake Extract] or Antiglyskin [INCI: Aqua, Helianthus Annuus Seed Extract] marketed by Silab; and combinations thereof.

**[0077]** The compositions of the invention may also include other active ingredients such as vitamins, minerals, proteins, peptides, fatty acids, antioxidants, anti-inflammatory agents, darkening agents and/or mixtures thereof. Particularly, the cosmetic composition may include other active ingredients for promoting hair growth, and/or preventing hair loss. Suitable, non limiting examples, in the context of this invention are Growth Oléoactif® [INCI: Helianthus Annuus (Sunflower) Seed Oil (and) Polyglyceryl-3 Diisostearate (and) Carthamus Tinctorius (Safflower) Flower Extract (and) Hibiscus Sabdariffa Flower Extract] marketed by Hallstar. Widelash™ [INCI: Glycerin, Water (Aqua), Panthenol, Biotinoyl Tripeptide-1] marketed by Sederma; SymPeptide® Xlash [INCI: Glycerin (and) Aqua (and) Myristoyl Pentapeptide-17], SymLash® 1631 [INCI: Pentylene Glycol (and) Isochrysis Galbana Extract] marketed by Symrise; Anargy™ [INCI: Water (and) Butylene Glycol (and) Oligopeptide-2 (and) Nicotiana Benthamiana Hexapeptide-40 sh-Polypeptide-9 (and) Nicotiana Benthamiana Hexapeptide-40 sh-Polypeptide-86] marketed by Lipotrue; Capixyl™ [INCI: Butylene Glycol (and) Aqua (and) Dextran (and) Acetyl Tetrapeptide-3 (and) Trifolium Pratense (Clover) Flower Extract] marketed by Lucas Meyer; Nano lashes [INCI: Aqua, Simmondsia Chinensis Seed Extract, Polysorbate 20, Hydroxipropyl guar, Sodium Benzoate, Potassium Sorbate] marketed by Nanovetores; Specped SC-MH16 [INCI: Aqua, Glycerin, Myristoyl Hexapeptide-16, Caprylyl Glycol, Ethylhexylglycerin], Myristoyl

Pentapeptide-17 [INCI: Myristoyl Pentapeptide-17], SpecPed® BT1 [INCI: Biotinoyl  
 Tripeptide-1], SpecPed® LashLD [INCI: Biotinoyl Tripeptide-1, Glycerin, Water, Caprylyl  
 Glycol & Ethylhexylglycerin, Panthenol, Trehalose], SpecPed® MP17P [INCI: Myristoyl  
 Pentapeptide-17] marketed by Spec-chem; Procapil™ [INCI: Butylene Glycol (and)  
 5 Aqua (and) PPG-26-Buteth-26 (and) PEG-40 Hydrogenated Castor Oil (and) Apigenin  
 (and) Oleanolic Acid (and) Biotinoyl Tripeptide-1] marketed by Sederma; AnaGain™  
 [INCI: Pisum Sativum (Pea) Sprout Extract (and) Isomalt (and) Aqua], PhytoCellTec™  
 Malus Domestica Hair [INCI: Malus Domestica Fruit Cell Culture Extract (and) Xanthan  
 Gum (and) Glycerin (and) Lecithin (and) Phenoxyethanol (and) Aqua / Water],  
 10 Santenergy™ [INCI: Bioflavonoids (and) Pentylene Glycol (and) Alcohol (and) Aqua],  
 RootBioTec™ HW [INCI: Ocimum Basilicum Hairy Root Culture Extract (and) Alcohol  
 (and) Aqua / Water], RootBioTec™ HO [INCI: Ocimum Basilicum Hairy Root Culture  
 Extract (and) Helianthus Annuus (Sunflower) Seed Oil (and) Cocos Nucifera (Coconut)  
 Oil] marketed by Mibelle; CAPILIA LONGA PPF [INCI: Curcuma Longa (Turmeric)  
 15 Callus Culture Conditioned Media (and) Water] marketed by Vytrus Biotech;  
 Cressatine® [INCI: Glycerin (and) Aqua (and) Nasturtium Officinale Extract (and)  
 Tropaeolum Majus Extract] marketed by Solabia; Alotide™ [INCI: Copper Ascorbyl  
 Phosphate Succinyl Tripeptide-34] marketed by Pepton; BURGEON-UP [INCI: Aqua  
 (and) Alcohol (and) Nasturtium Officinale Leaf/Stem Extract] marketed by Ichimaru  
 20 Pharcos; Capalgin® [INCI: Chondrus Crispus Extract] marketed by Exysmol;  
 Dermosaccharides® GY [INCI: Aqua (and) Glycerin (and) Glycogen (and)  
 Phenoxyethanol (and) Methylparaben], Trichogen™ VEG LS 9922 [INCI: Water (and)  
 Panax Ginseng Root Extract (and) Arginine (and) Acetyl Tyrosine (and) Arctium Majus  
 Root Extract (and) Hydrolyzed Soy Protein (and) Polyquaternium-11 (and) PEG-12  
 25 Dimethicone (and) Calcium Pantothenate (and) Zinc Gluconate (and) Niacinamide (and)  
 Ornithine HCl (and) Citrulline (and) Glucosamine HCl (and) Biotin] marketed by BASF;  
 Follicusan™ DP [INCI: Aqua (and) Alcohol Denat. (and) Panthenyl Ethyl Ether (and)  
 Inositol (and) Milk Protein (and) Lactose (and) Acetyl Cysteine (and) Acetyl Methionine  
 (and) Sodium Citrate (and) Citric Acid] marketed by CLR; Hairline® [INCI: Propanediol  
 30 (and) Water (aqua) (and) Lindera Strychnifolia Root Extract] marketed by Greentech;  
 Kerasalp™ [INCI: Propanediol (and) Glycerin (and) Phyllanthus Emblica Fruit Extract],  
 Baicapil™ [INCI: Propanediol (and) Water (and) Arginine (and) Lactic Acid (and) Glycine  
 Soja (Soybean) Germ Extract (and) Triticum Vulgare (Wheat) Germ Extract (and)  
 Scutellaria Baicalensis Root Extract (and) Sodium Benzoate (and) Gluconolactone (and)  
 35 Calcium Gluconate] marketed by Provital; BIOENERGIZER™ P BG PF [INCI:

Aqua/Water, Butylene Glycol, Panthenol, Propylene Glycol, Pelvetia Canaliculata Extract, Laminaria Digitata Extract] marketed by Seppic; Protectagen™ [INCI: Aqua (and) Glycerin (and) Hydrolyzed Rice Protein] marketed by Ashland; Redensyl® [INCI: Glycerin (and) Aqua (and) Sodium Metabisulfite (and) Larix Europaea Wood Extract (and) Glycine (and) Zinc Chloride (and) Camellia Sinensis Leaf Extract] marketed by Givaudan; GANOTHER® [INCI: Glycerin (and) Water (and) Ganoderma Lucidum (Mushroom) Mycelium Ferment Filtrate] marketed by B&G; Hairgenyl® [INCI: Saccharomyces Cerevisiae Extract], Anageline® [INCI: Hydrolyzed Lupine Protein] marketed by Silab; Hairdian AP [INCI: Propanediol (and) Thuja Orientalis Extract (and) Zingiber Officinale (Ginger) Root Extract (and) Trifolium Pratense (Clover) Leaf Extract (and) Artemisia Argyi Leaf Extract] marketed by Shanghai GREAF Biotech, Naturein™ Wheat Peptides (INCI: Water (aqua) (and) Hydrolyzed Wheat Protein) or from Givaudan, KeraMatch™ V (INCI: Aqua (and) Hydrolyzed Pea Protein (and) Hydrolyzed Vegetable Protein) from Croda Personal care.

### 15 Applications

**[0078]** The present invention is based on the finding that the protein hydrolysate of the invention is effective in alleviating and/or preventing adverse effects of the exposome on skin and/or hair. Adverse effects of the exposome include adverse effects due to air pollution, sunlight and/or greenhouse gases. It has also been found that the protein hydrolysate is effective in alleviating adverse effects of chemical treatment or heat stress on hair. In addition, it has been found that the protein hydrolysate is effective in maintaining and/or improving the physical barrier function of the skin. The physical barrier function of the skin is also referred to herein as the physical barrier of the skin, the skin's permeability barrier or simply the skin barrier. This barrier is provided by the stratum corneum and the tight junctions in the epidermis.

**[0079]** In one aspect, the invention provides for the use of the protein hydrolysate described herein, or a compound chosen from:

- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
- (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
- 30 (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);
- (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);
- (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5), and

stereoisomers, and cosmetically acceptable salts thereof, or a combination of said protein hydrolysate or said compound and a filtrate obtained by the fermentation of

Raphanus sativus root by the microorganism Leuconostoc, for the treatment and/or care of the skin and/or hair. In particular, the treatment and/or care of the skin and/or hair is cosmetic and non-therapeutic.

5       **[0080]**       The use of the protein hydrolysate, compounds or combinations described herein can be for the alleviation and/or prevention of adverse effects of the exposome on hair; the alleviation and/or prevention of adverse effects of air pollution on hair; the alleviation and/or prevention of adverse effects of sunlight on hair; the alleviation and/or prevention of the adverse effects of chemical stress on hair; the alleviation and/or prevention of adverse effects of heat stress on hair; and/or the alleviation or prevention  
10       of the adverse effects to the hair caused by peroxidation of lipids in hair and/or carbonylation of proteins in hair. The use of the protein hydrolysate, compounds or combinations described herein can be for the alleviation and/or prevention of adverse effects of air pollution and sunlight on hair. Adverse effects in hair can manifest themselves in the form of a damaged hair. Thus, the protein hydrolysate can be used for  
15       reducing hair damage. Adverse effects in hair can manifest themselves in the form of a hair with more split ends; reduced fiber alignment and/or increase in frizz. Therefore, the hair is less manageable. Thus, the protein hydrolysate can be used for increasing hair manageability, particularly by a reduction of split ends and/or an increase in fiber alignment and/or reduction of frizz and/or a reduction of volume.

20       **[0081]**       Thus there is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the hair, wherein the treatment and/or care of the hair is the alleviation or prevention of the adverse effects of the exposome on the hair.

25       **[0082]**       There is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the hair, wherein the treatment and/or care of the hair is the alleviation or prevention of the adverse effects of the air pollution on the hair, in particular, where the air pollution contains particulate matter.

30       **[0083]**       Air pollution includes particulate matter, for example. Particulate matter is an airborne pollutant that includes solid particles. Particulate matter may include dust, dirt, soot or smoke. Particulate matter can come from both anthropogenic and naturally occurring sources, including automobiles (e.g. exhaust emissions from combustion engines of automobiles), power plants, factories, fires, wood-burning stoves, dust from open land or construction, dust from roads (e.g. brake-, tyre- and road-wear particles),  
35       pollen (e.g. cedar pollen) and smoke from tobacco (e.g. cigarette smoke). The

particulate matter can have a size of 10 microns or less (also referred to as PM<sub>10</sub> and “dust”). The particulate matter can arise from automobile combustion engines (e.g. diesel particulate such as those encountered in Diesel particulate matters NIST1650B. The particular matter can comprise or pollen (e.g. cedar pollen).

5 **[0084]** There is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the hair, wherein the treatment and/or care of the hair is the alleviation or prevention of the adverse effects of the air pollution containing particulate matter on the hair, where the particulate matter is derived from road dust, derived from diesel, or is pollen.

10 **[0085]** There is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the hair, wherein the treatment and/or care of the hair is the alleviation or prevention of the adverse effects of sunlight on the hair. The sunlight can comprise UV-A radiation.

15 **[0086]** There is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the hair, wherein the treatment and/or care of the hair is the alleviation or prevention of the adverse effects of bleaching or dyeing on hair.

20 **[0087]** There is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the hair, wherein the treatment and/or care of the hair is the alleviation or prevention of the adverse effects of heat stress on hair.

25 **[0088]** There is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the hair, wherein the treatment and/or care of the hair is the alleviation or prevention of the adverse effects to the hair caused by peroxidation of lipids in hair and/or carbonylation of proteins in hair.

30 **[0089]** There is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the hair, wherein the treatment and/or care of the hair is the increase of hair manageability; and/or the increase in fiber alignment and/or reduction of frizz and/or the reduction of volume.

35 **[0090]** The use of the protein hydrolysate, compounds or combinations described herein can be for the alleviation and/or prevention of adverse effects of the exposome on skin; the the alleviation and/or prevention of adverse effects of air pollution on skin; the alleviation and/or prevention of adverse effects of sunlight on skin; the alleviation

and/or prevention of adverse effects caused by greenhouse gases; and/or the maintenance and/or improvement of the physical barrier function of the skin. The use of the protein hydrolysate, compounds or combinations described herein can be for the alleviation and/or prevention of adverse effects of air pollution and sunlight on skin.

5 Adverse effects to skin can manifest themselves in the form of a reduction in barrier function; a dull appearance of the skin; a loss of brightness, luminosity and/or glossiness of the skin; a loss in homogeneity in the texture of the skin; an increase in roughness of the skin; the appearance of dark spots; and/or erythema (skin redness).

**[0091]** Thus there is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the skin, wherein the treatment and/or care of the skin is the alleviation or prevention of the adverse effects of the exposome on the skin.

**[0092]** There is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the skin, wherein the treatment and/or care of the skin is the alleviation or prevention of the adverse effects of the air pollution containing particulate matter on the skin, where the particulate matter is derived from road dust, derived from diesel, or is pollen.

**[0093]** There is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the skin, wherein the treatment and/or care of the skin is the alleviation or prevention of the adverse effects of sunlight on the skin. The sunlight can comprise UV-A radiation.

**[0094]** Adverse effects can manifest themselves in the form of a loss in barrier function in the skin. Thus, there is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the skin, wherein the treatment and/or care of the skin is the maintenance and/or improvement of the physical barrier function of the skin.

**[0095]** Adverse effects can manifest themselves in the form of skin having a dull appearance; and/or having a less bright appearance; and/or having a less luminous appearance and/or a less glossy appearance. Thus, there is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the skin, wherein the treatment and/or care of the skin is increasing skin brightness, and/or increasing the gloss of the skin and/or increasing skin luminosity.

**[0096]** The adverse effects, may also lead to the appearance of dark spots. Thus, there is provided the use of the protein hydrolysate, compounds or combinations

35

described herein for the cosmetic, non-therapeutic treatment and/or care of the skin, wherein the treatment and/or care of the skin is reducing dark spots. In particular, the treatment and/or care can involve reducing melanin in the skin.

5 **[0097]** The adverse effects can manifest themselves in the form of a reduction of skin tone and/or skin texture homogeneity and/or an increase of skin roughness. Thus, there is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the skin, wherein the treatment and/or care of the skin is increasing skin tone and/or increasing the homogeneity of skin texture and/or reducing skin roughness.

10 **[0098]** The adverse effects can manifest themselves in the form of erythema or skin redness. Thus, there is provided the use of the protein hydrolysate, compounds or combinations described herein for the cosmetic, non-therapeutic treatment and/or care of the skin, wherein the treatment and/or care of the skin is reducing skin redness.

15 **[0099]** In one aspect, the invention relates to a method of treatment and/or care of the skin and/or hair comprising administering a protein hydrolysate of azuki beans according to the first aspect of the invention, or a compound chosen from:

- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
- (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
- (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);
- 20 (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);
- (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5), and

stereoisomers, and cosmetically acceptable salts thereof, or a combination of said compound and a filtrate obtained by the fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc* to a subject. The treatment and/or care may be a cosmetic, non-therapeutic treatment and/or care. The protein hydrolysate, compound or combination described above may be present in a cosmetic composition, for example a cosmetic composition as described herein. The method can be for the treatment and/or care of the skin and/or hair as described above in relation to applications (uses) of the protein hydrolysate, compounds and/or combinations of the invention.

25 **[00100]** The treatment and/or care is the alleviation or prevention of the adverse effects of the exposome on the skin and/or hair; the alleviation or prevention of the adverse effects of air pollution on the skin and/or hair; the alleviation or prevention of the adverse effects of sunlight on the skin and/or hair; the alleviation or prevention of the adverse effects of chemical treatment of the hair; the alleviation or prevention of the

adverse effects of heat stress on the hair; and/or the maintenance and/or improvement of the physical barrier function of the skin.

**[00101]** In the applications of the protein hydrolysate, compounds, and combinations of the invention, the treatment and/or care can be preventative in that it serves to protect the skin and/or hair from adverse effects. In this case, the protein hydrolysate, compounds, and combinations of the invention are applied to the skin and/or hair prior to exposure of the stressor. The treatment and/or care can be reparative or restorative, i.e. where alleviation has a reparative or restorative function, (e.g. with respect to aesthetic properties). In this case, the the protein hydrolysate, compounds, and combinations of the invention can be applied to the skin and/or hair during or after exposure of the stressor.

**[00102]** For the above described methods of the invention, topical or transdermal application can be carried out by iontophoresis, sonophoresis, electroporation, mechanical pressure, osmotic pressure gradient, occlusive cure, microinjections, by needle-free injections by means of pressure, by microelectric patches, face masks or any combination thereof.

**[00103]** For the above described methods of the invention, the frequency of application or administration can vary greatly, depending on the needs of each subject, with a recommendation of an application from once a month to ten times a day, preferably from once a week to four times a day, more preferably from three times a week to twice a day, even more preferably once a day.

**[00104]** The invention also provides for the use of the protein hydrolysate of the invention in a therapeutic treatment of the skin. Thus the invention provides a protein hydrolysate as disclosed herein for use as a medicament. In particular, the invention provides a protein hydrolysate as disclosed herein for use in the prevention or treatment of a disease or disorder associated with the exposome, exposure of the skin to air pollution, sunlight and/or greenhouse gases, and/or associated with an impaired physical barrier function of the skin. Further, the invention provides for the use of a compound of formula (I), its stereoisomers and/or its pharmaceutically acceptable salts for the manufacture of a medicament for the treatment or prevention of a disease or disorder.

**[00105]** The invention is further defined by the following non-limiting examples.

#### **EXAMPLE 1**

Preparation of ground azuki beans

**[00106]** The first step was the processing of the raw beans into a fine powder of brown granulated appearance. The aim of this stage is to reduce particle size as much as possible in order to facilitate protein solubilization. The azuki beans were ground into a flour and larger fragments were sifted out.

## 5 **EXAMPLE 2**

Enzymatic hydrolysis

2.1 Pre-treatment of the flour

**[00107]** Preliminary treatment of the ground azuki beans (also referred to herein as “flour”) increases the content of water-soluble protein in the hydrolysis media, prior to enzyme addition. The flour was suspended in deionized water at a concentration of 21.8 g of flour/Kg of water (w/w) and transferred into a suitable reactor vessel equipped with pH and temperature probes providing real-time monitoring. Initial pH was raised from 6.5-6.8 to 7.0 by addition of a basic solution (i.e. NaOH 1N) and, when needed, more solvent was added to reach a desired concentration. The conditions were maintained for 1 hour at room temperature ( $25 \pm 2^\circ\text{C}$ ). The resulting suspension is referred to as the “protein suspension” – it is a suspension of ground azuki beans in water containing proteins from the ground azuki beans that have been solubilised.

2.2. Enzymatic hydrolysis

**[00108]** Enzyme was added to the protein suspension and the mixture stirred for 1 hour at room temperature ( $25 \pm 2^\circ\text{C}$ ). Agitation conditions were adjusted to ensure homogenous and vigorous stirring, while keeping foam formation at minimum levels. Then, alcalase was added to the protein suspension. Alcalase is a subtilisin type enzyme. Preferred hydrolysis conditions are defined by the use of subtilisin-type Alcalase AF 2.4L (CAS no. 9014-01-1, EC 3.4.21.62, Novozymes®) enzymes.

**[00109]** Appropriate progress of hydrolysis was monitored by changes in pH. A drop in pH is associated with the release of peptide fragments and subsequent build up of acidic carboxylic acids. Typically, crude hydrolyzate suffers slight acidification during the process (0.2-0.3 drop in pH units).

30

2.3 Enzyme inactivation

**[00110]** After enzymatic hydrolysis, the enzyme was inactivated by increasing temperature to at least  $70^\circ\text{C}$  and maintaining the temperature for at least 20 min. The hydrolysate obtained is hereinafter referred as “crude hydrolysate”.

**EXAMPLE 3**Purification

5 [00111] With the aim to enrich the mixture in the desired active components (i.e. peptides) further clarification and dialysis steps can be performed as described below in more detail.

## 3.1. Centrifugation.

10 [00112] Hydrolyzed Azuki suspension (“crude hydrolyzate”) is optimally centrifuged in continuous flow (i.e. using Westfalia CSA-1 centrifuge or similar equipment) at 10.000xG, with an optimal flow of 50 l/h. Supernatant was collected, whereas pellet was discarded as waste material. Supernatant turbidity was controlled throughout the operation taking a representative sample, and maintained below 0.2 AU/g of protein at 600 nm (relative to an appropriate blank in these conditions).

## 15 3.2. Frontal filtration.

[00113] The supernatant obtained from step 3.1. was passed through filters of varying size under pump-generated pressure. First, a 1,2 µm pore filter was used to discard the bulk of large particles that were not eliminated during centrifugation. This first filtration step is optional and the purpose is to reduce the number of small pore size filters  
20 needed. Then, the obtained filtrate was subsequently filtered through 0.45 and 0.22 µm pore-sized filters.

## 3.3. Ultrafiltration.

25 [00114] The filtrate obtained in Example 3.2. was further ultrafiltered through ultrafiltration membrane (i.e. Millipore ref. P2PLBCC05, 0.5 m<sup>2</sup>/cassette) to remove fragments with molecular weight higher than 3 KDa. The resulting filtrate obtained is also referred to as “purified hydrolysate” or “permeate”.

**EXAMPLE 4**

## 30 Purified hydrolysate characterization

[00115] Twelve different batches of purified hydrolyzate obtained as described from Examples 1 to 3 were analyzed to determine the dry weight, protein content and antioxidant activity.

- 35 - Dry weight was determined by weight difference after drying at 110°C.  
- Protein content was determined by using a BCA Assay kit.

- Antioxidant activity was determined by DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay.

Briefly, 50  $\mu$ L of 100  $\mu$ M DPPH in EtOH were mixed with 50  $\mu$ L of purified hydrolysate and incubated in the dark at 37°C for 20 min and absorbance measured at 517 nm.

The inhibition percentage is calculated from the following formula:

$$\text{Inhibition (\%)} = \frac{(\text{Blank Absorbance} - \text{Sample Absorbance})}{\text{Blank absorbance}} \times 100$$

- Polyphenol content as determined by Folin-Ciocalteu method.

**[00116]** The dry weight of the different batches was on average from 2 to 5 mg of dry weight / purified hydrolysate.

The protein content ranged from 19 to 35% (w/w).

DPPH activity ranged from 74 to 80%.

Polyphenol content was from 0.15 to 0.30 mg/mL.

#### **EXAMPLE 5**

##### In vitro repair effect on follicle Dermal Papilla Cells from scalp

**[00117]** Human Follicle Dermal Papilla Cells (HFDPC) derived from scalp were cultivated with Follicle Dermal Papilla Cell Growth Medium at 37°C and 5% CO<sub>2</sub>. These cells were stressed with Particle Matters (PM) at 0.1  $\mu$ g/cm<sup>2</sup> (from European Reference Material; Ref.CZ100) followed by UV-A irradiation (LED source, emission peak at 365nm, 3 J/cm<sup>2</sup>) in 1X PBS buffer (Phosphate Buffered Saline, pH 7.4). The cells were treated with the stressors for 13 min. Just after stress exposure, cells were treated with purified hydrolysate of Example 3 at 1% (v/v) in culture medium. The anti-oxidant N-acetyl-cysteine (NAC) was used as a positive control. Two (2) hours after the intracellular levels of protein carbonylation were measured.

##### Analysis of Protein Carbonylation:

**[00118]** Carbonylated proteins in hair shafts or HFDPC were labeled in situ using a fluorescent probe functionalized to specifically bind carbonyl groups in proteins [Baraibar MA et al., "Proteomic quantification and identification of carbonylated proteins upon oxidative stress and during cellular aging" J Proteomics (2013) 30;92:63-70]. Specific fluorescent signals (relative fluorescent units) per experimental group (n=6) were

collected and analyzed with ImageJ software. A relative value (%) of Carbonylation Level per experimental group was obtained respect to basal control.

Repair value (%) was obtained for the experimental groups accordingly to the following formula:

$$5 \quad \text{Repair value} = [(\text{Carbonylation Stress} - \text{Carbonylation group X}) / (\text{Carbonylation Stress} - \text{Carbonylation Control})] \times 100$$

**[00119]** The control group was considered at maximum efficiency (100%) and the stress group at minimum efficiency (at 0%):

Exp. Group	Mean $\pm$ SEM	Repairing efficacy (%)
<i>Control</i>	100 $\pm$ 3.3	100%
<i>Stress (PM + UV-A)</i>	126.9 $\pm$ 3.0	0%
<i>Azuki 1% (v/v) + Stress</i>	119.4 $\pm$ 3.0	28%

Table 1

10

**[00120]** The purified hydrolyzate significantly repaired and protected from the deleterious effect of particle matters and UV-A radiation.

### EXAMPLE 6

#### In vitro protection evaluation on follicle Dermal Papilla Cells from scalp

15 **[00121]** Human Follicle Dermal Papilla Cells (HFDPC) derived from scalp were cultivated with HFDPC Growth Medium at 37°C and 5% CO<sub>2</sub>. These cells were treated with purified hydrolyzate according to Example 3 at 1% (v/v) in culture medium for 24 hours at 37°C and 5% CO<sub>2</sub> (prior to stress). Next day, cells were stressed with Particle Matters at 0.1  $\mu\text{g}/\text{cm}^2$  (from European Reference Material; Ref.CZ100) and Cedar pollen (Ref. Cedar Pollen-Cj\*; LG-5280 Euromedex; 5 ng/cm<sup>2</sup>) followed by UV-A irradiation (LED source, emission peak at 365nm, 3 J/cm<sup>2</sup>) in 1X PBS buffer (Phosphate Buffered Saline, pH 7.4). Stress treatment was conducted for 13 minutes. After treatments, cells were fixed and carbonyl protein labeled in situ. The detection of protein oxidation (carbonylation) was performed by epifluorescence microscopy on fixed cells.

25 **[00122]** Just after treatments and stress exposure, cells (HFDPC) were fixed on the plate with an Ethanol / Acetic Acid solution. carbonylated proteins were labeled in situ using a fluorescent probe (Ex = 647 nm / Em = 650 nm) functionalized to specifically bind carbonyl groups. Fluorescent images were collected with an epifluorescent

microscope (ThermoFisher, Evos M5000) and analyzed with ImageJ software (Schneider, 2012). Image collection for the different conditions was achieved using identical conditions of acquisition (40X objective).

5 **[00123]** The raw images (were collected in a range of intensity of specific signal from 0 to 65535 and analyzed with ImageJ software. The intensity of carbonylation was determined by integration of the specific fluorescence signal normalized by the evaluated area.

**[00124]** An efficacy value (%) was obtained for the experimental groups accordingly to the following formula:

10 Efficacy % of sample= [(Oxydation level Stress - oxidation level in sample tested)/(oxidation level Stress - oxidation level Control)] x 100

**[00125]** As reference, the control group is considered the maximum efficiency (100%) and the stress group the minimum efficiency (at 0%):

Exp. Group	Mean ± SEM	Protection efficacy (%)
Control	100 ± 0.4 ****	100%
Stress (PM + UV-A+ Cedar pollen)	130 ± 2.9	0%
Azuki 1% (V/V) + Stress	117 ± 1.0**	43%

p-Values were obtained by ANOVA variance analysis followed by Dunnett's post-hoc test for multi-comparisons in comparison with stress. \*\*\*\*p-Value < 0,0001 \*\*\*p-Value < 0,001 \*\*p-Value < 0,01 \*p-Value < 0,05

Table 2

15 **[00126]** The purified hydrolysate protected from the adverse effects (carbonylation) caused by stressors.

**EXAMPLE 7**

Ex vivo protection evaluation in Caucassian Hair Shafts (ex vivo)

20 **[00127]** Caucasian hair shafts (female donor; light brown) were treated with hydrolysate of Example 3 at 1% (v/v) (active ingredient solubilized in ultra-pure water, leave on (no washing steps)) for 30 minutes with gently movement at room temperature. After this time, hair shafts were dried at room temperature and were stressed with

Particle Matters at 150 µg/cm<sup>2</sup> (from European Reference Material; Ref. CZ100) followed by UV-A irradiation (LED source, emission peak at 365 nm, 84 J/cm<sup>2</sup>) for 6 h .

**[00128]** After stress exposure Proteins Carbonylation was determined as described in Example 5.

5

Carbonylation level %	MEAN ± SEM	Protection efficacy
BASAL CONTROL	100 ± 8.06 ****	100%
STRESS NON-TREATED	214 ± 9.01	0%
AZUKI 1% (v/v) (STRESS)	149.8 ± 5.71 ***	56.34%
Positive <b>control</b> NAC (stress)	166.8 ± 5.41**	41.39%
p-Values were obtained by ANOVA variance analysis followed by Dunnett's post-hoc test for multi-comparisons in comparison with stress. ****p-Value < 0.0001; ***p-Value < 0.001; **p-Value < 0.01		
Table 3		

**[00129]** Results confirm the protective effect of purified hydrolysates.

**EXAMPLE 8**

Ex vivo protection evaluation in Asian hair shafts

10 **[00130]** Asian hair shafts, were treated with purified hydrolysate according to Example 3 at 1% (v/v) (active ingredient solubilized in ultra-pure water, leave on (no washing steps)) for 30 minutes with a gently movement at room temperature. After this time, hair shafts were dried at natural evaporation at room temperature and were stressed with Cedar pollen (Ref. Cedar Pollen-Cj\*; LG-5280 Euromedex ; 500 ng/cm<sup>2</sup>),  
15 followed by Particulate Matter application (150 µg/cm<sup>2</sup>, applied as powder; from European Reference Material; Ref. ERM-CZ100) and UV-A irradiation (LED source, emission peak at 365 nm, 84 J/cm<sup>2</sup>; 6 hours of irradiation).

**[00131]** After stress exposure protein carbonylation was measured. Hair shafts from each experimental condition were sampled, cryo-preserved in Cryomatrix (OCT), snap-frozen into liquid nitrogen and kept at -80°C until OxiProteome View analysis. Hair shaft  
20

cross-sections (perpendicular to the hair axis, sagittal view) of 3 μm of thickness were obtained using a cryostat (Leica) for the analysis. For lateral (cuticle) view, carbonylated proteins were labeled on entire hair shafts. Carbonylated proteins were labeled in situ using a fluorescent probe (Ex = 647 nm / Em = 650 nm) functionalized to specifically bind carbonyl groups. Fluorescent images were collected with an epifluorescent microscope (ThermoFisher, Evos M5000) and analyzed with ImageJ software (Schneider, 2012). Image collection for the different conditions was achieved using identical conditions of acquisition (40X objective). The raw images were collected in a range of intensity of specific signal from 0 to 65535 and analyzed with ImageJ software. The intensity of carbonylation was obtained by the integration of the specific fluorescence signal normalized by the evaluated area. For hair shaft cross-sections, in each image, the quantification of carbonylation level was independently obtained for the different compartments (cuticle or cortex) and for the whole hair. Three (3) images per condition were used to quantify the carbonylation levels; the mean value and standard deviation were obtained. Data treatment and statistical analysis were achieved by using graphPad Prism (La Jolla, California, USA).

**[00132]** An efficacy value (%) was obtained for the experimental groups accordingly to the following formula.

$$\text{Efficacy \% (test group x)} = \frac{[\text{Oxidation level (Stress)} - \text{Oxidation level (group X)}]}{[\text{Oxidation level (Stress)} - \text{Oxidation level (Control)}]} * 100$$

As reference, the control group was considered the maximum efficiency (100%) and the stress group the minimum efficiency (at 0%):

WHOLE HAIR		
Exp. Group	Mean ± SEM	Protection efficacy (%)
Control	100 ± 10.2 ****	100%
Stress (PM + UV-A)	215 ± 4.1	0%
Azuki 1% + Stress	114 ± 2.9 ****	88%
CUTICLE		
Exp. Group	Mean ± SEM	Protection efficacy (%)
Control	100 ± 15.5 ***	100%
Stress (PM + UV-A)	250 ± 3.2	0%

Azuki 1% + Stress	164 ± 13.1 **	57%
CORTEX		
Exp. Group	Mean ± SEM	Protection efficacy (%)
Control	100 ± 5.7 ***	100%
Stress (PM + UV-A)	190 ± 10.1	0%
Azuki 1% + Stress	83 ± 3.8 ****	100%

p-Values were obtained by ANOVA variance analysis followed by Dunnett's post-hoc test for multi-comparisons in comparison with stress. \*\*\*\*p-Value < 0.0001 \*\*\*p-Value < 0.001 \*\*p-Value < 0.01 \*p-Value < 0.05

Table 4

**EXAMPLE 9**

Ex vivo protection evaluation in African hair shafts

**[00133]** African hair shafts, were treated with Azuki hydrolysate of Example 3 at 1% (v/v) (active ingredient solubilized in ultra-pure water, leave on (no washing steps)) during 30 minutes in a gently movement at room temperature. After this time, hair shafts were dried at room temperature and were stressed with Particulate Matter application (150 µg/cm<sup>2</sup>, applied as powder; from European Reference Material; Ref. ERM-CZ100) and UV-A irradiation (LED source, emission peak at 365 nm, 84 J/cm<sup>2</sup>). The stress treatment was conducted for 6h.

**[00134]** After stress exposure Proteins Carbonylation was measured as determined in Example 8.

WHOLE HAIR		
Exp. Group	Mean ± SEM	Protection efficacy (%)
Control	100 ± 2.0****	100%
Stress (PM + UV-A)	159 ± 5.7	0%
Azuki 1% + Stress	131 ± 2.9**	46%
CUTICLE		
Exp. Group	Mean SEM	Protection efficacy (%)

Control	100 ± 3.2****	100%
Stress (PM + UV-A)	177 ± 3.5	0%
Azuki 1% + Stress	148 ± 4.6**	38%
CORTEX		
Exp. Group	Mean ± SEM	Protection efficacy (%)
Control	100 ± 2.2**	100%
Stress (PM + UV-A)	138 ± 0.9	0%
Azuki 1% + Stress	121 ± 8.1 <sup>IS</sup>	44%

p-Values were obtained by ANOVA variance analysis followed by Dunnett's post-hoc test for multi-comparisons in comparison with stress. \*\*\*\*p-Value <0.0001; \*\*\*p-Value < 0.001; \*\*p-Value <0.01; \*p-Value < 0.05; IS p-Value < 0,10

Table 5

## EXAMPLE 10

### Ex vivo defense evaluation in Latin American Hair Shafts

5 [00135] Latin American curly hair shafts, were treated with a commercial dye product (chocolate color) with Azuki purified hydrolysate of Example 3 at 1% (v/v) (active ingredient solubilized in the dyeing product) for 30 minutes of contact.

10 [00136] In situ detection of fluorescein diffusion was performed by epifluorescence microscopy or hair cross sections. Hair shafts from each experimental condition were dipped into a fluorophore solution (fluorescein; Ex = 498 nm / Em = 517 nm). Then, the excess of fluorophore was eliminated by extensive wash steps with ultra-pure water and the hair shafts sampled, cryo-preserved in Cryomatrix (OCT), snap-frozen into liquid nitrogen and kept at -80°C until analysis. Hair shaft cross-sections (perpendicular to the hair axis, sagittal view) of 3 µm of thickness were obtained using a cryostat (Leica) for the analysis. Fluorescent images were collected with an epi-fluorescent microscope

15 (ThermoFisher, Evos M5000) and analyzed with ImageJ software (Schneider, 2012). Image collection for the different conditions was achieved using identical conditions of acquisition (40X objective).

[00137] The raw images were collected in a range of intensity of specific signal from 0 to 65535 and analyzed with ImageJ software. The maximum intensity of diffused

fluorophore was obtained and quantified by the integration of the specific fluorescence signal among the axis of penetration (from cuticle to cortex).

**[00138]** Three (3) images per condition were used to quantify the fluorescence intensity levels (R.F.U.); the mean value and standard deviation were obtained. Data treatment and statistical analysis were achieved by using graphPad Prism (La Jolla, California, USA) and included in the annexed file (Annex1- OxiProteomeView-D06CB.xlsx).

**[00139]** An efficacy value (%) was obtained for the experimental groups accordingly to the following formula, reported in the annexed excel file.

**Efficacy % (test group X) = [Fluorescence intensity (Stress) – Fluorescence intensity (test group)] / [Fluorescence intensity (Stress) – Fluorescence intensity (Control)] \* 100**

**[00140]** As references, the control group is considered at maximum efficiency (100%) and the stress group at minimum efficiency (at 0%):

Exp. Group	Mean ± SEM	Defense efficacy (%)
Control	100 ± 1.04 ****	100%
Dying	233 ± 11.96	0%
Azuki 1% + Dying	151 ± 8.31 ***	62%

p-Values were obtained by ANOVA variance analysis followed by Dunnett's post-hoc test for multi-comparisons in comparison with stress. \*\*\*\*p-Value < 0.001; \*\*\*p-Value < 0.001; \*\*p-Value < 0.01; \*p-Value < 0.05

Table 6

### EXAMPLE 11

**15** Ex vivo repairing efficacy in African hair Shafts

**[00141]** African hair shafts, were stressed heating at 95°C during 30 min. After the stress exposure hair shafts were treated with Azuki purified hydrolysate at 1% (v/v) (active ingredient solubilized in ultra-pure water) for 30 minutes.

**20** In situ detection of fluorescein diffusion was performed by epifluorescence microscopy or hair cross sections.

**[00142]** Just after treatments, hair shafts from each experimental condition were dipped into a fluorophore solution (fluorescein; Ex = 498 nm / Em = 517 nm). Then, the excess of fluorophore was eliminated by extensive wash steps with ultra-pure water and

the hair shafts sampled, cryo-preserved in Cryomatrix (OCT), snap-frozen into liquid nitrogen and kept at -80°C until analysis. Hair shaft cross-sections (perpendicular to the hair axis, sagittal view) of 3 µm of thickness were obtained using a cryostat (Leica) for the analysis. Fluorescent images were collected with an epi-fluorescent microscope (ThermoFisher, Evos M5000) and analyzed with ImageJ software (Schneider, 2012). Image collection for the different conditions was achieved using identical conditions of acquisition (40X objective).

**[00143]** The raw images were collected in a range of intensity of specific signal from 0 to 65535 and analyzed with ImageJ software. The maximum intensity of diffused fluorophore was obtained and quantified by the integration of the specific fluorescence signal among the axis of penetration (from cuticle to cortex). Three (3) images per condition were used to quantify the fluorescence intensity levels (R.F.U.); the mean value and standard deviation were obtained. Data treatment and statistical analysis were achieved by using graphPad Prism (La Jolla, California, USA)

**[00144]** An efficacy value (%) was obtained for the experimental groups accordingly to the following formula:

$$\text{Efficacy \% (test group X)} = \frac{[\text{Fluorescence intensity (Stress)} - \text{Fluorescence intensity (test group)}]}{[\text{Fluorescence intensity (Stress)} - \text{Fluorescence intensity (Control)}]} * 100$$

**[00145]** The control group is considered at maximum efficiency (100%) and the stress group at minimum efficiency (at 0%):

Exp. Group	Mean ± SEM	Repair efficacy (%)
Control	100 ± 11.0*	100%
Heating	212 ± 26.3	0%
Azuki 1% (v/v) + Heating	114 ± 24.5*	87%

p-Values were obtained by ANOVA variance analysis followed by Dunnett's post-hoc test for multi-comparisons in comparison with stress.

\*p-Value < 0.05

Table 7

## EXAMPLE 12

### Protection and defense against lipid peroxidation in hair samples

[00146] An aqueous solution of purified hydrolysate according to Example 3 was prepared at 2% (v/v in water) ("active ingredient").

5 [00147] For protection test: 300 mg of hair were weighted in Flacon Tubes (Test) and 3 mL of active ingredient were added and left on an orbital shaker for 2h. After the incubation time with the active ingredient, the hair was dried on cellulose paper for 30 min. After drying, 150 mg of Treated Hair were weighted in Petri Dishes and placed in a solar simulator for irradiation.

10 [00148] For protection and defense test: 300 mg of hair were weighted in Flacon Tubes (Test) and 3 mL active ingredient were added and left on an orbital shaker for 1h30min. After the incubation time with the active ingredient, the hair was dried on cellulose paper for 30 min. Once samples were dried, 150 mg of Treated Hair were weighted in Falcon tubes and 1.5 mL of active ingredient added again thereby being also present during the irradiation treatment. The solution was vortexed and all the contents (active ingredient+Hair) were transferred to a Petri dish for solar Irradiation

15 [00149] For defense test: 150 mg of untreated Hair were weighted in Falcon tubes and 1.5 mL of active ingredient added thereby being present during the irradiation treatment. The solution was vortexed and all the contents (active ingredinet+Hair) were transferred to a Petri dish for solar Irradiation.

20 [00150] For control stress samples (untreated with active ingredient but irradiated hair), 150 mg of hair were weighed on Petri dishes for solar irradiation.

[00151] All samples were Irradiated in a solar simulator (Solar simulator SOL500 Honle, 4-5 mW/cm<sup>2</sup>) for 2 h.

25 [00152] Sample preparation for HPLC Analysis: Samples from the protection test were directly used for HPLC analysis whereas samples of the "protection and defense"; and "defense" tests, were dried on cellulose paper for 30 min. 15-30 mg of hair samples were weighted and 1 mL of MeOH is added and sonicated for 15 min. After sonication, in 10 mL glass tubes, the following reagents are added: 500  $\mu$ L sample (extracted previously in MeOH), 750  $\mu$ L H<sub>3</sub>PO<sub>4</sub>, 250  $\mu$ L 2-Thiobarbituric Acid Solution at 42 mM in NaOH 1N and 450  $\mu$ L H<sub>2</sub>O-MilliQ. Tubes are vortexed and incubated at 90°C for 60 min.

30 Subsequently, 1.95 mL of Alkaline MeOH are added and vortexed before the HPLC analysis. A Kromasil 100 C-18, 5  $\mu$ m, 25 x 4.5 cm columns is used with a High Performance Liquid Chromatography (Shimadzu): an isocratic method of 40%B during 15min (A: 50 mM Buffer Phosphate pH 6.8, B:MeOH), 1mL/min, 50  $\mu$ L is injected at 36°C. The detector use is a fluorescence detector at  $\lambda_{ex}$  = 527 nm and  $\lambda_{em}$  = 551 nm.

35

PROTECTION			
	CONTROL	2h UV	2% AZUKI
MEAN	44.8	100.0	69.8
SEM	1.0	4.9	3.9
P VALUE			< 0.0001
% EFFICACY			30.2

Table 8A.

PROTECTION+DEFENSE			
	CONTROL	2h UV	2% AZUKI
MEAN	48.6	100,0	63,9
SEM	8.4	7.8	9.5
P VALUE			< 0,0001
% EFFICACY			36.1

Table 8B

DEFENSE			
	CONTROL	2h UV	2% AZUKI
MEAN	54.4	100.0	82.3
SEM	2.7	7.5	7.0
P VALUE			0.0181
% EFFICACY			17.7

Table 8C

5

**EXAMPLE 13**

Human dermal fibroblasts and human epidermal keratinocytes Cells (in vitro), protection and defense evaluation.

10 **[00153]** Human Dermal Fibroblasts (HDFa) and Human Epidermal Keratinocytes (HEKa) were co-cultivated in supplemented M106 medium and supplemented KGM2, respectively, at 37°C in 5% CO<sub>2</sub> humidified air. Next day, cells were treated with Azuki hydrolysate of Example 3 at 1% (v/v) in culture medium for 1 hour at 37°C in 5% CO<sub>2</sub>

humidified air. After the protection treatment, fresh culture medium was supplemented with Azuki hydrolysate of Example 3 at 1% (v/v) and cells were stressed with Particle Matters at 2.34 µg/cm<sup>2</sup> (Diesel particulate matters NIST1650B) followed by solar irradiation (Solar simulator SOL500 Honle, 32.4-36 J/cm<sup>2</sup>). Irradiation treatment was of 120 minutes. After solar irradiation, cells were incubated at 38°C in 20% CO<sub>2</sub> humidified air for 2 hours. Just after stress exposure cell viability was measured using Prestoblu<sup>TM</sup> reagent.

**[00154]** Analysis of cell viability: Resazurin, the active ingredient of PrestoBlue<sup>TM</sup> Reagent, is a nontoxic, cell-permeable compound that is virtually non-fluorescent. On entering live cells, the cellular reducing environment reduces resazurin to resorufin, a compound that is highly fluorescent. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence of the media surrounding the cells, being directly proportional to the number of viable cells in each well.

**[00155]** Briefly, medium was aspirated and 100ul/well of a 1/20 dilution of PrestoBlue<sup>TM</sup> in 1xDPBS was added. Then plates were incubated at 37°C in 5% CO<sub>2</sub> humidified air for 10 min, protected from light. The resulting fluorescence was read at excitation wavelength = 560 nm and emission wavelength = 590 nm in a microtiter plate reader (Clariostar, BMG LABTECH).

**[00156]** Each condition was tested at least in 5 replicates/wells and test items were assayed in 3 independent experiments.

% Viability	MEAN ± SEM	Protection efficacy (%)
CONTROL	133.5 ± 4.75****	100
Non-treated + stress	100.0 ± 3.44	0
Azuki 1% + stress	120.6 ± 4.73**	61.53
Azuki 2% + stress	125.0 ± 4.58****	74.51
p-Values were obtained by ANOVA variance analysis followed by Dunnett's post-hoc test for multi-comparisons in comparison with stress. ****p-Value < 0.0001; **p-Value < 0.01		

Table 9

**EXAMPLE 14**

Human dermal fibroblasts and human epidermal keratinocytes Cells (in vitro), repairing evaluation.

**[00157]** Human Dermal Fibroblasts (HDFa) and Human Epidermal Keratinocytes (HEKa) were co-cultivated in supplemented M106 medium and supplemented KGM2, respectively, at 37°C in 5% CO<sub>2</sub> humidified air. Next day, fresh culture medium was added, and cells were stressed with Particle Matters at 2.34 µg/cm<sup>2</sup> (Diesel particulate matters NIST1650B) followed by solar irradiation (Solar simulator SOL500 Honle, 24.3-36 J/cm<sup>2</sup>, 90-120 minutes of irradiation). After solar irradiation, cells were incubated at 38°C in 20% CO<sub>2</sub> humidified air for 22 hours. After the stress exposure, cells were washed and treated with azuki hydrolysate of Example 3 at 1% or 2% (v/v) in culture medium for 6 hours at 37°C in 5% CO<sub>2</sub> humidified air.

**[00158]** Then, cell viability was measured using Prestoblu reagent as disclosed in Example 13.

Viability(%)	MEAN ± SEM	Repairing efficacy (%)
CTR	142.6 ± 3.02****	100
Non-treated + stress	100 ± 3.44	0
Azuki 1% (v/v) + stress	123.4 ± 2.24****	54.78
Azuki 2% (v/v) + stress	116.9 ± 3.01****	39.69

p-Values were obtained by ANOVA variance analysis followed by Dunnett's post-hoc test for multi-comparisons in comparison with stress. \*\*\*\*p-Value < 0.0001

Table 10

**EXAMPLE 15**

In vitro protection from DNA oxidation in human epidermal keratinocytes Cells

**[00159]** At the nuclear level, DNA is susceptible to oxidative damage. Of the four DNA bases, 2'-deoxyguanosine (dG) has the lowest ionization potential and thus is the most susceptible to oxidation. One of the positions where oxidation of dG can occur is at the C8 position, giving rise to 8-oxo-dG. 8-oxo-dG is a useful biomarker of oxidative damage in DNA. If not repaired, this lesion, and/or the products derived from it, may be misread by a polymerase during replication and lead to mispairing with A. Then, during the next round of replication, this A will correctly pair with T leading to a G-T transversion mutation. This replication sequence demonstrates the mutagenic nature of 8-oxo-dG and

underscores the importance of protecting cells against oxidative DNA damage. [Edward Pelle et al. Ultraviolet-B-Induced Oxidative DNA Base Damage in Primary Normal Human Epidermal Keratinocytes and Inhibition by a Hydroxyl Radical Scavenger. J Invest Dermatol. 2003]

5 **[00160]** Human Epidermal Keratinocytes (HEKa) were cultivated in supplemented KGM2 at 37°C in 5% CO<sub>2</sub> humidified air. Then, cells were treated with azuki purified hydrolysate at 1% or 2% (v/v) in culture medium for 1 hour at 37°C in 5% CO<sub>2</sub> humidified air. Then, cells together with the purified hydrolysates were incubated at high temperature (38°C) and high levels of CO<sub>2</sub> (20% CO<sub>2</sub> humidified air) overnight to mimic  
10 the conditions of a “green house effect”. Fresh culture medium was added and cells were stressed with Particle Matters at 1.58 µg/cm<sup>2</sup> (Diesel particulate matters NIST1650B) followed by solar irradiation (Solar simulator SOL500 Honle, 12.15-36 J/cm<sup>2</sup>, 45-120 minutes of irradiation). After the stress exposure, the DNA extraction was carried out in Qiacube with the kit “DNeasy Blood & Tissue Kit” and DNA quantification was performed  
15 using Nanodrop™.

#### Enzymatic hydrolysis

**[00161]** DNA samples were digested to nucleosides using enzymatic hydrolysis. For each sample, 7µL 15mM 8-HQ (8-Hydroxyquinoline), and 50 µL buffer solution (50 mM ammonium acetate, 0.2 mM ZnCl<sub>2</sub>, pH 5.3) were added. Then 3 µL of Nuclease P1 (0.39  
20 unit/µL) and 4 µL of alkaline phosphatase (1 unit/µL in 10 mM Tris pH 7.4) were added, and the solution was incubated at 37 °C for 60 min. The solution was centrifuged for 5 min at 14,000 rpm before LC-MS/MS analysis.

#### HPLC–MS/MS analysis

25 **[00162]** The HPLC-ESI-MS/MS system was a SHIMADZU HPLC model Nexera XR in line with a SHIMADZU triple quadrupole mass spectrometer, model LCMS-8040, equipped with an electrospray ionization source. Nitrogen was used as the drying and nebulizing gas. Argon was used as the collision gas for CID.

30 **[00163]** A InfinityLab Poroshell 120-EC-C18 column (4.6 x 75mm x 2.7µm) were used at a flow rate of 0.5mL/min, maintained at 36°C. A gradient solvent system from water + 0.1% Acetic Acid (solvent A) to methanol (solvent B) was used for the separation. The gradient conditions were: 0 - 9min 5%B - 90%B, 9 – 12min 90%B, 12.1 – 17min 5%B. Analytes were quantified using tandem electrospray ionization mass spectrometry in  
35 positive-ion mode. Product ions were monitored in multiple-reaction monitoring mode.

Mass transition for 8-oxodG was  $m/z$  284.15 > 168.05 and for 8-Hydroxyguanosine  $m/z$  300.00 > 168.05. A secondary transition ( $m/z$  284.15 > 140.1 and  $m/z$  300.00 > 140.05, respectively) was used to confirm the identity of 8-oxodG and 8-Hydroxyguanosine respectively in samples, which are biomarkers of DNA oxidation. Injection volume was 5 25 $\mu$ L.

8-oxodG (%)	
	MEAN $\pm$ SEM
Control	60.70 $\pm$ 8.08**
Non-treated + stress	100.0 $\pm$ 8.90
Azuki 1% (v/v) + stress	67.28 $\pm$ 7.52*
Azuki 2% (v/v) + stress	53.67 $\pm$ 2.90***

p-Values were obtained by ANOVA variance analysis followed by Dunnett's post-hoc test for multi-comparisons in comparison with stress. \*\*\*p-Value < 0.001; \*\*p-Value < 0.01; \*p-Value < 0.05

Table 11A

8-Hydroxiguanosine (%)	
	MEAN $\pm$ SEM
Control	41.23 $\pm$ 5.90****
Non-treated + stress	100.0 $\pm$ 6.05
Azuki 1% + stress	79.15 $\pm$ 5.57*
Azuki 2% + stress	65.25 $\pm$ 3.19***

p-Values were obtained by ANOVA variance analysis followed by Dunnett's post-hoc test for multi-comparisons in comparison with stress. \*\*\*\*p-Value < 0.0001; \*\*\*p-Value < 0.001; \*\*p-Value < 0.01; \*p-Value < 0.05.

Table 11B

**[00164]** Both biomarkers of DNA oxidation (8-oxo-dG and 8-Hydroxiguanosine) were reduced after treatment with the purified hydrolysate compared to non-treated stressed cells, demonstrating the protective effect of the purified hydrolysate.

**EXAMPLE 16**

Antioxidant activity in combination with other ingredients

**[00165]** Suitable preservative [1% (w/w) Dermosoft 700B (INCI: evulinic Acid (and) Sodium Levulinate (and) Glycerin (and) Aqua), 4% (w/w) Leucidal™ SF Max from Active  
 5 Micro Technologies (AMT) (INCI: Lactobacillus Ferment) or 4% (w/w) Leucidal™ Liquid (INCI: Leuconostoc/Radish Root Ferment Filtrate)] was added onto deionized water under continuous stirring. Purified hydrolysate according to Example 3 was added on top of this solution to obtain a concentration of 44-88% (v/v), depending on sample dry weight. Final concentration of the purified protein hydrolysate (INCI: Phaseolus Angularis  
 10 Seed Extract) is then kept constant at 2.2 g per Kg of solution (0.22% (w/w)). Once the resulting solution becomes completely homogeneous, pH is adjusted with citric acid to a value of 4.5-5.0. Samples were maintained for 3 months at 40°C or at room temperature and antioxidant activity determined by DPPH assay.

	% DPPH INHIBITION		
	t=0	3M	
		40°C	room temperature
Purified azuki hydrolysate	73.76	Not tested	Not tested
1%(w/w) Dermosoft 700B	0		
0.5% (w/w) Sodium Benzoate	0		
Glycerin:H <sub>2</sub> O	0		
4% Leucidal™ Liquid	0		
Purified azuki hydrolysate and 1%(w/w) Dermosoft 700B + 4% (w/w) Leucidal™ SF Max	86.0	71.8 (-16.5)	86.5
Purified azuki hydrolysate and 0.5% (w/w) Sodium Benzoate at low pH	64.7	53.4 (-17.4)	61.6
Purified azuki hydrolysate and Glycerin:H <sub>2</sub> O (80:20)	76.0	77.1 (+1.4)	78.3
Purified azuki hydrolysate + 4% Leucidal™ Liquid	86.6	84.1 (-2.9)	87.0

Table 12

15

**[00166]** None of the preservatives demonstrated antioxidant activity. Combination with 4% Leucidal™ Liquid shown a synergistic effect between the purified hydrolysate and Leuconostoc/Radish Root Ferment in Leucidal™ liquid.

**EXAMPLE 17**Isolation and identification of active peptides

[00167] The purified extract according to Example 3 was further fractionated by using semi-preparative high-performance liquid chromatography (HPLC) and antioxidant activity assayed by DPPH for identifying the active fraction or peptides.

[00168] Semipreparative HPLC (Shimadzu Corporation) was used with a LC-8A binary pump, a SCL-8A system controller, a SIL-8A automatic sample injector (20 mL loop), a SPD-6A UV wavelength detector, a C-R6A data recording module, and a fraction collector. Appropriate dilutions of purified hydrolysate in water were loaded into a reverse-phase C<sub>18</sub> column (PuriFlash® PP-15C18-F0080, 31 x 205mm, 15µm; Interchim®). Peptides were separated using an eluent system consisting of eluents A (water/trifluoroacetic acid 1000:1) and B (acetonitrile/trifluoroacetic acid 1000:1). Elution was carried out at a flow of 35 ml/min using the following gradient composition: isocratic of 0% B in 3 min, 0 to 30% of B in 10 min, and 30 to 75% of B in 7 min, monitoring absorbance of eluting peaks at 220 nm.

[00169] Collected tubes were analyzed by analytical HPLC at 3.0 mg/ml using a Cortecs C18+ column (4.6 x 50 mm; 2.7 µm; Waters®) at a flow of 1.6 ml/min using the following gradient composition: isocratic of 0% B in 2 min, 0 to 30% of B in 5 min, and 30 to 75% of B in 5 min, monitoring absorbance of eluting peaks at 220 nm. Different fractions were obtained from the purified hydrolysate and lyophilized for further analysis and separation. Fractions were reconstituted in water and their antioxidant activity was determined by measuring the percentage of DPPH inhibition using the methodology described above.

[00170] Fractions showing the highest DPPH activity were further sub-fractionated. Peptides contained in the different fracts were dissolved in water at a concentration of 25 mg/ml and separated by loading 4 mL in the abovementioned semi-preparative HPLC system using the same PuriFlash column. Elution was performed at a flow of 35 ml/min using the following gradient composition: isocratic of 0% B in 3 min, and 5 to 15% of B in 15 min, monitoring absorbance of eluting peaks at 220 nm. Collected tubes were analyzed by means of similar analytical HPLC conditions as described above but using an isocratic gradient of 0% B in 2 min followed by 5 to 15% of B in 6 min. Different subfractions showing antioxidant activity were obtained.

Identification of antioxidant peptides by high resolution mass spectrometry

**[00171]** Subfraction were separated by reverse-phase nano-HPLC coupled to high resolution LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific). Peptide separation took place using a C18 nanocolumn (ID 75µm, 15cm, 3µm particle diameter, Nikkyo Technos Co. LTD) connected to a trap nanocolumn (ID 100 µm, 2cm, 5µm particle diameter, Thermo Fisher Scientific). A 120 min gradient in the following phases: 0.1% formic acid/ water (phase A), and 0.1% formic acid/ acetonitrile (phase B) was used, at a flow rate 300 nL/min. Real time ionization and peptide fragmentation was achieved by acquiring FT spectra in a resolution of 30.000 FHMW) followed by FT-MS/MS scan and selecting the 10 most intense ions with HCD fragmentation, collision energy of 35% and dynamic exclusion of 0.5 min. All spectra were acquired in positive ion mode.

**[00172]** To identify peptide sequences, the Proteome Discoverer 1.4.0.288 program (Thermo) with MASCOT 2.5 was used. The identified peptides were individually synthesized by applying standard solid phase peptide synthesis, using Fmoc/tBu chemistry protocols. As starting resin, CTC-PS or HO-Trt-Chem Matrix® resin was used. In the latter case, initial resin chlorination took place by overnight reaction with 37% HCl in dry THF. The first amino acid was introduced as a solution in DMF in presence of DIEA as base, followed by capping of the resin with MeOH. The resin was washed with DMF, prior to removal of Fmoc group by treatment with a 20% solution of piperidine in DMF, followed by washings in the same solvent. Subsequent residues were incorporated in DMF employing DIPCDI and Oxyma as coupling reagents. After verification of reaction completion using ninhydrin test, the solvent was filtrated away by suction. After this, the resin was washed with DMF and the Fmoc-deprotection step was repeated as abovementioned or in 2% DBU in DMF. Coupling-deprotection cycles were repeated for introduction of the remaining amino acids, with increasing Fmoc removal treatment times from the 6<sup>th</sup> residue. Oxyma (0.5M) was introduced in the cocktail whenever Asp residue was already incorporated into the growing peptide chain.

**[00173]** The peptidyl-resin was washed and dried using DMF, DCM and diethyl ether and finally left under vacuum overnight to get rid of solvent traces. Cleavage of the crude product from the solid support and removal of the side-chain protecting groups was achieved by acidolysis using TFA and 5% water as scavenger. The peptide was precipitated out of the acidic mixture with Et<sub>2</sub>O, followed by washes with the same solvent and drying under reduced pressure. Crude trifluoroacetate salt peptide was lyophilized and analyzed by analytical HPLC-MS, affording peptide purities over 80%.

**[00174]** Synthesized peptides were further evaluated for its capacity to inhibit DPPH as a reference for their antioxidant potential. The most active peptides as well as their antioxidant activity (DPPH) and presence in the purified hydrolysate are shown in Table 15. All synthesized sequences displayed the ability to inhibit DPPH, being SEQ. ID. NO 1, 2, 3, 4 and 5 the most antioxidant peptides found in the F'2 subfraction.

SEQ ID No.	Sequence	DPPH inhibition (%)	Concentration (ppm)
1	VAEQEQEQAKRQ	78.7	0.023
2	LPQQEQGHDR	77.5	0.033
3	VDAQPEEQQR	100	0.024
4	GINAENNQRN	96.3	0.068
5	EYTGDAAGR	83.9	0.007

Table 13

**EXAMPLE 18**In vitro proliferation assay on Human Epidermal Keratinocyte Cells

**[00175]** Human Epidermal Keratinocyte Cells from adult (HEKa) were cultivated with Keratinocyte Growth Medium II (*Promocell*) supplemented with Supplement Mix C39016-CaCl<sub>2</sub> solution (*Promocell*) at 37°C and 5% CO<sub>2</sub>. Cells were treated with purified hydrolysate of Example 3 at 1% (v/v) in culture medium. After 24 hours incubation, live cells were quantified with PrestoBlue™ Cell Viability Reagent (Thermo Fisher Scientific).

15 - Analysis of cell viability:

Resazurin, the active ingredient of PrestoBlue™ Reagent, is a nontoxic, cell-permeable compound that is virtually non-fluorescent. On entering live cells, the cellular reducing environment reduces resazurin to resorufin, a compound that is highly fluorescent. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence of the media surrounding the cells, being directly proportional to the number of viable cells in each well.

20 - Briefly, medium was aspirated and 100ul/well of a 1/20 dilution of PrestoBlue™ in 1xDPBS was added. Then plates were incubated at 37°C in 5% CO<sub>2</sub> humidified air for 10 min, protected from light. The resulting fluorescence was read at excitation wavelength = 560 nm and emission wavelength = 590 nm in a microtiter plate reader (Clariostar, BMG LABTECH).

25

[00176] Each condition was tested at least in 3 replicates/wells and test items were assayed in 3 independent experiments.

Dose Tested	Cell viability % (MEAN $\pm$ SEM)
CONTROL	100.00 $\pm$ 1.99
Azuki 1% (v/v)	127.62 $\pm$ 14.23 **

Statistical significance: \*\*  $p < 0.01$ , calculated using an unpaired Student's t test.

Table 18

5

### **EXAMPLE 19**

#### Lipid peroxidation repair from solar radiation (rinse-off)

[00177] Eight hundred milligrams (800 mg) of Asian hair are weighted in Petri Dishes and Irradiated in a Solar Simulator (Solar simulator SOL500 Honle, 32.4-36 J/cm<sup>2</sup>) for 2 h. Once the irradiation time is over, 600 mg of damaged hair is weighted in Flacon Tubes and 6 mL of azuki purified hydrolysate at 22  $\mu$ g/mL are added and left on an orbital shaker for 30 min. After the incubation time with the azuki purified hydrolysate, the hair is rinsed with water at 30°C for 30 seconds. Then, half of the hair is left to dry in cellulose paper and the remaining part (300 mg) is treated again with 3 mL azuki purified hydrolysate at 22  $\mu$ g/mL. Samples are left on an orbital shaker for 30 min and then hair is rinsed with water at 30°C for 30 seconds and left to dry until its analysis. Non-damaged (i.e.untreated) Asian hair is used as CONTROL sample. For damage control samples ("solar radiation damaged control"), damaged hair after Solar Irradiation application were used.

[00178] In Eppendorf tubes, 15-30 mg of hair samples are weighted, and 1 mL of methanol is added and sonicated for 15 min. After sonication, in 10 mL glass tubes, the following reagents are added: 500  $\mu$ L sample (extracted previously in methanol), 750  $\mu$ L H<sub>3</sub>PO<sub>4</sub>, 250  $\mu$ L 2-Thiobarbituric Acid Solution at 42 mM in NaOH 1N and 450  $\mu$ L H<sub>2</sub>O-MilliQ. Tubes are vortexed and incubated at 90°C for 60 min. Subsequently, 1.95 mL of alkaline methanol are added and vortexed before the HPLC analysis.

[00179] HPLC analysis is performed under the following conditions: Kromasil 100 C-18, 5  $\mu$ m, 25 x 4.5 cm, an isocratic method at 40%B was used and mobile phases are A: 50 mM Buffer Phosphate pH 6.8 and B: MeOH, a fluorescence detector was used at  $\lambda_{ex}$  = 527 nm and  $\lambda_{em}$  = 551 nm.

30 RESULTS:

**[00180]** When azuki purified hydrolysate is applied once at 22 µg/mL in Solar Radiation Damaged Hair, there is an inhibition of Lipid Peroxidation by 35.6%, as it can be observed in Fig. 1 and Table I.

	<b>Control</b>	<b>2h Solar Radiation</b>	<b>2h Solar Radiation + 22µg/mL azuki purified hydrolysate</b>
<b>MEAN</b>	44.8	100.0	64.4
<b>SEM</b>	1.0	5.3	2.8
<b>P Value</b>	-	-	< 0.001
<b>% Efficacy</b>	-	-	35.6

Table 18

**[00181]** Therefore, results demonstrate a protective effect of azuki purified hydrolysate against solar radiation.

**EXAMPLE 20**

Leave on serum

**[00182]** Leave-on serum was prepared including the following ingredients:

<b>Ingredient</b>	<b>wt %</b>	
WATER	95.20	
DISSOLVINE NA2 P	0.20	
Carbopol® Ultrez 21 polymer	0.35	
Active ingredient	WATER (AQUA)	1.96
	Leucidal™ liquid (LEUCONOSTOC/RADISH ROOT FERMENT FILTRATE)	0.04
	Azuki peptide hydrolysate (PHASEOLUS ANGULARIS SEED EXTRACT)	0.004
	Citric acid	0.0004
POLYSORBATE 20	1.05	
BABY ALOE VERA (PR155)	0.20	
EUXYL PE 9010 (BC042)	0.50	

SODIUM HYDROXIDE 20% w/w	0.50
--------------------------	------

Table 19

5 **EXAMPLE 21**  
Face cream

[00183] Face cream was prepared including the following ingredients:

Ingredient	wt %	
WATER (AQUA)	74.24	
ZEMEA PROPANEDIOL	7.00	
SCHERCEMOL CO	5.00	
GLYCERIN	2.00	
GLUCAM E-20 HUMECANT	2.00	
CAPRYLIC CAPRIC TRIGLYCERIDES	2.50	
NOVEMER EC-2	2.00	
DERMOSOFT 700B	1.00	
CETIOL SB-45	0.50	
DERMOSOFT GMCY	0.50	
CARBOPOL ULTREZ 20 POLYMER	0.30	
SODIUM HYDROXIDE 20% w/w	0.96	
Active ingredient	WATER (AQUA)	1.96
	Leucidal™ liquid (LEUCONOSTOC/RADISH ROOT FERMENT FILTRATE)	0.04
	Azuki peptide hydrolysate (PHASEOLUS ANGULARIS SEED EXTRACT)	0.004
	Citric acid	0.0004

Table 20

10

**EXAMPLE 22**  
In vivo efficacy in hair protection against exposome’s real outdoor conditions

15 [00184] The study was carried out for 14 days. Sixty-two (62) female volunteers aged between 19 and 49 years old were included in the study. Volunteers included in the study had different hair types according to Andre Walker Hair Typing System and at least 50% of them had their hair dyed. Subjects were divided into two groups. One of the groups applied the leave-on serum described in Example 20 (“Active leave-on serum”) and the

other applied a placebo leave-on serum having the same composition except azuki hydrolysate. Regardless of the group, the leave-on serum was applied for 14 days on the whole hair daily. During the study length, all subjects were required to spend daily 4 hours outdoors to expose hair to real outdoor conditions. Subjects served as their own reference and results obtained at 14 days were compared with those obtained at initial time.

**[00185]** Volunteers' hair was clinically evaluated by an expert at initial time and after 14 days of product application. The efficacy of the product against exposome's real outdoor conditions was assessed by:

- Volunteers' hair split ends were evaluated by an expert who performed a clinical evaluation grading split ends through a scale at initial time and 14 days of product application.

	Mean split ends variation (%)	
	Active leave-on serum	Placebo leave-on serum
$[(T14 \text{ days} - T0)/T0] \times 100$	- 12.9 *	-7.4

Table 21. Mean split ends variation of active and placebo leave-on serums after 14 days of product application. \* p <0.05 calculated using paired Wilcoxon test.

**[00186]** Results demonstrate that after 14 days of application of the active leave-on serum there is a significant decrease in split ends respect to initial time.

Volunteers' hair fiber alignment was evaluated by an expert who performed a clinical evaluation grading fiber alignment through a scale at initial time and 14 days of product application.

	Average fiber alignment variation (%)	
	Active leave-on serum	Placebo leave-on serum
$[(T14 \text{ days} - T0)/T0] \times 100$	22.2 **	16.1

Table 22. Average fiber alignment variation of active and placebo leave-on serums after 14 days of product application. \*\* p <0.01 calculated using paired Wilcoxon test.

**[00187]** Results demonstrate that after 14 days of application of the active leave-on serum there is a significant increase in fiber alignment respect to initial time.

- Volunteers' hair frizz was evaluated by an expert who performed a clinical evaluation grading frizz through a scale at initial time and 14 days of product application.

5

	Average frizz variation (%)	
	Active leave-on serum	Placebo leave-on serum
$[(T14 \text{ days} - T0)/T0] \times 100$	- 13.6 **	- 9.4 *

Table 23. Average frizz variation of active and placebo leave-on serums after 14 days of product application. \*\* p < 0.01 and \* p < 0.05 calculated using paired Wilcoxon test.

**[00188]** Results demonstrate that after 14 days of application of active leave-on serum there is a significant decrease in frizz respect to initial time.

10

**EXAMPLE 23**

In vivo efficacy in hair protection against exposome's real outdoor conditions in hair type 3 and 4.

15

**[00189]** The study was carried out for 14 days. Twenty-one (21) female volunteers aged between 23 and 44 years old were included in the study. Volunteers included in the study had hair types 3 (hair which is curly) and 4 (hair which is coiled) according to Andre Walker Hair Typing System and at least 50% of them had their hair dyed. Subjects were divided into two groups. One of the groups applied the leave-on serum described in example 20 ("active leave-on serum") and the other applied a placebo leave-on serum. Regardless of the group, the leave-on serum was applied for 14 days on the whole hair daily. During the study length, all subjects were required to spend daily 4 hours outdoors to expose hair to real outdoor conditions. Subjects served as their own reference and results obtained at 14 days were compared with those obtained at initial time.

20

25

**[00190]** Volunteers' hair was clinically evaluated by an expert at initial time and after 14 days of product application. The efficacy of the product against exposome's real outdoor conditions was assessed by volunteers' hair volume variation.

**[00191]** Hair volume was evaluated by an expert who performed a clinical evaluation grading hair volume through a scale at initial time and 14 days of product application.

	Mean hair volume variation (%)	
	Active leave-on serum	Placebo leave-on serum
$[(T14 \text{ days} - T0)/T0] \times 100$	- 19.9 (Is)	- 13.6

Table 24. Mean hair volume variation of active and placebo leave-on serums after 14 days of product application. Is  $p < 0.1$  calculated using paired Wilcoxon test.

5 **[00192]** Results demonstrate that after 14 days of application of the active leave on serum there is a significant decrease in hair volume respect to initial time in volunteers with hair type 3 and 4.

**EXAMPLE 24**

10 In vivo efficacy in skin protection against exposome's real outdoor conditions in multiethnic skin type volunteers.

**[00193]** The study was carried out for 28 days. Sixty-two (62) female volunteers of multiple ethnicities aged between 19 and 49 years old were included in the study. Subjects were divided into two groups. One of the groups applied the composition described in example 21 ("active cream") on the other applied a placebo cream having the same composition except azuki protein hydrolysate. Regardless of the group, the cream was applied for 28 days on the whole face twice a day at morning and afternoon. During the study length, all subjects were required to spend daily 4 hours outdoors to expose skin to real outdoor conditions. Subjects served as their own reference and results obtained at different study times were compared with those obtained at initial time. The efficacy of the product against exposome's real outdoor conditions was assessed by:

- Barrier function protection assessment by trans epidermal water loss (TEWL): When the barrier function of the skin is damaged an increase in TEWL can be expected. Tewameter® measurements on cheek were carried out at initial time, 14 days and 28 days of product application.

	TEWL variation (%)	
	Active cream	Placebo cream
	0.1	19.8

$[(T14 \text{ days} - T0)/T0] \times 100$		
$[(T28 \text{ days} - T0)/T0] \times 100$	- 0.8	12.2

Table 25. TEWL variation of active and placebo creams after 14 and 28 days of product application. Significance calculated using Wilcoxon paired test of active versus placebo cream at 14 days of application is \* p <0.05.

5 **[00194]** Results demonstrate that after 14 days of application of active cream there is a significant decrease in TEWL respect to placebo cream.

- Skin texture heterogeneity was assessed by the average perimeter of spots in volunteers' cheek. Images at initial time and 28 days of product application were acquired with Colorface® and perimeter average of spots in Region Of Interest (ROI) was measured with Framescan software

	Spot perimeter average variation (%)	
	Active cream	Placebo cream
$[(T28 \text{ days} - T0)/T0] \times 100$	- 3.2 *	- 0.2

Table 26. Spot perimeter average variation of active and placebo creams after 28 days of product application. \* p <0.05 calculated using a t-student test.

15 **[00195]** Results demonstrate that after 28 days of application of active cream there is a significant decrease in spot perimeter respect to initial time, leading to a decrease in skin texture heterogeneity.

- Skin tone homogeneity was evaluated by a dermatologist who performed a clinical evaluation grading skin's tone homogeneity through a scale at initial and 28 days of product application.

	Skin tone homogeneity average variation (%)	
	Active cream	Placebo cream
$[(T28 \text{ days} - T0)/T0] \times 100$	12.5 *	6.7

Table 27. Skin tone homogeneity average variation of active and placebo creams after 28 days of product application. \* p <0.05 calculated using a t-student test.

5 **[00196]** Results demonstrate that after 28 days of application of active cream there is a significant increase in skin's tone homogeneity according to the expert evaluation respect to initial time.

**EXAMPLE 25**

10 In vivo efficacy in skin protection against exposome's real outdoor conditions in Asian ethnic volunteers.

**[00197]** The study was carried out for 28 days. Seven (7) female volunteers of Asian ethnicity aged between 23 and 49 years old were included in the study. Four (4) subjects applied active cream and three (3) placebo cream for 28 days on the whole face twice a day in the morning and afternoon. Subjects applying active cream applied the composition described in example 21 and the subjects applying placebo cream applied the same composition except azuki peptide hydrolysate. During the study length, all subjects were required to spend daily 4 hours outdoors to expose skin to real outdoor conditions. Subjects served as their own reference and results obtained at 28 days were compared with those obtained at initial time.

20 **[00198]** Volunteers' images of forehead were acquired at initial time and 28 days of product application with Colorface®. The efficacy of the product against exposome's real outdoor conditions in Asian volunteers was assessed by different parameters obtained from image analysis:

- Skin erythema was assessed by the average variation of a\* parameter which is used for measuring color related to erythema.

	a* parameter average variation (%)	
	Active cream	Placebo cream
$[(T28 \text{ days} - T0)/T0] \times 100$	- 8.3 *	- 1.7

Table 28. a\* parameter average variation of active and placebo creams after 28 days of product application. \* p <0.05 calculated using paired t-student test.

- Skin melanin was evaluated by the average variation b\* parameter which is used for measuring color related to melanin.

b* parameter average variation (%)		
	Active cream	Placebo cream
$[(T28 \text{ days} - T0)/T0] \times 100$	- 10.5 (ls)	- 2.7

Table 29. b\* parameter average variation of active and placebo creams after 28 days of product application. ls p <0.1 calculated using paired t-student test.

5 **[00199]** Results demonstrate that after 28 days of application of active there is a significant decrease in a\* and b\* parameters respect to placebo cream in Asian volunteers. According to this, skin erythema and skin melanin decreases after 28 day product application in Asian volunteers. This parameters are related with a visual reduction of skin redness and an improving of skin brightnes, respectively.

10 **EXAMPLE 26**

In vivo efficacy in skin protection against exposome's real outdoor conditions in Caucasian ethnic volunteers.

15 **[00200]** The study was carried out for 28 days. Twenty-one (21) female volunteers of Caucasian ethnicity aged between 19 and 39 years old were included in the study. Ten (10) subjects applied active cream and eleven (11) placebo cream for 28 days on the whole face twice a day in the morning and afternoon. Subjects applying active cream applied the composition described in example 21 and the subjects applying placebo cream having the same composition except azuki hydrolysate. During the study length, all subjects were required to spend daily 4 hours outdoors to expose skin to real outdoor conditions. Subjects served as their own reference and results obtained at 28 days were compared with those obtained at initial time.

20 **[00201]** Volunteers' images of cheeks were acquired at initial time and 28 days of product application with Colorface®. The efficacy of the product against exposome's real outdoor conditions in Caucasian volunteers was assessed by a\* parameter obtained from image analysis.

25 **[00202]** Skin erythema was evaluated at initial time and after 28 days of product application. Skin erythema is assessed by the mean variation of a\* parameter which is used for measuring color related to erythema.

a* parameter mean variation (%)
---------------------------------

	Active cream	Placebo cream
$[(T28 \text{ days} - T0)/T0] \times 100$	- 4.5 *	- 0.5

Table 30. a\* parameter mean variation of active and placebo creams after 28 days of product application. \* p <0.05 calculated using paired t-student test.

5 [00203] Results that after 28 days of product application there is a significant decrease in a\* parameter respect to placebo cream in Caucasian volunteers leading to a skin showing less erythema (i.e.skin redness).

**EXAMPE 27**

10 In vivo efficacy in skin protection against exposome’s real outdoor conditions in African ethnic volunteers.

[00204] The study was carried out for 28 days. Nineteen (19) female volunteers of African ethnicity aged between 23 and 44 years old were included in the study. Nine (9) subjects applied active cream and ten (10) placebo cream for 28 days on the whole face twice a day in the morning and afternoon. Subjects applying active cream applied the composition described in example 21 and the subjects applying placebo cream applied the same composition except azuki hydrolysate. During the study length, all subjects were required to spend daily 4 hours outdoors to expose skin to real outdoor conditions. Subjects served as their own reference and results obtained at 28 days were compared with those obtained at initial time.

20 [00205] Volunteers’ images of cheeks were acquired at initial time and 28 days of product application with Colorface®. The efficacy of the product against exposome’s real outdoor conditions in African volunteers was assessed by b\* parameter obtained from image analysis.

25 [00206] Skin melanin was evaluated at initial time and after 28 days of product application. Skin melanin is assessed by the average variation of b\* parameter which is used for measuring color related to melanin.

	b* parameter average variation (%)	
	Active cream	Placebo cream
$[(T28 \text{ days} - T0)/T0] \times 100$	- 4.6 *	- 1.3

Table 31. b\* parameter average variation of active and placebo creams after 28 days of product application. \* p <0.05 calculated using paired t-student test.

- 5 Results demonstrate that after 28 days of product application there is a significant decrease in b\* parameter respect to placebo cream in African volunteers leading to a skin with less melanin (i.e. higher brightness).

**CLAIMS**

1. A protein hydrolysate of azuki beans comprising at least one compound (i.e. 1, 2, 3 or 4 compounds) chosen from:
  - (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
  - 5 (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
  - (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and
  - (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4).
  
2. A protein hydrolysate according to claim 1 comprising:
  - 10 (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1); and
  - (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2).
  
3. A protein hydrolysate according to claim 1 comprising:
  - (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and
  - 15 (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);
  
4. A protein hydrolysate according to claim 3 comprising:
  - (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
  - (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and
  - 20 (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4).
  
5. A protein hydrolysate according to claim 3 comprising:
  - (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
  - (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and
  - 25 (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4).
  
6. A protein hydrolysate of azuki beans according to any one of the preceding claims comprising:
  - (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
  - 30 (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
  - (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3); and
  - (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4).
  
7. A protein hydrolysate of azuki beans according to anyone of the preceding claims further comprising:  
35

- (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5).
8. A protein hydrolysate according to any one of the preceding claims, wherein said hydrolysate does not contain any components having a molecular weight of greater than 3 kDa.
9. A protein hydrolysate according to any one of the preceding claims, wherein, when present, each of the following compounds is present in an amount of from 0.001 to 0.15 ppm or 0.005 to 0.08 ppm:
- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
- (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
- (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);
- (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4); and
- (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5).
10. A protein hydrolysate according to any one of the preceding claims which further comprises at least 0.05 mg, preferably 0.15 mg of polyphenols per milliliter of protein hydrolysate as determined by Folin-Ciocalteu method.
11. A protein hydrolysate according to any one of the preceding claims, wherein said hydrolysate is obtained by enzymatically hydrolysing protein of azuki beans in the presence of a serine endoprotease.
12. A protein hydrolysate according to claim 11, wherein the endoprotease is subtilisin A.
13. A protein hydrolysate according to any one of the preceding claims, wherein the hydrolysate is obtained by:
- a) providing a suspension of ground azuki beans in water;
- b) treating the suspension with alkali so as to solubilize proteins in the ground azuki beans;
- c) enzymatically treating the suspension obtained in b) with a serine endoprotease;
- d) deactivating the serine endoprotease in the suspension obtained in c) to form a crude hydrolysate; and

- e) removing components from the crude hydrolysate having a molecular weight of greater than 3 KDa.
14. A protein hydrolysate according to claim 13, wherein e) involves
- 5 f) recovering a supernatant from the the crude hydrolysate by solid/liquid separation; and
- g) removing components from the supernatant having a molecular weight of greater than 3 KDa.
- 10 15. A protein hydrolysate according to claim 13 or claim 14, wherein b) involves adjusting the pH of the suspension to a pH of from about 6.5 to about 7.5, preferably 7.
- 15 16. A protein hydrolysate according to any one of claims 13 to 15, wherein c) is carried out at a temperature of from 20 to 30°C, preferably 23 to 27°C.
17. A protein hydrolysate according to any one of claims 13 to 16, wherein d) comprises heating the suspension obtained in c) to a temperature of at least 70°C, or from 70 to 90 °C, or from 75 to 85°C.
- 20 18. A compound chosen from:
- (i) Val-Ala-Glu-Gln-Glu-Gln-Glu-Gln-Ala-Lys-Arg-Gln (SEQ ID No.1);
- (ii) Leu-Pro-Gln-Gln-Glu-Gln-Gly-His-Asp-Arg (SEQ ID No.2);
- (iii) Val-Asp-Ala-Gln-Pro-Glu-Glu-Gln-Gln-Arg (SEQ ID No.3);
- 25 (iv) Gly-Ile-Asn-Ala-Glu-Asn-Asn-Gln-Arg-Asn (SEQ ID No.4);
- (v) Glu-Tyr-Thr-Gly-Asp-Ala-Ala-Gly-Arg (SEQ ID No.5), and stereoisomers, and cosmetically acceptable salts thereof.
19. A combination of a protein hydrolysate according to any one of claims 1 to 16 or at least one compound according to claim 17, with filtrate obtained by the
- 30 fermentation of *Raphanus sativus* root by the microorganism *Leuconostoc*.
20. A process for preparing a protein hydrolysate of azuki beans, wherein the hydrolysate is obtained by:
- 35 a) providing a suspension of ground azuki beans in water;

- b) treating the suspension with alkali so as to solubilize proteins in the ground azuki beans;
- c) enzymatically treating the suspension obtained in b) with a serine endoprotease;
- 5 d) deactivating the serine endoprotease in the suspension obtained in c) to form a crude hydrolysate; and
- e) removing components from the crude hydrolysate having a molecular weight of greater than 3 KDa.
- 10 21. A process according to claim 20, wherein e) involves
- f) recovering a supernatant from the the crude hydrolysate by solid/liquid separation; and
- g) removing components from the supernatant having a molecular weight of greater than 3 KDa.
- 15 22. A process according to claim 20 or 21, wherein the serine endoprotease is subtilisin A.
- 20 23. A process according to any one of claims 20 to 22, wherein b) involves adjusting the pH of the suspension to a pH of from about 6.5 to about 7.5, preferably 7.
- 25 24. A process according to any one of claims 20 to 23, wherein c) is carried out at a temperature of from 20 to 30 °C, preferably 23 to 27°C, preferably for a period of about 30 to about 120, about 45 to about 90, or about 60 minutes.
- 30 25. A process according to any one of claims 20 to 24, wherein d) comprises heating the suspension obtained in c) to a temperature of at least 70°C, or from 70 to 90 °C, or from 75 to 85°C, preferably for at least about 20 minutes.
- 35 26. A process according to claim 21, wherein e) involves centrifugation.
27. A process according to claim 21, wherein f) involves ultrafiltration.
28. A process according to any one of claims 20 to 27, wherein the enzyme activity per g of of protein in the ground azuki beans is 0.1 to 0.5 AU/g.

29. A process according to any one of claims 20 to 28, wherein the percentage of protein in the ground azuki beans is 15 to 25 % w/w.
- 5 30. A process according to any one of claims 20 to 29, wherein the ground azuki beans in the suspension of ground azuki beans in water is present in an amount of from 20 to 100 g per Kg of water.
31. A protein hydrolysate obtainable by the process of any one of claims 20 to 30.
- 10 32. A process according to any one of claims 20 to 30, wherein the protein hydrolysate is a protein hydrolysate according to any one of claims 1 to 17.
33. A personal care or cosmetic composition comprising: a protein hydrolysate of azuki beans according to any one of claims 1 to 17 or 31; or a compound according to claim 18 or a stereoisomer, or a cosmetically acceptable salt thereof; or a combination according to claim 19; and at least one cosmetically acceptable excipient or ingredient.
- 15 34. A personal care or cosmetic composition according to claim 33 comprising a protein hydrolysate of azuki beans according to any one of claims 1 to 17 or 31 in an amount from about 0.001 to 10 wt %, preferably from about 0.004 to 5 wt%, based on the total weight of the personal care or cosmetic composition.
- 20 35. A personal care or cosmetic composition according to claim 33 or claim 34, wherein the at least one cosmetically acceptable excipient or ingredient is selected from surfactants, hair conditioning agents, emollients, emulsifiers, humectants, rheology modifiers, vitamins, hair growth promoters, anti-dandruff agents, moisturizing agents, hair fixatives, hair colorants, oxidizing agents, hair dyes, hair bleaching agents, pigment; and combinations thereof.
- 25 36. A personal care or cosmetic composition according to any one of claims 33 to 35, wherein the cosmetic composition is a form selected from the group of creams, multiple emulsions, solutions, liquid crystals, anhydrous compositions, aqueous dispersions, oils, milks, balsams, foams, lotions, gels, cream gels, hydroalcoholic
- 30 35

solutions, hydroglycolic solutions, hydrogels, liniments, soaps, shampoos, conditioners, serums, polysaccharide films, ointments, mousses, pomades, powders, bars, pencils, sprays or aerosols.

- 5      37. Use of a protein hydrolysate of azuki beans according to any one of claims 1 to 17 or 31; or a compound according to claim 18 or a stereoisomer, or a cosmetically acceptable salt thereof; or a combination according to claim 19, for the cosmetic, non-therapeutic treatment and/or care of the skin and/or hair.
- 10     38. Use according to claim 37, wherein the cosmetic, non-therapeutic treatment and/or care is the alleviation or prevention of the adverse effects of the exposome on the skin and/or hair.
- 15     39. Use according to claim 37 or claim 38 wherein the cosmetic, non-therapeutic treatment and/or care is the alleviation or prevention of the adverse effects of air pollution containing particulate matter on the skin and/or hair.
- 20     40. Use according to claim 39, wherein the particulate matter is derived from road dust, derived from diesel or is pollen (e.g. cedar pollen).
- 25     41. Use according to claim 37 or claim 38 wherein the cosmetic, non-therapeutic treatment and/or care is the alleviation or prevention of the adverse effects of sunlight on the skin and/or hair.
- 30     42. Use according to claim 41, wherein the sunlight comprises UV-A irradiation.
43. Use according to claim 41 or claim 42, wherein the adverse effects include the lightening of the colour of the hair due to sunlight.
44. Use according to claim 37 or 38, wherein the cosmetic, non-therapeutic treatment and/or care is the alleviation or prevention of the adverse effects of bleaching or dyeing on hair.

45. Use according to claim 37 or 38, wherein the cosmetic, non-therapeutic treatment and/or care is the alleviation or prevention of the adverse effects of heat stress on hair.
- 5 46. Use according to any one of claims 37 to 45, wherein the adverse effects include damage to the hair caused by peroxidation of lipids in hair and/or carbonylation of proteins in hair.
- 10 47. Use according to any one of claims 37 to 42, wherein the adverse effects include damage to the skin caused by the oxidation of the skin.
48. Use according to any one of claims 37 to 42, wherein the cosmetic, non-therapeutic treatment and/or care is the maintenance and/or improvement of the physical barrier function of the skin.
- 15 49. Use according to any one of claims 37 to 42, claim 47 or claim 48 wherein said use involves increasing keratinocyte proliferation in the skin.
- 20 50. Use according to any one of claims 37 to 42, claim 47 or claim 49 wherein said use comprises increasing skin brightening, and/or increasing the gloss of the skin and/or increasing skin luminosity, and/or reducing dark spots.
- 25 51. Use according to any one of claims 37 to 42, claim 47 or claim 49 wherein said use comprises a reduction of skin redness.
52. Use according to any one of claims 37 to 42, claim 47 or claim 49 wherein said use comprises increasing skin tone and/or increasing skin texture homogeneity and/or reducing skin roughness.
- 30 53. Use according to any one of claims 37 to 46 wherein said use comprises increasing hair maneagability.
- 35 54. Use according to claim 53 wherein the increase of hair maneagability includes a reduction of split ends and/or an increase in fiber alignment and/or reduction of frizz and/or reduction of volume.

55. A (cosmetic, non-therapeutic) method of treatment and/or care of the skin and/or hair comprising administering a protein hydrolysate of azuki beans according to any one of claims 1 to 12, or a compound according to claim 13 or a stereoisomer, or a cosmetically acceptable salt thereof, or a combination according to claim 14 to a subject, wherein the treatment and/or care is the alleviation or prevention of the adverse effects of the exposome on the skin and/or hair; the alleviation or prevention of the adverse effects of air pollution containing particulate matter on the skin and/or hair; the alleviation or prevention of the adverse effects of sunlight on the skin and/or hair; the alleviation or prevention of the adverse effects of dyeing on hair; and/or the maintenance and/or improvement of the physical barrier function of the skin.
56. A method of treatment and/or care according to claim 55 wherein the administration is topical.

# INTERNATIONAL SEARCH REPORT

International application No <b>PCT/IB2023/052524</b>
--

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
<b>INV.</b>	<b>A61K8/64</b>	<b>A61K8/9789</b>
	<b>A61Q5/00</b>	<b>A61Q17/00</b>
	<b>A61Q19/08</b>	<b>A61Q17/04</b>
<b>ADD.</b>		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols)		
<b>A61K A61Q</b>		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
<b>EPO-Internal, BIOSIS, Sequence Search, EMBASE, EMBL, INSPEC, CHEM ABS Data, SCISEARCH, WPI Data</b>		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
<b>X</b>	<b>US 2019/142897 A1 (HWANG JAE KWAN [KR] ET AL) 16 May 2019 (2019-05-16)</b>  <b>paragraphs [0002] - [0021], [0079], [0081], [0085], [0086]; example 2</b> <div style="text-align: center;">----- -/--</div>	<b>1-7, 9-12, 33-36</b>
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <span style="margin-left: 200px;"><input checked="" type="checkbox"/> See patent family annex.</span>		
* Special categories of cited documents :		
"A" document defining the general state of the art which is not considered to be of particular relevance	"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	
"E" earlier application or patent but published on or after the international filing date	"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	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"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	
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search	Date of mailing of the international search report	
<b>26 May 2023</b>	<b>05/06/2023</b>	
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  <b>Steffen, Pierre</b>	

**INTERNATIONAL SEARCH REPORT**

International application No <b>PCT/IB2023/052524</b>
--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SHI ZHENXING ET AL: "Peptides Released from Extruded Adzuki Bean Protein through Simulated Gastrointestinal Digestion Exhibit Anti-inflammatory Activity", JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 69, no. 25, 17 June 2021 (2021-06-17), pages 7028-7036, XP093049803, US ISSN: 0021-8561, DOI: 10.1021/acs.jafc.1c01712 cited in the application page 7029, left-hand column, paragraph 2 - page 7030, left-hand column, paragraph 1; figure 1; table 1; compounds KQS-1</p> <p align="center">-----</p>	20,21, 23-31
A	<p>WO 2012/175868 A2 (LIMOUSINE D APPLIC BIOLOG SOC IND [FR]; PAUFIQUE JEAN [FR]) 27 December 2012 (2012-12-27) page 2, line 10 - page 4, line 10 page 5, line 4 - line 17 page 6 page 11, line 18 - page 16, line 3; claims</p> <p align="center">-----</p>	1-56
A	<p>JP 6 571321 B2 (KYOEI CHEMICAL IND) 4 September 2019 (2019-09-04) paragraphs [0006] - [0022]; claims; examples</p> <p align="center">-----</p>	1-56

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2023/052524

### Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a.  forming part of the international application as filed.
  - b.  furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).
    - accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2.  With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2023/052524

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 2019142897	A1	16-05-2019	CN 109475588 A	15-03-2019
			EP 3453398 A1	13-03-2019
			JP 2019520319 A	18-07-2019
			KR 20170124473 A	10-11-2017
			US 2019142897 A1	16-05-2019
-----				
WO 2012175868	A2	27-12-2012	EP 2720677 A2	23-04-2014
			FR 2976490 A1	21-12-2012
			WO 2012175868 A2	27-12-2012
-----				
JP 6571321	B2	04-09-2019	JP 6571321 B2	04-09-2019
			JP 2016074609 A	12-05-2016
-----				