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(54) **DETERGENT COMPOSITION**

REINIGUNGSMITTELZUSAMMENSETZUNG

COMPOSITION DE DÉTERGENT

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Description**Field of Invention**

5 **[0001]** The invention concerns a detergent composition, more specifically a laundry detergent composition, said composition comprising a specific esterase enzyme.

Background of the Invention

10 **[0002]** Sebum is an oily soil which has remained a difficult stain to remove from worn garments. With a drive to encourage consumers to wash at lower temperatures, the challenge for effective removal of sebum remains demanding. Sebum consists of a number of fats and esters including wax esters, cholesterol esters, squalene and many free fatty acids/ alcohols. Sebum is liquid at body temperature, but solid at ambient temperature.

15 **[0003]** These properties are particularly important for collar/cuff soil removal because it is easier to remove a liquid body oil than solids from clothes. Current laundry enzymes are not able to degrade all the components of the sebum which makes removal from fabric difficult. US5698508 A and EP 0399681 A2 are both concerned with the removal of sebum soil on fabrics by using a detergent composition comprising an esterase, i.e. a lipase or cutinase.

[0004] There is a problem with sebum removal in that detergents including current commercial enzymes do not remove sebum adequately.

Summary of the Invention

[0005] We have found that the incorporation of a specific esterase enzyme in detergent compositions improve the sebum removal from fabrics.

25 **[0006]** In one aspect the present invention provides a detergent composition comprising:

(i) from 1 to 60 wt.%, preferably from 2 to 50 wt.%, more preferably from 3 to 45 wt.%, even more preferably from 5 to 40 wt.%, most preferably from 6 to 40 wt.% of a surfactant; and,

30 (ii) from 0.0005 to 5 wt.%, preferably from 0.005 to 2.5 wt.%, more preferably from 0.01 to 1 wt.% of an esterase enzyme of enzyme class EC 3.1.1.1, having at least 60% sequence identity to any one of SEQ ID NO: 1 to 4.

[0007] Preferably the esterase enzyme has at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95%, most preferably at least 97%, at least 98% or even at least 99% sequence identity to any one of SEQ ID NO: 1 to 4.

35 **[0008]** Most preferably the esterase enzyme has 100% sequence identity to any one of SEQ ID NO: 1 to 4.

[0009] Preferably the detergent composition comprises from 0.1 to 10 wt.%, preferably from 0.2 to 9 wt.%, more preferably from 0.25 to 8, even more preferably from 0.5 to 6 wt.%, most preferably from 1 to 5 wt.% of a soil release polymer, more preferably a polyester based soil released polymer.

40 **[0010]** Preferably the polyester soil release polymer is a polyethylene and/or polypropylene terephthalate based soil release polymer, preferably a polypropylene terephthalate based soil release polymer.

[0011] Preferably the detergent composition comprises an alkoxyated polyamine, preferably at a level of from 0.1 to 8 wt.%, more preferably from 0.2 to 6 wt.%, most preferably from 0.5 to 5 wt.%.

[0012] Preferably the detergent composition is a laundry detergent composition. Preferably the laundry detergent composition is a liquid or a powder, most preferably a liquid detergent.

45 **[0013]** Preferably the surfactant in the detergent composition comprises anionic and/or nonionic surfactant, in one case comprising both anionic and nonionic surfactant.

[0014] Preferred detergent compositions, particularly laundry detergent compositions additionally comprise a further enzyme selected from the group consisting of: lipases, proteases, cellulases, alpha-amylases, peroxidases/oxidases, pectate lyases, and/or mannanases.

50 **[0015]** Preferred detergent compositions, particularly laundry detergent compositions additionally comprise a further ingredient selected from fluorescent agent, perfume, shading dyes and polymers, and mixtures thereof.

[0016] In another aspect the present invention provides a method of treatment of a fabric substrate with a sebum stain, said method comprising incorporation of an esterase enzyme of enzyme class EC 3.1.1.1, having at least 60%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95%, most preferably at least 97%, at least 98% or even at least 99% sequence identity, most preferably 100%, sequence identity to any one of SEQ ID NO: 1 to 4 into a detergent composition comprising from 1 to 60 wt.% of a surfactant; and subsequent treatment of a fabric substrate with a sebum stain, with said composition.

[0017] In another aspect the present invention provides the use of an esterase enzyme of enzyme class EC 3.1.1.1, having at least 60%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95%, most preferably at least 97%, at least 98% or even at least 99% sequence identity, most preferably 100%, sequence identity to any one of SEQ ID NO: 1 to 4, to improve cleaning of sebum stains on fabric.

Detailed Description of the Invention

[0018] The indefinite article "a" or "an" and its corresponding definite article "the" as used herein means at least one, or one or more, unless specified otherwise.

[0019] All % levels of ingredients in compositions (formulations) listed herein are in wt.% based on total formulation unless other stated.

[0020] It is understood that any reference to a preferred ingredient of the detergent composition is envisaged to be combinable subject matter with any other preferred ingredient of the detergent composition disclosed herein.

[0021] The detergent composition may take any suitable form, for example liquids, solids (including powders) or gels.

[0022] The detergent composition can be applied to any suitable substrate. Particularly preferred substrates are textiles. Particularly preferred detergent compositions are laundry detergent compositions.

[0023] Laundry detergent compositions may take any suitable form. Preferred forms are liquid or powder, with liquid being most preferred.

Sequence Information

[0024] The sequences disclosed herein are SEQ ID NO 1 to 4.

SEQ ID 1 is from *Thermogutta terrifontis*
The sequence is:

```
maqrvkitt atpgeielaf edtgtglpvl lvhgfpldrt mwkaqreelc defrvivpdl rgfgesqvip gvatmeamad
dla glcnhlg Itgkivlggl smggyvafaf arkyrdrlag lilcdtrarp dspeakenrr rvaervrreg pgfaeemip
rlccestfrn hpeviekirq milsappegv aaaalgmaer pdstdllpal scptlvlggq fdaisppeer eamartipqs
qfvvipdagh lppmeqperv tqairewlrk vhte
```

SEQ ID 2 is from *Thermogutta terrifontis*
The sequence is:

```
maqrvkitt atpgeielaf edtgtglpvl lvhgfpAdrt mwkaqreelc defrvivpdl rgfgesqvip gvatmeamad
dla glcnhlg Itgkivlggl smggyvafaf arkyrdrlag lilcdtrarp dspeakenrr rvaervrreg pgfaeemip
rlccestfrn hpeviekirq milsappegv aaaalgmaer pdstdllpal scptlvlggq fdaisppeer eamartipqs
qfvvipdagh lppmeqperv tqairewlrk vhte
```

This sequence is a mutant of SEQ ID 1 and differs at the 37 position.

SEQ ID 3 is from *Thermogutta terrifontis*
The sequence is:

```
maqrvkitt atpgeielaf edtgtglpvl lvhgfpldrt mwkaqreelc defrvivpdl rgfgesqvip gvatmeamad
dla glcnhlg Itgkivlggl smggyvafaf arkyrdrlag lilcdtrarp dspeakenrr rvaervrreg pgfaeemip
rlccestfrn hpeviekirq milsappegv aaaalgmaer pdstdllpal scptlvlggq fdaisppeer eamartipqs
qfvvipdagh Appmeqperv tqairewlrk vhte
```

This sequence is a mutant of SEQ ID 1 and differs at the 251 position.

SEQ ID 4 is from *Archaeoglobus fulgidus*

The sequence is:

MLERVFIDVDGVKVSLLKGRERKVFYIHSSGSDATQWVNQLTAIGGYAIDLPNHGQSDTVE
VNSVDEYAYYASESLKKTVGKAVVVGHSLGGAVAQKLYLRNPEICLALVLVGTGARLRVLP
EILEGLKKEPEKAVDLMLSMAFASKGEEYEKKRREFLDRVDVLHLDLSLCDRFDLLEDYRN
GKLKIGVPTLVIVGEEDKLTPKYHEFFHKHIPNSELVVIPGASHMVMLEKHVEFNEALEKFL
KKVGV

Esterase enzyme

[0025] The esterase enzyme has at least 60% sequence identity to any one of SEQ ID NO: 1 to 4.

[0026] Preferably the esterase enzyme has at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95%, most preferably at least 97%, at least 98% or even at least 99% sequence identity to any one of SEQ ID NO: 1 to 4.

[0027] Most preferably the esterase enzyme has 100% sequence identity to any one of SEQ ID NO: 1 to 4.

[0028] The esterase can be described as being of enzyme class EC 3.1.1.1, known as carboxylesterase.

[0029] Preferred esterases are from *Thermogutta terrifontis* or *Archaeoglobus fulgidus*.

Surfactant

[0030] The detergent composition comprises surfactant (which may include a single surfactant or a mixture of two or more surfactants). The composition comprises from 1 to 60 wt.%, preferably from 2 to 50 wt.%, more preferably from 3 to 45 wt.%, even more preferably from 5 to 40 wt.%, most preferably from 6 to 40 wt.% of surfactant.

[0031] The detergent composition (preferably a laundry detergent composition) comprises anionic and/or nonionic surfactant, preferably comprising both anionic and nonionic surfactant.

[0032] Suitable anionic detergent compounds which may be used are usually water-soluble alkali metal salts of organic sulphates and sulphonates having alkyl radicals containing from about 8 to about 22 carbon atoms, the term alkyl being used to include the alkyl portion of higher alkyl radicals.

[0033] Examples of suitable synthetic anionic detergent compounds are sodium and potassium alkyl sulphates, especially those obtained by sulphating higher C₈ to C₁₈ alcohols, produced for example from tallow or coconut oil, sodium and potassium alkyl C₉ to C₂₀ benzene sulphonates, particularly sodium linear secondary alkyl C₁₀ to C₁₅ benzene sulphonates; and sodium alkyl glyceryl ether sulphates, especially those ethers of the higher alcohols derived from tallow or coconut oil and synthetic alcohols derived from petroleum.

[0034] The anionic surfactant is preferably selected from: linear alkyl benzene sulphonate; alkyl sulphates; alkyl ether sulphates; soaps; alkyl (preferably methyl) ester sulphonates, and mixtures thereof.

[0035] The most preferred anionic surfactants are selected from: linear alkyl benzene sulphonate; alkyl sulphates; alkyl ether sulphates and mixtures thereof. Preferably the alkyl ether sulphate is a C₁₂-C₁₄ n-alkyl ether sulphate with an average of 1 to 3EO (ethoxylate) units.

[0036] Sodium lauryl ether sulphate is particularly preferred (SLES). Preferably the linear alkyl benzene sulphonate is a sodium C₁₁ to C₁₅ alkyl benzene sulphonates. Preferably the alkyl sulphates is a linear or branched sodium C₁₂ to C₁₈ alkyl sulphates. Sodium dodecyl sulphate is particularly preferred, (SDS, also known as primary alkyl sulphate).

[0037] In liquid formulations preferably two or more anionic surfactant are present, for example linear alkyl benzene sulphonate together with an alkyl ether sulphate.

[0038] In liquid formulations, preferably the laundry composition in addition to the anionic surfactant comprises alkyl ethoxylated non-ionic surfactant, preferably from 2 to 8 wt.% of alkyl ethoxylated non-ionic surfactant.

[0039] Suitable nonionic detergent compounds which may be used include, in particular, the reaction products of compounds having an aliphatic hydrophobic group and a reactive hydrogen atom, for example, aliphatic alcohols, acids or amides, especially ethylene oxide either alone or with propylene oxide. Preferred nonionic detergent compounds are the condensation products of aliphatic C₈ to C₁₈ primary or secondary linear or branched alcohols with ethylene oxide.

[0040] Most preferably the nonionic detergent compound is the alkyl ethoxylated non-ionic surfactant is a C₈ to C₁₈ primary alcohol with an average ethoxylation of 7EO to 9EO units.

[0041] Preferably the surfactants used are saturated.

Soil release polymer

[0042] The soil release polymer is preferably present at a level of from 0.1 to 10 wt.%. Preferred levels of inclusion of the soil release polymer are preferably from 0.2 to 9 wt.%, more preferably from 0.25 to 8 wt.%, even more preferably from 0.5 to 6 wt.%, most preferably from 1 to 5 wt.%.

[0043] Preferably the soil release polymer is a polyester based soil released polymer. More preferably the polyester soil release polymer is a polyethylene and/or polypropylene terephthalate based soil release polymer, most preferably a polypropylene terephthalate based soil release polymer.

[0044] Suitable polyester based soil release polymers are described in WO 2014/029479 and WO 2016/005338.

Alkoxylated polyamine

[0045] The detergent composition preferably comprises an alkoxylated polyamine. Especially when the detergent composition is in the form of a laundry composition, it is preferred that an alkoxylated polyamine is included.

[0046] Preferred levels of alkoxylated polyamine range from 0.1 to 8 wt.%, preferably from 0.2 to 6 wt.%, more preferably from 0.5 to 5 wt.%. Another preferred level is from 1 to 4 wt.%.

[0047] The alkoxylated polyamine may be linear or branched. It may be branched to the extent that it is a dendrimer. The alkoxylation may typically be ethoxylation or propoxylation, or a mixture of both. Where a nitrogen atom is alkoxylated, a preferred average degree of alkoxylation is from 10 to 30, preferably from 15 to 25.

[0048] A preferred material is alkoxylated polyethylenimine, most preferably ethoxylated polyethylenimine, with an average degree of ethoxylation being from 10 to 30 preferably from 15 to 25, where a nitrogen atom is ethoxylated.

Additional Enzymes

[0049] Additional enzymes, other than the specified lipase may be present in the detergent composition. It is preferred that additional enzymes are present in the preferred laundry detergent composition.

[0050] If present, then the level of each enzyme in the laundry composition of the invention is from 0.0001 wt.% to 0.1 wt.%.

[0051] Levels of enzyme present in the composition preferably relate to the level of enzyme as pure protein.

[0052] Preferred further enzymes include those in the group consisting of: lipases, proteases, cellulases, alpha-amylases, peroxidases/oxidases, pectate lyases, and/or mannanases. Said preferred additional enzymes include a mixture of two or more of these enzymes.

[0053] Preferably the further enzyme is selected from: lipases, proteases, cellulases, and/or alpha-amylases.

[0054] Suitable lipases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful lipases include lipases from *Humicola* (synonym *Thermomyces*), e.g. from *H. lanuginosa* (*T. lanuginosus*) as described in EP 258 068 and EP 305 216 or from *H. insolens* as described in WO 96/13580, a *Pseudomonas* lipase, e.g. from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), *P. cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012), a *Bacillus* lipase, e.g. from *B. subtilis* (Dartois et al. (1993), Biochemica et Biophysica Acta, 1131, 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422).

Other examples are lipase variants such as those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079 and WO 97/07202, WO 00/60063.

[0055] Preferred commercially available lipase enzymes include Lipolase™ and Lipolase Ultra™, Lipex™ and Lipoclean™ (Novozymes A/S).

[0056] The method of the invention may be carried out in the presence of phospholipase classified as EC 3.1.1.4 and/or EC 3.1.1.32. As used herein, the term phospholipase is an enzyme which has activity towards phospholipids.

[0057] Phospholipids, such as lecithin or phosphatidylcholine, consist of glycerol esterified with two fatty acids in an outer (sn-1) and the middle (sn-2) positions and esterified with phosphoric acid in the third position; the phosphoric acid, in turn, may be esterified to an amino-alcohol. Phospholipases are enzymes which participate in the hydrolysis of phospholipids. Several types of phospholipase activity can be distinguished, including phospholipases A₁ and A₂ which hydrolyze one fatty acyl group (in the sn-1 and sn-2 position, respectively) to form lysophospholipid; and lysophospholipase (or phospholipase B) which can hydrolyze the remaining fatty acyl group in lysophospholipid. Phospholipase C and phospholipase D (phosphodiesterases) release diacyl glycerol or phosphatidic acid respectively.

[0058] Protease enzymes hydrolyse bonds within peptides and proteins, in the laundry context this leads to enhanced removal of protein or peptide containing stains. Examples of suitable proteases families include aspartic proteases;

cysteine proteases; glutamic proteases; asparagine peptide lyase; serine proteases and threonine proteases. Such protease families are described in the MEROPS peptidase database (<http://merops.sanger.ac.uk/>). Serine proteases are preferred. Subtilase type serine proteases are more preferred. The term "subtilases" refers to a sub-group of serine protease according to Siezen et al., Protein Engng. 4 (1991) 719-737 and Siezen et al. Protein Science 6 (1997) 501-523. Serine proteases are a subgroup of proteases characterized by having a serine in the active site, which forms a covalent adduct with the substrate. The subtilases may be divided into 6 subdivisions, i.e. the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family.

[0059] Examples of subtilases are those derived from *Bacillus* such as *Bacillus lentus*, *B. alkalophilus*, *B. subtilis*, *B. amyloliquefaciens*, *Bacillus pumilus* and *Bacillus gibsonii* described in; US7262042 and WO09/021867, and subtilisin *lentus*, subtilisin *Novo*, subtilisin *Carlsberg*, *Bacillus licheniformis*, subtilisin *BPN'*, subtilisin 309, subtilisin 147 and subtilisin 168 described in WO 89/06279 and protease PD138 described in (WO 93/18140). Other useful proteases may be those described in WO 92/175177, WO 01/016285, WO 02/026024 and WO 02/016547. Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270, WO 94/25583 and WO 05/040372, and the chymotrypsin proteases derived from *Cellulomonas* described in WO 05/052161 and WO 05/052146.

[0060] Most preferably the protease is a subtilisin (EC 3.4.21.62).

[0061] Examples of subtilases are those derived from *Bacillus* such as *Bacillus lentus*, *B. alkalophilus*, *B. subtilis*, *B. amyloliquefaciens*, *Bacillus pumilus* and *Bacillus gibsonii* described in; US7262042 and WO09/021867, and subtilisin *lentus*, subtilisin *Novo*, subtilisin *Carlsberg*, *Bacillus licheniformis*, subtilisin *BPN'*, subtilisin 309, subtilisin 147 and subtilisin 168 described in WO89/06279 and protease PD138 described in (WO93/18140). Preferably the subtilisin is derived from *Bacillus*, preferably *Bacillus lentus*, *B. alkalophilus*, *B. subtilis*, *B. amyloliquefaciens*, *Bacillus pumilus* and *Bacillus gibsonii* as described in US 6,312,936 B1, US 5,679,630, US 4,760,025, US7,262,042 and WO 09/021867. Most preferably the subtilisin is derived from *Bacillus gibsonii* or *Bacillus Lentus*.

[0062] Suitable commercially available protease enzymes include those sold under the trade names names Alcalase[®], Blaze[®], Duralase[™], Durazym[™], Relase[®], Relase[®] Ultra, Savinase[®], Savinase[®] Ultra, Primase[®], Polazyme[®], Kan-nase[®], Liquanase[®], Liquanase[®] Ultra, Ovozyme[®], Coronase[®], Coronase[®] Ultra, Neutrase[®], Everlase[®] and Esperase[®] all could be sold as Ultra[®] or Evity[®] (Novozymes A/S).

[0063] The composition may use cutinase, classified in EC 3.1.1.74. The cutinase used according to the invention may be of any origin. Preferably cutinases are of microbial origin, in particular of bacterial, of fungal or of yeast origin.

[0064] Suitable amylases (alpha and/or beta) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from *Bacillus*, e.g. a special strain of *B. licheniformis*, described in more detail in GB 1,296,839, or the *Bacillus* sp. strains disclosed in WO 95/026397 or WO 00/060060. Commercially available amylases are Duramyl[™], Termamyl[™], Termamyl Ultra[™], Natalase[™], Stainzyme[™], Amplify[™], Fungamyl[™] and BAN[™] (Novozymes A/S), Rapidase[™] and Purastar[™] (from Genencor International Inc.).

[0065] Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g. the fungal cellulases produced from *Humicola insolens*, *Thielavia terrestris*, *Myceliophthora thermophila*, and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757, WO 89/09259, WO 96/029397, and WO 98/012307. Commercially available cellulases include Celluzyme[™], Carezyme[™], Celluclean[™], Endolase[™], Renozyme[™] (Novozymes A/S), Clazinase[™] and Puradax HA[™] (Genencor International Inc.), and KAC-500(B)[™] (Kao Corporation). Celluclean[™] is preferred.

[0066] Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus*, e.g. from *C. cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257. Commercially available peroxidases include Guardzyme[™] and Novozym[™] 51004 (Novozymes A/S).

[0067] Further enzymes suitable for use are discussed in WO 2009/087524, WO 2009/090576, WO 2009/107091, WO 2009/111258 and WO 2009/148983.

[0068] The aqueous solution used in the method preferably has an enzyme present. The enzyme is preferably present in the aqueous solution used in the method at a concentration in the range from 0.01 to 10ppm, preferably 0.05 to 1ppm.

Enzyme Stabilizers

[0069] Any enzyme present in the composition may be stabilized using conventional stabilizing agents, e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in e.g. WO 92/19709 and WO 92/19708.

Further materials

[0070] Further optional but preferred materials that may be included in the detergent compositions (preferably laundry detergent compositions) include fluorescent agent, perfume, shading dyes, polymers and chelating agents.

Fluorescent Agent

[0071] The composition preferably comprises a fluorescent agent (optical brightener). Fluorescent agents are well known and many such fluorescent agents are available commercially. Usually, these fluorescent agents are supplied and used in the form of their alkali metal salts, for example, the sodium salts.

[0072] The total amount of the fluorescent agent or agents used in the composition is generally from 0.0001 to 0.5 wt.%, preferably 0.005 to 2 wt.%, more preferably 0.01 to 0.1 wt.%.

[0073] Preferred classes of fluorescer are: Di-styryl biphenyl compounds, e.g. Tinopal (Trade Mark) CBS-X, Di-amine stilbene di-sulphonic acid compounds, e.g. Tinopal DMS pure Xtra and Blankophor (Trade Mark) HRH, and Pyrazoline compounds, e.g. Blankophor SN.

[0074] Preferred fluorescers are fluorescers with CAS-No 3426-43-5; CAS-No 35632-99-6; CAS-No 24565-13-7; CAS-No 12224-16-7; CAS-No 13863-31-5; CAS-No 4193-55-9; CAS-No 16090-02-1; CAS-No 133-66-4; CAS-No 68444-86-0; CAS-No 27344-41-8.

[0075] Most preferred fluorescers are: sodium 2 (4-styryl-3-sulfophenyl)-2H-naphthol[1,2-d]triazole, disodium 4,4'-bis[[[4-anilino-6-(N methyl-N-2 hydroxyethyl) amino 1,3,5-triazin-2-yl]]amino]stilbene-2-2' disulphonate, disodium 4,4'-bis[[[4-anilino-6-morpholino-1,3,5-triazin-2-yl]]amino] stilbene-2-2' disulphonate, and disodium 4,4'-bis(2-sulphostyryl)biphenyl.

[0076] The aqueous solution used in the method has a fluorescer present. The fluorescer is present in the aqueous solution used in the method preferably in the range from 0.0001 g/l to 0.1 g/l, more preferably 0.001 to 0.02 g/l.

Perfume

[0077] The composition preferably comprises a perfume. Many suitable examples of perfumes are provided in the CTFA (Cosmetic, Toiletry and Fragrance Association) 1992 International Buyers Guide, published by CFTA Publications and OPD 1993 Chemicals Buyers Directory 80th Annual Edition, published by Schnell Publishing Co.

[0078] Preferably the perfume comprises at least one note (compound) from: alpha-isomethyl ionone, benzyl salicylate; citronellol; coumarin; hexyl cinnamal; linalool; pentanoic acid, 2-methyl-, ethyl ester; octanal; benzyl acetate; 1,6-octadien-3-ol, 3,7-dimethyl-, 3-acetate; cyclohexanol, 2-(1,1-dimethylethyl)-, 1-acetate; delta-damascone; beta-ionone; verdyl acetate; dodecanal; hexyl cinnamic aldehyde; cyclopentadecanolide; benzeneacetic acid, 2-phenylethyl ester; amyl salicylate; beta-caryophyllene; ethyl undecylenate; geranyl anthranilate; alpha-irone; beta-phenyl ethyl benzoate; alpha-santalol; cedrol; cedryl acetate; cedryl formate; cyclohexyl salicylate; gamma-dodecalactone; and, beta phenylethyl phenyl acetate.

[0079] Useful components of the perfume include materials of both natural and synthetic origin. They include single compounds and mixtures. Specific examples of such components may be found in the current literature, e.g., in Fenaroli's Handbook of Flavour Ingredients, 1975, CRC Press; Synthetic Food Adjuncts, 1947 by M. B. Jacobs, edited by Van Nostrand; or Perfume and Flavour Chemicals by S. Arcander 1969, Montclair, N.J. (USA).

[0080] It is commonplace for a plurality of perfume components to be present in a formulation. In the compositions of the present invention it is envisaged that there will be four or more, preferably five or more, more preferably six or more or even seven or more different perfume components.

[0081] In perfume mixtures preferably 15 to 25 wt% are top notes. Top notes are defined by Poucher (Journal of the Society of Cosmetic Chemists 6(2):80 [1955]). Preferred top-notes are selected from citrus oils, linalool, linalyl acetate, lavender, dihydromyrcenol, rose oxide and cis-3-hexanol.

[0082] The International Fragrance Association has published a list of fragrance ingredients (perfumes) in 2011. (<http://www.ifraorg.org/en-us/ingredients#.U7Z4hPldWzk>)

[0083] The Research Institute for Fragrance Materials provides a database of perfumes (fragrances) with safety information.

[0084] Perfume top note may be used to cue the whiteness and brightness benefit of the invention.

[0085] Some or all of the perfume may be encapsulated, typical perfume components which it is advantageous to encapsulate, include those with a relatively low boiling point, preferably those with a boiling point of less than 300, preferably 100-250 Celsius. It is also advantageous to encapsulate perfume components which have a low CLog P (ie. those which will have a greater tendency to be partitioned into water), preferably with a CLog P of less than 3.0. These materials, of relatively low boiling point and relatively low CLog P have been called the "delayed blooming" perfume ingredients and include one or more of the following materials: allyl caproate, amyl acetate, amyl propionate, anisic

aldehyde, anisole, benzaldehyde, benzyl acetate, benzyl acetone, benzyl alcohol, benzyl formate, benzyl iso valerate, benzyl propionate, beta gamma hexenol, camphor gum, laevo-carvone, d-carvone, cinnamic alcohol, cinamyl formate, cis-jasmone, cis-3-hexenyl acetate, cuminic alcohol, cyclal c, dimethyl benzyl carbinol, dimethyl benzyl carbinol acetate, ethyl acetate, ethyl aceto acetate, ethyl amyl ketone, ethyl benzoate, ethyl butyrate, ethyl hexyl ketone, ethyl phenyl acetate, eucalyptol, eugenol, fenchyl acetate, flor acetate (tricyclo decenyl acetate), frutene (tricyclo decenyl propionate), geraniol, hexenol, hexenyl acetate, hexyl acetate, hexyl formate, hydratropic alcohol, hydroxycitronellal, indone, isoamyl alcohol, iso menthone, isopulegyl acetate, isoquinolone, ligustral, linalool, linalool oxide, linalyl formate, menthone, menthyl acetphenone, methyl amyl ketone, methyl anthranilate, methyl benzoate, methyl benyl acetate, methyl eugenol, methyl heptenone, methyl heptine carbonate, methyl heptyl ketone, methyl hexyl ketone, methyl phenyl carbonyl acetate, methyl salicylate, methyl-n-methyl anthranilate, nerol, octalactone, octyl alcohol, p-cresol, p-cresol methyl ether, p-methoxy acetophenone, p-methyl acetophenone, phenoxy ethanol, phenyl acetaldehyde, phenyl ethyl acetate, phenyl ethyl alcohol, phenyl ethyl dimethyl carbinol, prenyl acetate, propyl bornate, pulegone, rose oxide, safrole, 4-terpinenol, alpha-terpinenol, and /or viridine. It is commonplace for a plurality of perfume components to be present in a formulation. In the compositions of the present invention it is envisaged that there will be four or more, preferably five or more, more preferably six or more or even seven or more different perfume components from the list given of delayed blooming perfumes given above present in the perfume.

[0086] Another group of perfumes with which the present invention can be applied are the so-called aromatherapy materials. These include many components also used in perfumery, including components of essential oils such as Clary Sage, Eucalyptus, Geranium, Lavender, Mace Extract, Neroli, Nutmeg, Spearmint, Sweet Violet Leaf and Valerian.

[0087] It is preferred that the laundry treatment composition does not contain a peroxygen bleach, e.g., sodium percarbonate, sodium perborate, and peracid.

Shading Dye

[0088] Preferably when the composition is a laundry detergent composition, then it comprises a shading dye. Preferably the shading dye is present at from 0.0001 to 0.1 wt.% of the composition.

[0089] Dyes are described in Color Chemistry Synthesis, Properties and Applications of Organic Dyes and Pigments, (H Zollinger, Wiley VCH, Zurich, 2003) and, Industrial Dyes Chemistry, Properties Applications. (K Hunger (ed), Wiley-VCH Weinheim 2003).

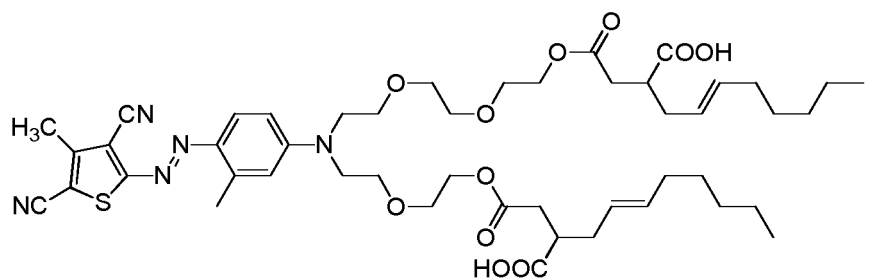
[0090] Shading Dyes for use in laundry compositions preferably have an extinction coefficient at the maximum absorption in the visible range (400 to 700nm) of greater than 5000 L mol⁻¹ cm⁻¹, preferably greater than 10000 L mol⁻¹ cm⁻¹. The dyes are blue or violet in colour.

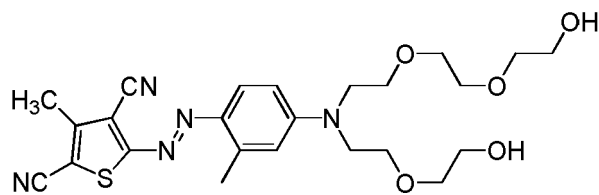
[0091] Preferred shading dye chromophores are azo, azine, anthraquinone, and triphenylmethane.

[0092] Azo, anthraquinone, phthalocyanine and triphenylmethane dyes preferably carry a net anionic charged or are uncharged. Azine preferably carry a net anionic or cationic charge. Blue or violet shading dyes deposit to fabric during the wash or rinse step of the washing process providing a visible hue to the fabric. In this regard the dye gives a blue or violet colour to a white cloth with a hue angle of 240 to 345, more preferably 250 to 320, most preferably 250 to 280. The white cloth used in this test is bleached non-mercerised woven cotton sheeting.

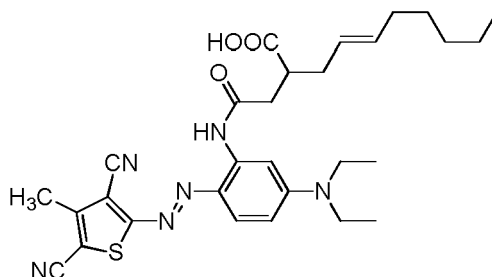
[0093] Shading dyes are discussed in WO 2005/003274, WO 2006/032327(Unilever), WO 2006/032397(Unilever), WO 2006/045275(Unilever), WO 2006/027086(Unilever), WO 2008/017570(Unilever), WO 2008/141880 (Unilever), WO 2009/132870(Unilever), WO 2009/141173 (Unilever), WO 2010/099997(Unilever), WO 2010/102861(Unilever), WO 2010/148624(Unilever), WO 2008/087497 (P&G), WO 2011/011799 (P&G), WO 2012/054820 (P&G), WO 2013/142495 (P&G) and WO 2013/151970 (P&G).

[0094] Mono-azo dyes preferably contain a heterocyclic ring and are most preferably thiophene dyes. The mono-azo dyes are preferably alkoxyated and are preferably uncharged or anionically charged at pH=7. Alkoxyated thiophene dyes are discussed in WO/2013/142495 and WO/2008/087497. Preferred examples of thiophene dyes are shown below:



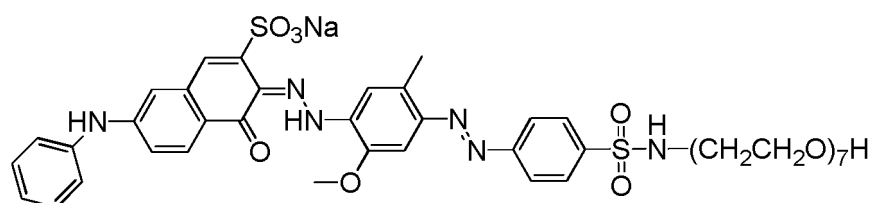


and,



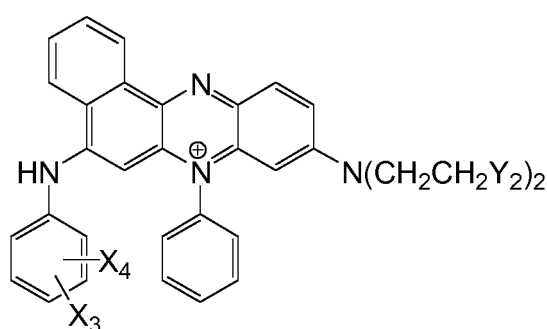
Bis-azo dyes are preferably sulphonated bis-azo dyes. Preferred examples of sulphonated bis-azo compounds are direct violet 7, direct violet 9, direct violet 11, direct violet 26, direct violet 31, direct violet 35, direct violet 40, direct violet 41, direct violet 51, Direct Violet 66, direct violet 99 and alkoxyated versions thereof. Alkoxyated bis-azo dyes are discussed in WO2012/054058 and WO2010/151906.

[0095] An example of an alkoxyated bis-azo dye is :



[0096] Thiophene dyes are available from Milliken under the tradenames of Liquitint Violet DD and Liquitint Violet ION.

[0097] Azine dye are preferably selected from sulphonated phenazine dyes and cationic phenazine dyes. Preferred examples are acid blue 98, acid violet 50, dye with CAS-No 72749-80-5, acid blue 59, and the phenazine dye selected from:



wherein:

X_3 is selected from: -H; -F; -CH₃; -C₂H₅; -OCH₃; and, -OC₂H₅;

X_4 is selected from: -H; -CH₃; -C₂H₅; -OCH₃; and, -OC₂H₅;

Y_2 is selected from: -OH; -OCH₂CH₂OH; -CH(OH)CH₂OH; -OC(O)CH₃; and, C(O)OCH₃.

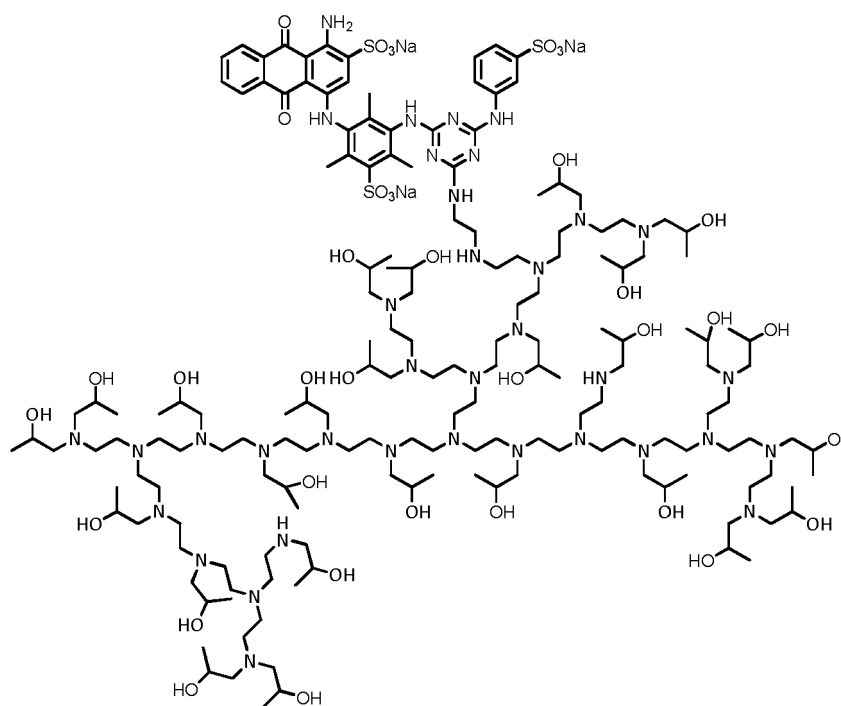
[0098] The shading dye is present in the composition in range from 0.0001 to 0.5 wt %, preferably 0.001 to 0.1 wt%. Depending upon the nature of the shading dye there are preferred ranges

depending upon the efficacy of the shading dye which is dependent on class and particular efficacy within any particular class. As stated above the shading dye is a blue or violet shading dye.

[0099] A mixture of shading dyes may be used.

[0100] The shading dye is most preferably a reactive blue anthraquinone dye covalently linked to an alkoxyated polyethyleneimine. The alkoxylation is preferably selected from ethoxylation and propoxylation, most preferably propoxylation. Preferably 80 to 95 mol% of the N-H groups in the polyethylene imine are replaced with iso-propyl alcohol groups by propoxylation. Preferably the polyethylene imine before reaction with the dye and the propoxylation has a molecular weight of 600 to 1800.

[0101] An example structure of a preferred reactive anthraquinone covalently attached to a propoxylated polyethylene imine is:



(Structure I).

Polymers

[0102] The composition may comprise one or more further polymers. Examples are carboxymethylcellulose, poly(ethylene glycol), poly(vinyl alcohol), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

Chelating Agent

[0103] Chelating agents may be present or absent from the detergent compositions.

[0104] If present, then the chelating agent is present at a level of from 0.01 to 5 wt. %.

[0105] Example phosphonic acid (or salt thereof) chelating agents are: 1-Hydroxyethylidene-1,1-diphosphonic acid (HEDP); Diethylenetriaminepenta(methylenephosphonic acid) (DTPMP); Hexamethylenediaminetetra(methylenephosphonic acid) (HDTMP); Aminotris(methylenephosphonic acid) (ATMP); Ethylenediaminetetra(methylenephosphonic acid) (EDTMP); Tetramethylenediaminetetra(methylenephosphonic acid) (TDTMP); and, Phosphonobutanetricarboxylic acid (PBTC).

Examples

[0106] The invention will be demonstrated by the following non-limiting examples.

ExamplesCloning & expression including sequence information**TtEst *Thermogutta terrifontis*:**

[0107] The DNA sequence encoding a protein with putative hydrolytic activity was identified using the ANASTASIA halaxy pipeline from the HotZyme database. The gene was amplified from genomic DNA and cloning was performed using the aLICator LIC Cloning and Expression Kit for an N-terminal His₆-tag (pLATE31, *E. coli* ArcticExpress (DE3)RIL was transformed (heat-shock) and used as an expression strain for protein production.

[0108] TtEst mutants L37A and L251A were prepared using the QuikChange Lightning Site-Directed Mutagenesis Kit in accordance with the manufacturer's instructions. The mutant constructs were over-expressed in the same way as the native protein.

TtEst (SEQ ID NO 1)

maqrvkittt atpgeielaf edtgtglpvl lvhgfpldrt mwkaqreelc defrvivpdl rgfgesqvip gvatmeamad
 dlaglnhlg Itgkivlggl smggyvafaf arkyrdrlag lilcdtrarp dspeakenrr rvaervrreg pgfiaemip
 rlccstfrn hpeviekirq milsappegv aaaalgmaer pdstdllpal scptlvlggq fdaisppeer eamartipqs
 qfvvipdagh lppmeqperv tqairewlrk vhte

TtEst L37A (SEQ ID NO 2)

maqrvkittt atpgeielaf edtgtglpvl lvhgfpAdrt mwkaqreelc defrvivpdl rgfgesqvip gvatmeamad
 dlaglnhlg Itgkivlggl smggyvafaf arkyrdrlag lilcdtrarp dspeakenrr rvaervrreg pgfiaemip
 rlccstfrn hpeviekirq milsappegv aaaalgmaer pdstdllpal scptlvlggq fdaisppeer eamartipqs
 qfvvipdagh lppmeqperv tqairewlrk vhte

TtEst L251A (SEQ ID NO 3)

maqrvkittt atpgeielaf edtgtglpvl lvhgfpldrt mwkaqreelc defrvivpdl rgfgesqvip gvatmeamad
 dlaglnhlg Itgkivlggl smggyvafaf arkyrdrlag lilcdtrarp dspeakenrr rvaervrreg pgfiaemip
 rlccstfrn hpeviekirq milsappegv aaaalgmaer pdstdllpal scptlvlggq fdaisppeer eamartipqs
 qfvvipdagh Appmeqperv tqairewlrk vhte

AfEst2: Esterase from *Archaeoglobus fulgidus*:

The gene encoding AF-Est2 (locus tag: AF1537; Uniprot accession number: O28735) was PCR-amplified, without its stop codon, using chromosomal DNA of *A. fulgidus* as a template and introducing respectively a *Nco*I and *Xho*I restriction site. The generated PCR product was digested by *Nco*I and *Xho*I and the product was purified and ligated into the protein expression vector pET24d digested with the same restriction enzymes, resulting in the plasmid pWUR365 for the expression of the C-terminal 6x-His-tag AF-Est2 protein. *E. coli* strain BL21-CodonPlus (DE3)-RIPL was transformed with the expression plasmid.

AfEst2 (SEQ ID NO 4)

MLERVFIDVDGVKVSLLKGRERKVFYIHSSGSDATQWVNQLTAIGGYAIDLPHNGQSDTVE
 VNSVDEYAYYASESLKKTVGKAVVVGHSLGGAVAQKLYLRNPEICLALVLVGTGARLRVLP
 5 EILEGLKKEPEKAVDLMLSMAFASKGEEYEKKRREFLDRVDVLHLDLSLCDFDLLEDYRN
 GKLKIGVPTLVIVGEEDKLTPLKYHEFFHKHIPNSELVVIPGASHMVMLEKHVEFNEALEKFL
 KKVGV

10 Fermentation (harvest) & purification

TtEst *Thermogutta terrifontis*:

15 **[0109]** Protein production was performed in 2L Erlenmeyer flasks with 1L LB-medium and the appropriate antibiotic for plasmid selection (Ampicillin, 100 µg/mL, Gentamycin 20 µg/mL). The LB-medium containing only Ampicillin was inoculated with 1-3% (v/v) of preculture and incubated at 37°C and 180rpm until reaching OD₆₀₀ = 0.4. Subsequent the incubation temperature was lowered to 12°C for 1h. The gene expression was induced by addition of IPTG to final 1mM and carried out for 2d at 12°C and 180rpm. Cells were harvested by centrifugation (4750 x g, 20 min, 4 °C) and stored at -80°C.

20 **[0110]** Cell lysis was performed by resuspension of the cell paste in equilibration buffer (25 mM Tris-HCl, pH 8.0, 500 mM NaCl, 20 mM Imidazole, 10mL buffer for 1g cell wet weight) and sonication on ice to break the cells. The protein purification was performed using a 1mL HisTrap FF (GE Healthcare) column and AKTA purifier system (GE Healthcare) for affinity chromatography via the poly Histidine-tag. Elution of the protein was performed via a linear gradient for 30 min using buffer with increased imidazole concentration (25 mM Tris-HCl, pH 8.0, 500 mM NaCl, 500 mM Imidazole).
 25 Elution fractions were identified via absorbance (280nm) and applied to an SDS-PAGE. Fractions containing the protein of interest were pooled and dialysed overnight against 5 L of buffer without imidazole (25 mM Tris-HCl, pH 8.0, 500 mM NaCl). The dialysed protein was supplemented with 0.005% (v/v) sodium azide and 10% (v/v) glycerol for freezing and storage at -80 °C.

30 AfEst2: Esterase from *Archaeoglobus fulgidus*:

[0111] Protein production was performed in Luria-Bertani (LB) medium containing 50 µ g/ml each of kanamycin, chloramphenicol and streptomycin. The LB-medium containing kanamycin and chloramphenicol with 1-3% (v/v) of pre-culture and incubated at 37°C and 180rpm until reaching OD₆₀₀ = 0.6. The gene expression was induced by addition of
 35 IPTG to final 1mM and carried out for overnight 30°C and 180rpm. Cells were harvested by centrifugation (4750 x g, 20 min, 4 °C) and stored at -80°C.

[0112] Cell lysis was performed by resuspension of the cell paste in equilibration buffer (25 mM Tris-HCl, pH 8.0, 500 mM NaCl, 20 mM Imidazole, 10mL buffer for 1g cell wet weight) and sonication on ice to break the cells. The protein purification was performed using a 1mL HisTrap FF column using an AKTA purifier. for affinity chromatography via the
 40 poly Histidine-tag. Elution of the protein was performed via a linear gradient for 30 min using buffer with increased imidazole concentration (25 mM Tris-HCl, pH 8.0, 500 mM NaCl, 500 mM Imidazole). Elution fractions were identified via absorbance (280nm) and applied to an SDS-PAGE. Fractions containing the protein of interest were pooled and dialysed overnight against 5 L of buffer without imidazole (25 mM Tris-HCl, pH 8.0, 500 mM NaCl). The dialysed protein was supplemented with 0.005% (v/v) sodium azide and 10% (v/v) glycerol for freezing and storage at -80 °C.

45 Bioanalytics

Determination of protein concentration

50 **[0113]** The total amount of protein of enzyme samples was estimated by using Sigma-Aldrich (bicinchoninic acid) BCA assay kit and the working reagent was prepared as instructed in the user's manual. BCA reagent was prepared by mixing solution A [1% (w/v) bicinchoninic acid in sodium salt form, 2% (w/v) sodium carbonate, 0.16% (w/v) sodium tartrate, 0.4% (w/v) sodium hydroxide, 0.95% (w/v) sodium hydrogen carbonate, pH 11.5] with solution B [4% (w/v) copper sulphate] at 50:1 (v/v) ratio. A serial dilution of bovine serum albumin (2mg/mL) was carried out in deionised water to
 55 create 7 points of a standard curve. To perform the assay, BCA reagent (200µL) was added into the wells of 96-well plate, followed by sample protein dilutions (20µL). The microtitre plates (MTP) were sealed and incubated at 37°C for 30min. After incubation, the absorbance at 540nm was measured on a spectrophotometer.

Determination of esterase purity

[0114] Esterase-containing protein samples (20 μ L) were prepared with SDS-PAGE sample loading buffer and heated at 70°C for 10min before running on 4-12% NuPage Bis-Tris gels with MOPS buffer at 170V. PageRulerPlus molecular weight marker were run alongside samples for the determination of the molecular mass. Each gel was then stained using GelCode Blue Safe protein stain following the manufacturers protocol.

Biochemical determination of esterase activity

[0115] Esterase activity was determined by a colorimetric method using 4-nitrophenyl-valerate (C5) and 4-nitrophenyl-dodecanoate (C12) as substrates. 4-nitrophenyl-dodecanoate (25mg) or 4-nitrophenyl-valerate (18mg) were dissolved in 10mL solvent (methanol) to prepare 8mM stock solutions. Before carrying out the assay, 1mL of stock solution was added in 7mL of acidified water (pH 4.5), to give a final concentration of 1mM. In 96-well microtitre plates, 60 μ L dH₂O, 115 μ L Tris-HCl buffer (pH 8.5, 50mM), 5 μ L of diluted enzyme solution and 20 μ L substrate (multi-channel at the end) were added. For blanks, enzyme solution was replaced with dH₂O. Following the addition of reagents, the release of product (4-nitrophenol) was monitored at 405nm for 15min at ambient temperature.

APPLICATION TESTINGComposition of model human-like sebum and application to fabric

[0116] Table 1A shows the composition of human-like sebum to be used in the wash studies, and which is comparable to human sebum analysed in the literature (table 1B). Macrolex violet dye (0.4% w/w) was added to the model sebum, and then 100 μ L applied to a 10x10cm swatch of polycotton which was pre-heated to 60°C. Wicking of the stain was facilitated by leaving the stain to dry o/n at 60°C. Uniformity of staining was confirmed by colourimetric determination of SRI values across the swatch which was subsequently cut into smaller 30 mm diameter circles, enabling a fit in 6-well microtitre plates for subsequent wash trials.

Table 1: (A) Composition of the human-like sebum tested. Shown in comparison (B) is the composition of human sebum as proposed by Nikkari 1974, In Ro 2005, Stefaniak 2010. Model human-like sebum was designed to mimic the literature description.

(A) Model human-like sebum tested		
Ingredient	Type	% inclusion
Oleic Acid	Fatty acids (12%)	8
Isostearic Acid		4
Tricaprin	Triglycerides (39.2%)	1.8
Triolein		28.2
Triisostearin		9.2
Oleyl oleate	Wax esters (29.8%)	11.9
Myristyl myristate		11.9
Isostearyl isostearate		6
Squalene	Squalene (13.8%)	13.8
Cholesterol oleate	Cholesterol (esters) (5.1%)	3.4
Cholesterol		1.7
Total		99.9
(B) Human sebum (literature)		
Type	% inclusion median (range)	
Fatty acids	28.3 (2.3 - 38.3)	
Triglycerides	32.5 (14.8 - 44)	

(continued)

(B) Human sebum (literature)	
Type	% inclusion median (range)
Wax esters	25 (10 - 26)
Squalene	10.6 (3.3 - 20)
Cholesterol (esters)	6 (1 - 9.5)

Wash studies for enzymatic cleaning performance against human-like sebum

[0117] Pre-wash readings were taken for the 30 mm diameter sebum stains to measure stain intensity. Wash studies were conducted either in a 5 mL volume (within a 6 well plate, at 40 °C for 1 hour at 100 rpm) or in 100mL (within glass bottles, at 40 °C for 1 hour at 100 rpm). Enzymes were present at 25 mg/L within 2 g/L of a 7.5% surfactancy formulation. The stains were then rinsed three times post wash to completely remove the wash liquor and any residual enzyme. After drying, the stain plates were digitally scanned and their deltaE measured. This value is used to express cleaning effect and is defined as the colour difference between a white cloth and that of the stained cloth after being washed.

[0118] Mathematically, the definition of deltaE is:

$$\text{deltaE} = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

wherein ΔL is a measure of the difference in darkness between the washed and white cloth; Δa and Δb are measures for the difference in redness and yellowness respectively between both cloths. From this equation, it is clear that the lower the value of deltaE, the whiter the cloth will be. With regard to this colour measurement technique, reference is made to Commission International de l'Eclairage (CIE); Recommendation on Uniform Colour Spaces, colour difference equations, psychometric colour terms, supplement no. 2 to CIE Publication, no. 15, Colorimetry, Bureau Central de la CIE, Paris 1978.

[0119] Herein the cleaning effect is expressed in the form of a stain removal index (SRI):

$$\text{SRI} = 100 - \text{deltaE}.$$

[0120] The higher the SRI the cleaner the cloth, SRI = 100 (white).

Enzymatic cleaning performance against human-like sebum

[0121] Wash studies in a 5mL wash volume identified that the esterase enzymes of SEQ ID 1 to 4 the enzymes denoted TtEst, TtEst37, TtEst151, AfEst2, all show improved performance towards removal of the human-like sebum than the control samples which includes the laundry esterase benchmark (Cutinase) and the laundry lipase benchmark (Lipase Evtity). The 3-5 units SRI increase for the experimental enzymes shown is a clearly visualised cleaning improvement above that of the control enzyme cutinase and the laundry lipase benchmark (Lipase Evtity). Test was carried out in triplicate at 40°C for 1h. Formulation applied contains 7.5% total surfactant.

[0122] The >3 units SRI increase for the lipase enzyme of the invention is a clearly visualised cleaning improvement compared to Cutinase and Lipex Evtity (table 2).

Table 2: Cleaning performance of esterase enzymes of SEQ ID 1 to 4 (towards model human-like sebum) shown in comparison to controls of washes in either: water, or formulation plus benchmark commercial esterase (Cutinase) or formulation plus benchmark commercial laundry lipase (Lipex Evtity)

Sample	Wash performance (SRI)
Negative Control (water)	68.5 ± 1.03
Positive Control (formulation + Lipex Evtity)	70.6 ± 0.6
Positive Control (formulation + Cutinase)	73.9 ± 0.98
Invention (formulation + TtEst esterase of SEQ ID 1)	76.1 ± 1.1

(continued)

Sample	Wash performance (SRI)
Invention (formulation + TtEst37 esterase of SEQ ID 2)	76.2 $\pm$ 1.2
Invention (formulation + TtEst251 esterase of SEQ ID 3)	78.8 $\pm$ 2.3
Invention (formulation + AfEst2 esterase of SEQ ID 4)	77.2 $\pm$ 1.0

[0123] The stain removal index (SRI) indicating wash performance was measured. The $\pm$ statistics relates to 95% confidence level. The test shows that the esterases of SEQ ID 1 to 4 had much better performance against sebum than the commercial enzymes esterase (Cutinase) and lipase (Lipex Evely).

Enzymatic cleaning performance against human-like sebum

[0124] Wash studies in a 100mL volume confirm that the lipase of SEQ ID 4 shows improved performance towards removal of the human-like sebum than the control samples which includes the current commercial enzymes esterase (Cutinase) and lipase (Lipex Evely) (Table 3). Test was carried out in triplicate at 40°C for 1h. Formulation applied contains 7.5% total surfactant.

Table 3: Cleaning performance of esterase enzyme of SEQ ID 4 (towards model human-like sebum) shown in comparison to controls of washes in either: water, or formulation plus benchmark commercial esterase (Cutinase) or formulation plus benchmark commercial laundry lipase (Lipex Evely)

Sample	Wash performance (SRI)
Negative Control (water)	72.6 $\pm$ 1.2
Positive Control (formulation + Lipex Evely)	79.7 $\pm$ 1.28
Positive Control (formulation + Cutinase)	81.4 $\pm$ 1.65
Invention (formulation + AfEst2 esterase of SEQ ID 4)	84.2 $\pm$ 0.12

SEQUENCE LISTING

[0125]

<110> Unilever

<120> Detergent Composition

<130> C30306

<160> 4

<170> BiSSAP 1.3.6

<210> 1

<211> 274

<212> PRT

<213> thermogutta terrifontis

<400> 1

EP 3 884 025 B1

	Met	Ala	Gln	Arg	Val	Lys	Ile	Thr	Thr	Thr	Ala	Thr	Pro	Gly	Glu	Ile
	1				5					10					15	
	Glu	Leu	Ala	Phe	Glu	Asp	Thr	Gly	Thr	Gly	Leu	Pro	Val	Leu	Leu	Val
				20					25					30		
5	His	Gly	Phe	Pro	Leu	Asp	Arg	Thr	Met	Trp	Lys	Ala	Gln	Arg	Glu	Glu
			35					40					45			
	Leu	Cys	Asp	Glu	Phe	Arg	Val	Ile	Val	Pro	Asp	Leu	Arg	Gly	Phe	Gly
		50					55					60				
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Claims

- 35 1. A detergent composition comprising:
 - (i) from 1 to 60 wt.%, preferably from 2 to 50 wt.%, more preferably from 3 to 45 wt.%, even more preferably from 5 to 40 wt.%, most preferably from 6 to 40 wt.% of a surfactant; and,
 - (ii) from 0.0005 to 5 wt.%, preferably from 0.005 to 2.5 wt.%, more preferably from 0.01 to 1 wt.% of an esterase enzyme of enzyme class EC 3.1.1.1, having at least 60% sequence identity to any one of SEQ ID NO: 1 to 4.
- 40 2. A detergent composition according to claim 1, wherein the esterase enzyme has at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95%, most preferably at least 97%, at least 98% or even at least 99% sequence identity to any one of SEQ ID NO: 1 to 4.
- 45 3. A detergent composition according to claim 1 or claim 2, wherein the esterase enzyme has 100% sequence identity to any one of SEQ ID NO: 1 to 4.
- 50 4. A detergent composition according to any preceding claim, comprising from 0.1 to 10 wt.%, preferably from 0.2 to 9 wt.%, more preferably from 0.25 to 8, even more preferably from 0.5 to 6 wt.%, most preferably from 1 to 5 wt.% of a soil release polymer, more preferably a polyester based soil released polymer.
- 55 5. A detergent composition according to claim 4, wherein the polyester soil release polymer is a polyethylene and/or polypropylene terephthalate based soil release polymer, preferably a polypropylene terephthalate based soil release polymer.
6. A detergent composition according to any preceding claim, wherein the detergent composition comprises an alkox-

ylated polyamine, preferably at a level of from 0.1 to 8 wt.%, more preferably from 0.2 to 6 wt.%, most preferably from 0.5 to 5 wt.%.

7. A detergent composition according to any preceding claim, wherein the detergent composition is a laundry detergent composition, preferably the laundry detergent composition is a liquid or a powder, most preferably a liquid detergent.
8. A laundry detergent composition according to claim 7, wherein the surfactant comprises anionic and/or nonionic surfactant, preferably comprising both anionic and nonionic surfactant.
9. A detergent composition according to any preceding claim, preferably a laundry detergent composition, additionally comprising a further enzyme selected from the group consisting of: lipases, proteases, cellulases, alpha-amylases, peroxidases/oxidases, pectate lyases, and/or mannanases.
10. A detergent composition according to any preceding claim, preferably a laundry detergent composition, additionally comprising a further ingredient selected from fluorescent agent, perfume, shading dyes and polymers, and mixtures thereof.
11. A method of treatment of a fabric substrate with a sebum stain, said method comprising incorporation of an esterase enzyme of enzyme class EC 3.1.1.1, having at least 60%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95%, most preferably at least 97%, at least 98% or even at least 99% sequence identity, most preferably 100%, sequence identity to any one of SEQ ID NO: 1 to 4 into a detergent composition comprising from 1 to 60 wt.% of a surfactant; and subsequent treatment of a fabric substrate with a sebum stain, with said composition.
12. Use of an esterase enzyme of enzyme class EC 3.1.1.1, having at least 60%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95%, most preferably at least 97%, at least 98% or even at least 99% sequence identity, most preferably 100%, sequence identity to any one of SEQ ID NO: 1 to 4 to improve cleaning of sebum stains on fabric.

Patentansprüche

1. Reinigungsmittelzusammensetzung, umfassend:

(i) 1 bis 60 Gew.-%, vorzugsweise 2 bis 50 Gew.-%, bevorzugter 3 bis 45 Gew.-%, noch bevorzugter 5 bis 40 Gew.-%, höchst bevorzugt 6 bis 40 Gew.-% eines Tensids; und
(ii) 0,0005 bis 5 Gew.-%, vorzugsweise 0,005 bis 2,5 Gew.-%, bevorzugter 0,01 bis 1 Gew.-% eines Esterase-Enzyms der Enzymklasse EC 3.1.1.1 mit mindestens 60% Sequenzidentität mit irgendeiner der SEQ ID NO: 1 bis 4.

2. Reinigungsmittelzusammensetzung nach Anspruch 1, wobei das Esterase-Enzym mindestens 70%, bevorzugter mindestens 75%, bevorzugter mindestens 80%, bevorzugter mindestens 85%, noch bevorzugter mindestens 90%, noch bevorzugter mindestens 95%, höchst bevorzugt mindestens 97%, mindestens 98% oder sogar mindestens 99% Sequenzidentität mit irgendeiner der SEQ ID NO: 1 bis 4 aufweist.
3. Reinigungsmittelzusammensetzung nach Anspruch 1 oder Anspruch 2, wobei das Esterase-Enzym 100% Sequenzidentität mit irgendeiner der SEQ ID NO: 1 bis 4 aufweist.
4. Reinigungsmittelzusammensetzung nach irgendeinem vorhergehenden Anspruch, umfassend 0,1 bis 10 Gew.-%, vorzugsweise 0,2 bis 9 Gew.-%, bevorzugter 0,25 bis 8 Gew.-%, noch bevorzugter 0,5 bis 6 Gew.-%, höchst bevorzugt 1 bis 5 Gew.-% eines Soil-Release-Polymers, bevorzugter eines Soil-Release-Polymers auf Basis von Polyester.
5. Reinigungsmittelzusammensetzung nach Anspruch 4, wobei das Polyester-Soil-Release-Polymer ein Soil-Release-Polymer auf Basis von Polyethylen- und/oder Polypropylenterephthalat, vorzugsweise auf Basis von Polypropylenterephthalat ist.

6. Reinigungsmittelzusammensetzung nach einem vorhergehenden Anspruch, wobei die Reinigungsmittelzusammensetzung ein alkoxyliertes Polyamin umfasst, vorzugsweise in einem Anteil von 0,1 bis 8 Gew.-%, bevorzugter von 0,2 bis 6 Gew.-%, höchst bevorzugt von 0,5 bis 5 Gew.-%.
- 5 7. Reinigungsmittelzusammensetzung nach einem vorhergehenden Anspruch, wobei die Reinigungsmittelzusammensetzung eine Wäschereinigungsmittelzusammensetzung ist, vorzugsweise die Wäschereinigungsmittelzusammensetzung eine Flüssigkeit oder ein Pulver darstellt, höchst bevorzugt ein flüssiges Reinigungsmittel.
- 10 8. Wäschereinigungsmittelzusammensetzung nach Anspruch 7, wobei das Tensid anionisches und/oder nichtionisches Tensid, vorzugsweise sowohl anionisches als auch nichtionisches Tensid umfasst.
9. Reinigungsmittelzusammensetzung nach einem vorhergehenden Anspruch, vorzugsweise Wäschereinigungsmittelzusammensetzung, die zusätzlich ein weiteres Enzym umfasst, ausgewählt aus der Gruppe, bestehend aus: Lipasen, Proteasen, Cellulasen, alpha-Amylasen, Peroxidasen/Oxidasen, Pectatlyasen und/oder Mannanasen.
- 15 10. Reinigungsmittelzusammensetzung nach einem vorhergehenden Anspruch, vorzugsweise eine Wäschereinigungsmittelzusammensetzung, die zusätzlich einen weiteren Bestandteil umfasst, ausgewählt aus Fluoreszenzmittel, Parfüm, Schattierungsfarbstoffen und Polymeren und Mischungen davon.
- 20 11. Verfahren zur Behandlung eines textilen Substrats mit einem Talgfleck, wobei das Verfahren umfasst die Einverleibung eines Esterase-Enzyms der Enzymklasse EC 3.1.1.1 mit mindestens 60%, vorzugsweise mindestens 70%, bevorzugter mindestens 75%, bevorzugter mindestens 80%, bevorzugter mindestens 85%, noch bevorzugter mindestens 90%, noch bevorzugter mindestens 95%, höchst bevorzugt mindestens 97%, mindestens 98% oder sogar mindestens 99% Sequenzidentität, höchst bevorzugt 100% Sequenzidentität mit irgendeiner der SEQ ID NO: 1 bis 4 in einer Reinigungsmittelzusammensetzung, umfassend 1 bis 60 Gew.-% eines Tensids und nachfolgende Behandlung eines textilen Substrats mit einem Talgflecken mit dieser Zusammensetzung.
- 25 12. Verwendung eines Esterase-Enzyms der Enzymklasse EC 3.1.1.1 mit mindestens 60%, vorzugsweise mindestens 70%, bevorzugter mindestens 75%, bevorzugter mindestens 80%, bevorzugter mindestens 85%, noch bevorzugter mindestens 90%, noch bevorzugter mindestens 95%, höchst bevorzugt mindestens 97%, mindestens 98% oder sogar mindestens 99% Sequenzidentität, höchst bevorzugt 100% Sequenzidentität mit irgendeiner der SEQ ID NO: 1 bis 4, um die Reinigung von Talgflecken auf dem Textil zu verbessern.
- 30

35 Revendications

1. Composition de détergent comprenant :
 - (i) de 1 à 60 % en masse, de préférence de 2 à 50 % en masse, encore mieux de 3 à 45 % en masse, bien mieux encore de 5 à 40 % en masse, particulièrement de préférence de 6 à 40 % en masse d'un tensioactif ; et,
 - (ii) de 0,0005 à 5 % en masse, de préférence de 0,005 à 2,5 % en masse, encore mieux de 0,01 à 1 % en masse d'une enzyme estérase de classe d'enzyme EC 3.1.1.1, présentant au moins 60 % d'identité de séquence à l'une quelconque de SEQ ID NO : 1 à 4.
- 40 2. Composition de détergent selon la revendication 1, dans laquelle l'enzyme estérase présente au moins 70 %, encore mieux au moins 75 %, encore mieux au moins 80 %, encore mieux au moins 85 %, bien mieux encore au moins 90 %, bien mieux encore au moins 95 %, particulièrement de préférence au moins 97 %, au moins 98 % ou même au moins 99 % d'identité de séquence à l'une quelconque de SEQ ID NO : 1 à 4.
- 45 3. Composition de détergent selon la revendication 1 ou revendication 2, dans laquelle l'enzyme estérase présente 100 % d'identité de séquence à l'une quelconque de SEQ ID NO : 1 à 4.
- 50 4. Composition de détergent selon l'une quelconque des revendications précédentes, comprenant de 0,1 à 10 % en masse, de préférence de 0,2 à 9 % en masse, encore mieux de 0,25 à 8 %, bien mieux encore de 0,5 à 6 % en masse, particulièrement de préférence de 1 à 5 % en masse d'un polymère de libération des salissures, encore mieux d'un polymère de libération des salissures à base de polyester.
- 55 5. Composition de détergent selon la revendication 4, dans laquelle le polymère de libération des salissures de polyester

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est un polymère de libération des salissures à base de polyéthylène et/ou poly(propylène téréphtalate), de préférence un polymère de libération des salissures à base de poly(propylène téréphtalate).

- 5 **6.** Composition de détergent selon l'une quelconque des revendications précédentes, dans laquelle la composition de détergent comprend une polyamine alcoylée, de préférence à une teneur de 0,1 à 8 % en masse, encore mieux de 0,2 à 6 % en masse, bien mieux encore de 0,5 à 5 % en masse.
- 10 **7.** Composition de détergent selon l'une quelconque des revendications précédentes, dans laquelle la composition de détergent est une composition de détergent de lessive, la composition de détergent de lessive est de préférence un liquide ou une poudre, encore mieux un détergent liquide.
- 15 **8.** Composition de détergent de lessive selon la revendication 7, dans laquelle le tensioactif comprend un tensioactif anionique et/ou non ionique, comprenant de préférence un tensioactif à la fois anionique et non ionique.
- 20 **9.** Composition de détergent selon l'une quelconque des revendications précédentes, de préférence composition de détergent de lessive, comprenant de plus une autre enzyme choisie dans le groupe consistant en : lipases, protéases, cellulases, alpha-amylases, peroxydases/oxydases, pectate lyases, et/ou mannanases.
- 25 **10.** Composition de détergent selon l'une quelconque des revendications précédentes, de préférence composition de détergent de lessive, comprenant de plus un autre ingrédient choisi parmi un agent fluorescent, parfum, des colorants d'ombrage et polymères, et mélanges de ceux-ci.
- 30 **11.** Procédé de traitement d'un substrat de textile avec une tache de sébum, ledit procédé comprenant l'incorporation d'une enzyme estérase de classe d'enzyme EC 3.1.1.1, présentant au moins 60 %, de préférence au moins 70 %, encore mieux au moins 75 %, encore mieux au moins 80 %, encore mieux au moins 85 %, bien mieux encore au moins 90 %, bien mieux encore au moins 95 %, particulièrement de préférence au moins 97 %, au moins 98 % ou même au moins 99 % d'identité de séquence, de préférence 100 %, d'identité de séquence à l'une quelconque de SEQ ID NO : 1 à 4 dans une composition de détergent comprenant de 1 à 60 % en masse d'un tensioactif ; et le traitement subséquent d'un substrat de textile avec une tache de sébum, avec ladite composition.
- 35 **12.** Utilisation d'une enzyme estérase de classe d'enzyme EC 3.1.1.1, présentant au moins 60 %, de préférence au moins 70 %, encore mieux au moins 75 %, encore mieux au moins 80 %, encore mieux au moins 85 %, bien mieux encore au moins 90 %, bien mieux encore au moins 95 %, particulièrement de préférence au moins 97 %, au moins 98 % ou même au moins 99 % d'identité de séquence, particulièrement de préférence 100 %, d'identité de séquence à l'une quelconque de SEQ ID NO : 1 à 4 pour améliorer le nettoyage de taches de sébum sur du textile.

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