



(51) International Patent Classification:

A61K 8/64 (2006.01) A61Q 19/00 (2006.01)

A61Q 17/04 (2006.01) A61Q 19/08 (2006.01)

(21) International Application Number:

PCT/EP2018/080472

(22) International Filing Date:

07 November 2018 (07.11.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

PCT/CN2017/112116

21 November 2017 (21.11.2017) CN

17208941.9 20 December 2017 (20.12.2017) EP

(71) Applicant (for all designated States except AE, AG, AU, BB, BH, BN, BW, BZ, CA, CY, EG, GB, GD, GH, GM, IE, IL, IN, JO, KE, KN, KW, LC, LK, LS, MT, MW, MY, NA, NG, NZ, OM, PG, QA, RW, SA, SC, SD, SG, SL, SZ, TT, TZ, UG, US, VC, ZA, ZM, ZW): **UNILEVER N.V.** [NL/NL]; Weena 455, 3013 AL Rotterdam (NL).

(71) Applicant (for AE, AG, AU, BB, BH, BN, BW, BZ, CA, CY, EG, GB, GD, GH, GM, IE, IL, IN, JO, KE, KN, KW, LC, LK, LS, MT, MW, MY, NA, NG, NZ, OM, PG, QA, RW, SA, SC, SD, SG, SL, SZ, TT, TZ, UG, VC, ZA, ZM, ZW only): **UNILEVER PLC** [GB/GB]; a company registered in England and, Wales under company no. 41424 of, Unilever House, 100 Victoria Embankment, London Greater London EC4Y 0DY (GB).

(71) Applicant (for US only): **CONOPCO, INC., D/B/A UNILEVER** [US/US]; 700 Sylvan Avenue (A4), Englewood Cliffs, New Jersey 07632 (US).

(72) Inventors: **HUANG, Nan**; 224-402, 2688 Hunan Road Pudong District, Shanghai, 201315 (CN). **PATIL, Nivedita, Jagdish**; Hindustan Unilever Ltd, Research Centre, 64, Main Road, Whitefield, Bangalore 560 066 (IN). **RAJKUMAR, Savitha**; Hindustan Unilever Ltd, Research Centre, 64, Main Road, Whitefield, Bangalore 560 066 (IN). **TIWARI, Jyoti, Kumar**; Hindustan Unilever Ltd, Research Centre, 64, Main Road, Whitefield, Bangalore 560 066 (IN).

(74) Agent: **ASKEW, Sarah, Elizabeth**; Unilever NV, Unilever Patent Group, Olivier van Noortlaan 120, 3133 AT Vlaardingen (NL).

(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, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, 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, 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:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

Published:

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

(54) Title: A PERSONAL CARE COMPOSITION COMPRISING SERICIN

(57) Abstract: The invention relates to a personal care composition which delivers one or more of benefits selected from anti-aging, hydration, anti-inflammation, anti-oxidant and anti-pollution. This is achieved through a composition comprising 0.1 to 5 wt.% sericin having specific structure.

A PERSONAL CARE COMPOSITION COMPRISING SERICIN

Field of the invention

- 5 The present invention relates to compositions that provide benefits to skin like anti-aging, hydration, anti-inflammation, anti-oxidant and anti-pollution.

Background of the invention

- 10 Cosmetic products for skin care are formulated to deliver a variety of benefits, like sun protection, moisturisation, skin lightening, anti-aging, anti-inflammation and even skin appearance. A variety of synthetically prepared and/or naturally available actives have been used to deliver one or more of such benefits. Such actives may be available in various physical forms like particulate, liquid, emulsion, or as beads and may be of various chemical entities like inorganic powders, polysaccharides, proteins, peptides, salts etc.
- 15 Each active or a combination of actives gives the benefit to the desired extent only if delivered in an optimised delivery medium.

- The present inventors have been working to deliver benefits to skin while researching the proteins derivable from silkworm. They found that specific fractions of sericin (having
- 20 high proportion of beta sheet in the secondary structure), a protein present in silk worm *Bombyx mori*, when included in an emulsion or a gel based skin composition is able to provide highly desirable benefits like anti-aging, hydration, anti-inflammation, anti-oxidant and anti-pollution.

- 25 Sericin, a component of silk, is a protein. Silk is a natural protein fibre derived from the silkworm *Bombyx mori* and is typically composed of two proteins - fibroin (70 to 80 %) and sericin (20 to 30 %), the remainder being a mixture of wax, carbohydrate and inorganic matter. Fibroin is a fibrous glycoprotein, present as a delicate twin thread, enveloped by layers of sericin that help in the formation of a cocoon. To manufacture silk
- 30 from the dried cocoons of silkworm, fibroin is separated from sericin by a so-called 'degumming' process and sericin is discarded in the wastewater. Degumming by heat or heat under pressure has an advantage because it results in fewer impurities. Many

industrial removal methods involve extraction with soaps and detergents, enzymatic hydrolysis, acid or alkaline hydrolysis.

Until recently, sericin was seen as a waste product from silk production. Sericin exists in
5 a wide range of molecular weights, from 10 to over 400 kDa. Sericin occurs mostly in amorphous random coil and to a lesser extent in a beta-sheet structure (Padamwar et al., *Silk sericin and its applications: A review*, Journal of Scientific & Industrial Research, 2004, 63, pp 323-329).

10 US 2009/176965 discloses that the molecular structure of isolated sericin can be modified by irradiating a sericin solution to produce high molecular weight sericin having improved radical scavenging ability and Tyrosinase inhibitory ability. A maximum of 50% beta-sheet structure is obtained (Fig.2, 200 kGy dose radiation). Sericin having modified
15 molecular structure can be used in manufacturing a variety of products for improvement of antioxidant and tyrosinase inhibitory abilities, which include food products, cosmetics and/or pharmaceutical products and medicines.

The above publications do not disclose that certain formulations for skin care comprising specific types of sericin which are high in beta sheet provide highly useful benefits like
20 anti-aging, hydration, anti-inflammation, anti-oxidant and anti-pollution.

It is thus an object of the present invention to provide for a personal care composition especially useful to the skin by delivering anti-aging, hydration, anti-inflammation, anti-oxidant and anti-pollution benefits.

25

Summary of the invention

According to the first aspect of the present invention there is provided a personal care composition comprising (i) 0.1 to 5 % sericin having a proportion of beta-sheet in the
30 secondary structure higher than 70% by weight of the composition, and (ii) a cosmetically acceptable vehicle comprising an emulsion or a gel.

According to another aspect of the present invention there is provided a method of delivering benefits to skin comprising the step of applying a composition of the first aspect on to the desired skin surface wherein the benefits are selected from one or more of anti-aging, hydration, anti-inflammation, anti-oxidant and anti-pollution.

5

Detailed description of the invention

Except in the examples, or where otherwise explicitly indicated, all numbers in this description indicating amounts of material or conditions of reaction, physical properties
10 of materials and/or use may optionally be understood as modified by the word “about”.

All amounts are by weight of the final composition, unless otherwise specified.

It should be noted that in specifying any ranges of values, any particular upper value can
15 be associated with any particular lower value.

For the avoidance of doubt, the word “comprising” is intended to mean “including” but not necessarily “consisting of” or “composed of”. In other words, the listed steps or options need not be exhaustive.

20

The disclosure of the invention as found herein is to be considered to cover all embodiments as found in the claims as being multiply dependent upon each other irrespective of the fact that claims may be found without multiple dependency or redundancy.

25

Where a feature is disclosed with respect to a particular aspect of the invention (for example a composition of the invention), such disclosure is also to be considered to apply to any other aspect of the invention (for example a method of the invention) *mutatis mutandis*.

30

By ‘A topical composition’ or a ‘skin care composition’ as used herein, is meant to include a composition for topical application to the skin of mammals, especially humans. Such a composition could be of the leave-on or of the wash-off/ rinse-off type. By a leave-on

composition is meant a composition that is applied to the desired skin surface and left on for a period of time (say from one minute to 24 hours) after which it may be wiped or rinsed off with water, usually during the regular course of personal washing. By a wash-off/ rinse off composition is meant a composition that is applied to the desired skin surface for a shorter period of time say of the order of seconds or minutes and usually contains sufficient surfactants that aids in cleaning the surface which may be rinsed off with copious amounts of water. The composition may also be formulated into a product which is applied to a human body for improving the appearance, cleansing, odor control or general aesthetics. The composition of the present invention can be in the form of a liquid, lotion, cream, foam, scrub, gel or stick form and may be delivered through a roll-on device or using a propellant containing aerosol can. The composition however comprises an emulsion or a gel as part of the cosmetically acceptable vehicle. "Skin" as used herein is meant to include skin on any part of the body e.g., neck, chest, back, arms, underarms, hands, legs, buttocks and scalp. When the product is used for the underarms it is usually called a deodorant product or a deo product. A class of deodorant product is the so called anti-perspirant (AP) product which contains an AP active which when applied to the axilla of an individual delivers anti-perspirancy and deodorancy benefits.

The present invention relates to a composition comprising sericin having a proportion of beta-sheet in the secondary structure higher than 70% by weight of the composition, and a cosmetically acceptable vehicle comprising an emulsion or a gel.

The term "sericin" as used herein, unless indicated otherwise, refers to sericin polypeptide obtained from the *Bombyx mori* silkworm, said polypeptide having a molecular weight of at least 2 kDa. Sericin from *Bombyx mori* is identified by the UniProtKB database identifier P07856. The term "sericin" also encompasses partially hydrolysed forms of native sericin.

The term "secondary structure" as used herein, unless indicated otherwise, refers to the spatial arrangement of local segments of sericin polypeptides, denoted as alpha helix, beta-sheet or random coil.

The term "alpha helix" as used herein, unless indicated otherwise, refers to a portion of molecular structure that is a rodlike structure in which a tightly coiled backbone forms the inner part of the rod and the side chains extend outward in a helical array. The alpha helix is stabilized by hydrogen bonds between the NH and CO groups of the main chain.

5 In particular, the CO group of each amino acid forms a hydrogen bond with the NH group of the amino acid that is situated four residues ahead (i+4) in the backbone sequence (Berg JM, Tymoczko JL, Stryer L., Biochemistry 5th Ed. New York, W H Freeman, 2002, 3.3.1).

10 The term "beta-sheet" as used herein, unless indicated otherwise, refers to a portion of molecular structure formed by linking two or more beta-strands by hydrogen bonds. A beta-strand is a stretch of polypeptide chain typically 3 to 10 amino acids long with backbone in an extended conformation. Adjacent chains in a beta-sheet can run in opposite directions (antiparallel β sheet) or in the same direction (parallel β sheet). In the
15 antiparallel arrangement, the NH group and the CO group of each amino acid are respectively hydrogen bonded to the CO group and the NH group of a partner on the adjacent chain in the parallel arrangement, the hydrogen-bonding scheme is more complicated. For each amino acid, the NH group is hydrogen bonded to the CO group of one amino acid on the adjacent strand, whereas the CO group is hydrogen bonded to
20 the NH group on the amino acid two residues farther along the chain (Berg JM, Tymoczko JL, Stryer L., Biochemistry 5th Ed. New York, W H Freeman, 2002, 3.3.2).

The term "random coil" as used herein, unless indicated otherwise, refers to a portion of molecular structure lacking a defined hydrogen bonding pattern such as alpha helix or
25 beta-sheet.

The sericin secondary structure can be assigned using circular dichroism spectroscopy in the "far-UV" spectral region (190 to 250 nm). At these wavelengths, the chromophore is the peptide bond, and the signal arises when it is located in a regular, folded
30 environment. A maximum ellipticity per residue (theta) at circa 195 nm and a minimum at circa 220 nm is characteristic of a beta-sheet structure. A maximum ellipticity per residue (theta) at circa 190 nm and double minima at circa 205 nm and 225 nm is

characteristic of alpha helix. A minimum ellipticity at circa 200 nm and a broad maximum between 210 and 220 nm is characteristic of a random coil structure.

5 In accordance with a particularly preferred embodiment, the sericin in the composition of the present invention has a proportion of beta-sheet in the secondary structure that exceeds 70%, more preferably exceeds 80%, even more exceeds 85% and most preferably exceeds 90%.

10 In one preferred embodiment, the sericin has a molecular weight of in the range of 2kDa to 400 kDa, preferably in the range 25 kDa to 300 kDa, more preferably 40 kDa to 250 kDa, even more preferably in the range 60 to 200 kDa.

15 In a preferred embodiment of the invention, the composition contains 0.1 to 4%, preferably 0.1 to 3 % even more preferably 0.1 to 2% sericin by weight of the composition.

20 Preferably, at least 80 wt.% of the sericin contained in the composition is present in the structured aqueous phase. More preferably, at least 90 wt.% of the sericin, most preferably at least 95 wt.% of the sericin contained in the composition is present in the structured aqueous phase.

25 The inventors have discovered that the water structuring capability of the sericin is optimal when the structured aqueous phase has a pH in the range from 2.0 to 4.0 or from 5.5 to 10.5. Preferably, the structured aqueous phase has a pH in the range from 5.8 to 9.5, even more preferably in the range from 6 to 8.5.

30 The composition of the present invention can be provided such that the cosmetically acceptable vehicle comprises an emulsion or a gel and in an especially preferred aspect the product form is an emulsion or a gel.

The gel is usually formed by including a hydrocolloid in water. The gel preferably contains at least 50 wt.% water, more preferably at least 60 wt.% water, even more preferably at

least 70 wt.% water, further preferably at least 80 wt.% water and most preferably at least 85 wt.% water.

According to a particularly preferred embodiment, the present composition is an emulsion comprising 1 to 80 wt.% of a hydrophobic phase (oil) and 20 to 99 wt.% of the water. More preferably, the emulsion comprises 5 to 70 wt.% of oil and 30 to 95 wt.% of water. Most preferably, the emulsion contains 10 to 50 wt.% oil and 50 to 90 wt.% of water.

The inventors have found that the water structuring capability of sericin can be enhanced by the presence of multivalent metal cations. Consequently, in yet another preferred embodiment, the composition comprises at least 0.08 mmol of multivalent metal cation, said multivalent metal cation being selected from Zn^{2+} , Cu^{2+} , Ca^{2+} and combinations thereof. More preferably, the composition comprises at least 0.1 mmol of said multivalent metal cation, even more preferably in the range of 0.2 to 0.9 mmol of said multivalent metal cation, more preferably 0.3 to 0.8 mmol of said multivalent metal cation and most preferably 0.4 to 0.6 mmol of said multivalent metal cation.

The sericin employed in accordance with the present invention preferably is a highly pure sericin quality. Accordingly, in another preferred embodiment, the present composition contains fibroin and sericin in a weight ratio of fibroin:sericin of not more than 1:5, preferably of not more than 1:10.

Unlike the sericins described in US 2016/036241, the sericin of the present invention is not a gluteraldehyde cross-linked sericin. Even more preferably, the sericin is not a cross-linked sericin.

Unlike the sericins described in US 2009/176965, the sericin of the present invention is not an irradiated sericin that has been subjected to radiation at an absorption dose of 10 kGy to 500 kGy.

The structured phase of the present composition preferably contains less than 0.1wt.% ethanol, more preferably less than 0.01 wt.% ethanol.

In a particular embodiment, the present composition comprises at least 0.3% by weight of water of sericin having a molecular weight of at least 10 kDa and having a proportion of beta-sheet in the secondary structure that exceeds 70%.

5

In an even more preferred embodiment, the composition comprises at least 0.3% by weight of water of sericin having a molecular weight of at least 15 kDa and having a proportion of beta-sheet in the secondary structure that exceeds 75%.

- 10 Besides water and/or oil, suitable additives in the cosmetically acceptable vehicle include silicones, polyhydric alcohols, hydrocarbons, triglycerides and thickening powders.

The composition can be either a leave-on product such as skin lotions, creams, antiperspirants, deodorants, sunless tanners and sunscreen lotions or a rinse-off product
15 such as shower gels and toilet bars. It is preferred that the composition is a skin lotion or a cream.

The composition may comprise an emollient oil that act as a co-solvent. Suitable emollient oils include, for example, ester of alkoxyated aromatic alcohol with fatty
20 carboxylic acid, esters of polyglycols or diols with fatty carboxylic acid such as caprylic/capric triglyceride, ester of fatty alcohol and fatty acid, alkoxyated derivative of benzyl alcohol and mixtures thereof. Preferably the emollient oil is caprylic/capric triglyceride.

- 25 Typically, such compositions comprise co-solvent in an amount from 0.01 to 10%, more preferably from 0.1 to 8%, most preferably from 1 to 6%, based on the total weight of the sunscreen composition and including all ranges subsumed therein.

The composition may additionally comprise sunscreen agents such as inorganic
30 sunscreens. For example, zinc oxide, titanium dioxide, iron oxide, silica such as fumed silica. The amount of such sunscreen agents is preferably incorporated from 0.1 to 5% by total weight of the sunscreen composition.

The composition of the invention may comprise a UV-A sunscreen agent selected from the group consisting of a dibenzoylmethane derivative, a triazine derivative, a benzophenone derivative and mixtures thereof. In a preferred embodiment, the UV-A sunscreen agent comprises or is a dibenzoylmethane derivative, for example, butyl methoxydibenzoylmethane (sold under the trade name Parsol 1789).

Typically, the sunscreen composition of the present invention comprises from 0.1 to 15% by weight of the UV-A sunscreen agent, more preferably from 0.1 to 10%, most preferably from 1 to 5%, based on the total weight of the composition and including all ranges subsumed therein.

The composition of the invention may also comprise a UV-B sunscreen agent. Suitable UV-B sunscreen agent of the invention is selected from the group consisting of a benzophenone, an anthranilate, a salicylate, a cinnamate, a camphor, benzylidene malonate, a triazone, and derivatives thereof. In a preferred embodiment, the UV-B sunscreen agent comprises or is a cinnamate derivative, for example, ethylhexyl methoxycinnamate (sold under the trade name Parsol MCX).

Typically, the composition comprises from 0.1 to 20% by weight of the UV-B sunscreen agent, more preferably from 0.5 to 18%, most preferably from 1 to 15%, based on the total weight of the composition and including all ranges subsumed therein.

A skin lightening agent may also be incorporated into the composition of the invention. Suitable skin lightening agents other than Vitamin B3 and its derivatives (e.g. niacin, nicotinic acid, niacinamide) are kojic acid, arbutin, tranexamic acid, placental extract, ascorbic acid and its derivatives (e.g. magnesium ascorbyl phosphate, sodium ascorbyl phosphate, ascorbyl glucoside, and ascorbyl tetraisopalmitates), aloe extract, ammonium lactate, azelaic acid, citrate esters, ellagic acid, glycolic acid, green tea extract, hydroquinone, lemon extract, linoleic acid, vitamins like vitamin B6, vitamin B12, vitamin C, vitamin A, a dicarboxylic acid, resorcinol derivatives, hydroxycarboxylic acid like lactic acid and their salts (e.g. sodium lactate) or a mixture thereof. Typically the skin lightening agent is present in an amount from 0.1 to 10%, more preferably from 0.2 to 5%, most preferably from 0.3 to 3% by total weight of the composition including all ranges subsumed therein.

The composition may also comprise other ingredients which are common in the art to enhance physical properties and performance. Suitable ingredients include but are not limited to humectants, thickeners, opacifiers, binders, colorants and pigments, pH
5 adjusting agents, preservatives, optics, perfumes, viscosity modifiers, biological additives, buffering agents, conditioners, natural extracts, essential oils and skin benefit agents including anti-inflammatory agents, cooling agents, antiperspirant agents, anti-aging agents, anti-acne agents, anti-microbial agents and antioxidants.

10

The sericin for use in the composition of the present invention, i.e. sericin having more than 70% beta-sheet structure, can be isolated from silkworm cocoons by a process that comprises the steps of:

- cutting the cocoons in pieces of appr. 5x5 mm;
- 15 • subjecting the cocoons to a first washing treatment with de-mineralized water (demi-water) at approximately 25 °C;
- subjecting the cocoons to a second washing treatment with demi-water at approximately 70 °C;
- subjecting the cocoons to a second washing treatment with ethanol at approximately
20 25 °C;
- removing the ethanol from the washed cocoons by evaporation at a temperature below 30 °C;
- combining 1 party by weight of the dry washed cocoons with approximately 20 parts by weight hot water (≈ 95 °C) to form an aqueous suspension;
- 25 • keeping the aqueous suspension at a temperature of approximately 120 °C for about 5 minutes to about 30 minutes;
- centrifugating the heated suspension at 4000 G for 20 minutes, followed by decanting to recover the sericin protein extract.

30

In the above process it is preferred that the aqueous suspension is kept at a temperature of approximately 120 °C for up to 15 minutes, preferably 10 minutes, and most preferably up to 5 minutes. The present inventors have determined that when the aqueous suspension is maintained at a temperature of approximately 120 °C for up to about 20 minutes, the sericin thus produced has a proportion of beta-sheet in the secondary

structure that exceeds 80%, which reduces to about 70% when the reaction time is increased to about 30 minutes. The amount of sericin in the beta sheet form reduces further as the temperature is maintained for longer times.

- 5 Another aspect of the present invention relates to a process of preparing a composition as defined herein before, said process comprising mixing 0.1 to 5% sericin having a proportion of beta-sheet in the secondary structure that exceeds 70% with an emulsion or a gel.
- 10 Another aspect of the process comprises mixing with additionally one or more other components preferably selected from the group consisting of fillers, dyes, pH modifiers, solvents, polymers and thickening agents.

According to a particularly preferred embodiment, the process comprises the addition of
15 a water-soluble salt of a multivalent metal cation selected from Zn^{2+} , Cu^{2+} , Ca^{2+} and combinations thereof.

According to another aspect of the present invention there is provided a method of delivering benefits to skin comprising the step of applying a composition as of the
20 invention wherein the benefits are selected from one or more of anti-aging, hydration, anti-inflammation, anti-oxidant and anti-pollution. The method is preferably cosmetic i.e. non-therapeutic.

The invention will now be demonstrated with the help of the following non-limiting
25 examples.

Examples

30

Example 1: sericin extraction

Sericin was extracted from *Bombyx mori* cocoons (sourced from Bangalore, India) by means of the following procedure:

- i) The cocoons were soaked in ultrapure and degassed water at ambient temperature for 15 minutes, with shaking of the contents every 5 minutes. Cocoons were separated from water using forceps.
- ii) The prewashed pieces were soaked again in degassed and ultrapure water at 70°C, for 30 minutes (with shaking every 5 minutes). Cocoons were separated from water using forceps.
- iii) The cocoons were soaked in ethanol at room temperature for 15 minutes (shaking every 3 minutes). Cocoons were separated from ethanol using forceps.

The cocoons were dried over Whatman filter paper at ambient temperature and atmospheric pressure. The dried cocoons were used as stock material for the extraction process.

Known amounts of water were introduced into containers and heated to 95 °C (using bottom stirred autoclave). Prewashed cocoons (1:30 cocoon to water ratio) were added into the water and the suspensions so obtained were subjected to stirring and heated to 120 °C for 30 minutes. At the end of the heating period, the heat was turned off and the pressure was released immediately (this allows faster cooling of the slurry).

The sericin was separated from fibroin by centrifugation (6000 rpm (4,000 G) for 30 min) and decanting). The sericin content of the extracts was about 1.1 wt.%. The molecular weight was about 70 kDa.

Secondary structure

The secondary structure of the sericin protein in Example 1 was analysed using circular dichroism (CD) measurements. Standard CD spectra of protein secondary structures was recorded using 195 to 240 nm wavelength scan (see Figure 1) and then the data was fitted using online analysis software "K2D3".

A folded protein consists of secondary structures α -helix; β -sheet; loops (linker residues interconnecting secondary structure domains. The software "K2D3" takes 200 to 240 nm as the scan wavelength. The mean residue ellipticity value from Circular dichroism refers to percent of amino acids that contribute to the secondary structures (α -helix & β -sheet) and does not include residues in the linker region. The maximum sum of all secondary

structures (46%) was taken from computational model of sericin protein. The sericin beta sheet structure was estimated using results of K2D3 software.

The CD data are presented in terms of mean residue ellipticity (MRE, express as deg
5 cm² dmol⁻¹, as a function of wavelength, using the following equation:

$$[\theta]_{MRE} = \frac{MRW \times [\theta]_{obs}}{10 \times d \times c}$$

Where $[\theta]_{MRE}$ is the calculated mean residue ellipticity (deg cm² mol⁻¹); MRW, the mean
10 residue weight for the peptide bond [MRW is calculated as $MRW = M/N^{-1}$, where M is molecular mass of the peptide chain (Da) and N is the number of amino acids in the chain]; $[\theta]_{obs}$, the observed ellipticity (expressed in degrees); d, the path length (cm); c, the protein concentration (g L⁻¹). All CD spectra were corrected for buffer concentrations and secondary structure were calculated using web based K2D3 analysis tool K2D3:
15 Kelly et al. (2005) *Biochimica et Biophysica Acta* 1751: 119-39. PMID:16027053 neural network software package.

The %beta sheet in the sericin sample of Example – 1 was determined to be 71%. This sample of sericin was tested in various in-vitro bioassays in comparison with a
20 commercially available sericin from Xintiansi (Example A). In the sericin sample of Example A the percentage of beta sheet in the secondary structure was < 2.3 %.

The various assays to which these samples were subjected are summarized below. The model used was In-vitro cell culture. Primary dermal fibroblasts cells were treated
25 with sericin as per this invention (Example - 1) and commercial sericin (Example A) at different doses for 3 consecutive days. The respective markers were tested by ELISA method. Effect of both the sericins was evaluated at basal condition and under UV stress. Depending on the relevant marker, the following benefit areas as mentioned below, were tested.

30

Anti-aging Benefit

Biomarker used- Procollagen

Primary HDFa (Human dermal fibroblast from adult donor) were obtained from Gibco and cultured in Fibroblast growth media (FGM) from Cascade Biologics with growth factors and antibiotics at 5% CO₂/37°C. For bioassays, fibroblasts were seeded in a 48 well plate (10,000 cells/well) in FGM. After 24 hours, the media was removed and thin layer of PBS was added. Half of the wells were exposed to UVA radiation (325Mj/CM²). Different doses of sericin were added in UVA exposed and un-exposed cells. Control wells were not exposed to UVA (sham). Exposure and sericin addition was repeated for 3 consecutive days. Cell supernatant was collected after 24 hours and procollagen ELISA was performed using Takara kit.

The data on % pro-collagen-1 of UV treated samples when treated with sample of Example-1 and Example A is shown in the table below.

Example - 1	Control	Conc of sericin = 0.83 µg/ml	Conc of sericin = 8.3 µg/ml	Conc of sericin = 83 µg/ml
% Procollagen	76	72	85	91
Example - A	Control	Conc of sericin = 0.5 µg/ml	Conc of sericin = 5.0 µg/ml	Conc of sericin = 50 µg/ml
% Procollagen	78	78	76	76

The data in the table above indicates that sericin as per the invention (Example -1) provides improved anti-aging benefit (by exhibiting higher %procollagen with increased amounts of active), as compared to sericin outside the invention (Example A).

Skin hydration Benefit

Biomarker used- Hyaluronidase

Primary HDFa (Human dermal fibroblast from adult donor) were obtained from Gibco and cultured in Fibroblast growth media (FGM) from Cascade Biologics with growth factors and antibiotics at 5% CO₂/37°C. For bioassay, fibroblasts were seeded in a 48

well plate (10,000 cells/well) in FGM. After 24 hours, the media was removed and thin layer of PBS was added. Half of the wells were exposed to UVA radiation (325Mj/CM²). Different doses of sericin were added in UVA exposed and un-exposed cells. Control wells were not exposed to UVA (sham). Exposure and sericin addition was repeated for 3 consecutive days. Cell supernatant was collected after 24 hours and hyaluronidase ELISA was performed (MBS703230-48T).

The data on % hyaluronidase for the samples is shown in the table below:

Example - 1	Control	Conc of sericin = 0.83 µg/ml	Conc of sericin = 8.3 µg/ml	Conc of sericin = 83 µg/ml
% hyaluronidase	140	138	123	112
Example - A	Control	Conc of sericin = 0.5 µg/ml	Conc of sericin = 5.0 µg/ml	Conc of sericin = 50 µg/ml
% hyaluronidase	152	153	150	162

The data in the table above indicates that sericin as per the invention (Example -1) provides improved skin hydration benefit (by exhibiting lower % hyaluronidase with increased amounts of active), as compared to sericin outside the invention (Example A).

Anti-inflammatory Benefit

Biomarker used- Cytokine-IL-1

Primary NHEK (Neonatal epidermal human keratinocytes) were obtained from Invitrogen and were maintained in keratinocyte growth media (KGM- EpiLife M154) from Cascade Biologics. Cells were used from passage 3 to 5 for all experiments. For bioassays, keratinocytes were seeded in 24 well plate (50,000 cells/well) in KGM. After 24 hours incubation, different dilutions of sericins were added to keratinocytes. After 24 hours incubation, media was removed and cells were washed with PBS. Thin layer of PBS was added to each well and cells were exposed to UVA light (~22.5mJ/cm²). Control wells were not exposed to UVA (sham). After exposure fresh media was added with sericin. Cell supernatant was collected after 6 hours for IL-1a ELISA (R&D Systems).

The data on % change in IL-1 levels for the samples is shown in the table below:

Example - 1	Control	Conc of sericin = 0.83 µg/ml	Conc of sericin = 8.3 µg/ml	Conc of sericin = 83 µg/ml
% change	129	112	99	97
Example - A	Control	Conc of sericin = 0.5 µg/ml	Conc of sericin = 5.0 µg/ml	Conc of sericin = 50 µg/ml
% change	145	135	129	134

- 5 The data in the table above indicates that sericin as per the invention (Example -1) provides improved anti-inflammatory benefit (by exhibiting lower %change in IL-1 with increasing sericin concentration), as compared to sericin outside the invention (Example A).

10 Anti-oxidant Benefit

Marker used- Lipid peroxidation (LPO)

- Primary HDFa (Human dermal fibroblast from adult donor) were obtained from Gibco and cultured in Fibroblast growth media (FGM) from Cascade Biologics with growth factors and antibiotics at 5% CO₂/37°C. For bioassay, fibroblasts were seeded in a 48 well plate (10,000 cells/well) in FGM. After 24 hours, the media was removed and thin layer of PBS was added. Half of the wells were exposed to UVA radiation (325Mj/CM²). Different doses of sericin were added in UVA exposed and un-exposed cells. Control wells were not exposed to UVA (sham). Exposure and sericin addition was repeated for 3 consecutive days. Cell lysate was prepared after 24 hours and LPO was estimated using TBARS method.

The data on % change in LPO levels for the samples is shown in the table below:

Example - 1	Control	Conc of sericin = 0.83 µg/ml	Conc of sericin = 8.3 µg/ml	Conc of sericin = 83 µg/ml
% change	195	154	112	110

Example - A	Control	Conc of sericin = 0.5 µg/ml	Conc of sericin = 5.0 µg/ml	Conc of sericin = 50 µg/ml
% change	156	128	110	127

The data in the table above indicates that sericin as per the invention (Example -1) provides improved anti-oxidant benefit (by exhibiting lower %change in LPO with increasing sericin concentration), as compared to sericin outside the invention (Example A).

Anti-pollution benefit

Marker used – luminance

- 10 Pigmented living skin equivalent (with primary human keratinocytes and melanocytes) were acquired from Biocell (Xi'an, China) 6 days after air-lifting. Bioactives were applied to the media and refreshed on daily basis. on day 9, topical application of BaP (benzo-a-pyrene, as model pollutant) was conducted in squalene as solvent, at 10 µM. On day 12, the models were harvested, for L* measurement using a CM-700d spectrometer.
- 15 Sericin samples were applied through media treatment at 50 µg/mL.

The L* value for the samples is indicated in the table below:

Example - 1	L*
Control	55.6
Treatment with BaP	45.7
Treatment with BaP + Sericin as per Example - 1	53.5

The data in the table above indicates that pollutant tends to darken the skin while treatment with sericin as per the invention (Example -1) provides improved lightness.

Claims

1. A personal care composition comprising (i) 0.1 to 5 % sericin having a proportion of beta-sheet in the secondary structure higher than 70% by weight of the composition, and
(ii) a cosmetically acceptable vehicle comprising an emulsion or a gel.
2. A composition as claimed in claim 1, wherein the sericin has a molecular weight in the range of 2kDa to 400 kDa.
3. A composition as claimed in claim 1 or claim 2, wherein the composition comprises 0.1 to 2% sericin by weight of water.
4. A composition as claimed in any one of the preceding claims wherein the composition has a pH in the range from 2.0 to 4.0 or from 5.5 to 10.5.
5. A composition as claimed in any one of the preceding claims wherein the composition comprises at least 0.08 mmol of multivalent metal cation, said multivalent metal cation being selected from Zn^{2+} , Cu^{2+} , Ca^{2+} and combinations thereof.
6. A composition as claimed in any one of the preceding claims comprising at least 50 wt.% water.
7. A composition as claimed in any one of the preceding claims, comprising at least 0.4 wt% of said sericin having a molecular weight of at least 10 kDa.
8. A method of delivering benefits to skin comprising the step of applying a composition as claimed in any one of the preceding claims wherein the benefits are selected from one or more of anti-aging, hydration, anti-inflammation, anti-oxidant and anti-pollution.

ANY REFERENCE TO FIGURES
SHOULD BE CONSIDERED
AS NON-EXISTENT

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/080472

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K8/64 A61Q17/04 A61Q19/00 A61Q19/08
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61Q

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)

EPO-Internal, BIOSIS, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/176965 A1 (LEE JU-WOON [KR] ET AL) 9 July 2009 (2009-07-09) paragraphs [0003], [0010], [0011], [0032] - [0041]; claims; examples -----	1-8
X	US 2005/130857 A1 (MEESILPA PRATEEP [TH] ET AL) 16 June 2005 (2005-06-16) paragraphs [0004] - [0008]; claims -----	1-8
X	GB 888 625 A (LOUIS ROMAIN DUMAS) 31 January 1962 (1962-01-31) page 1, line 29 - line 51; examples page 1, line 51 ----- -/-	1-8



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

13 February 2019

Date of mailing of the international search report

19/02/2019

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

Mitchell, Gemma

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2018/080472

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	VOEGELI RAINER ET AL: "Sericin silk protein:unique structure and properties", COSMETICS & TOILETRIES, ALLURED PUBLISHING CORP, US , vol. 108, no. 12 1 January 1993 (1993-01-01), pages 101-108, XP009504839, ISSN: 0361-4387 Retrieved from the Internet: URL:https://epo.summon.serialssolutions.com/2.0.0/link/0/eLvHCXMwtV3NS8MwFA80L3oQP_E TethtFLokXTpvY7ipIMIoXkeaNKNYVlnX_9_32vRjg qAHL2n7WlKaX_ry8vLye4T0_aFg1FPwf3NtXC60dKU eea4RTHkr2BNwRJ_ulIdzsZhjtoY66lor-1ekQQZY4 87ZP6DdVAoCOAfMoQTUofwV7ujuUsl6kCfp-6BkYUj K4I2iYmqT-GLrVYNPdmVvtnUgYc1YkOUfcUPfHGag0 DDtVmN9v2W page 104; figures 10, 11 page 107	1-8
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X	----- EP 0 841 065 A2 (SEIREN CO LTD [JP]) 13 May 1998 (1998-05-13) page 3, line 37 - page 4, line 24; claims; examples 1, 2 -----	1-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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