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(54) Title: USE OF POLYPEPTIDES HAVING DNASE ACTIVITY

(57) Abstract: The invention relates to detergent compositions comprising an enzyme having DNase activity and at least one perfume compound, as well as to use of a DNase in detergent compositions comprising a perfume for improving the effect of the perfume.

WO 2022/084303 A2

USE OF POLYPEPTIDES HAVING DNASE ACTIVITY

Reference to a Sequence Listing

This application contains a Sequence Listing in computer readable form, which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

Field of the invention

The present invention relates to compositions such as cleaning compositions comprising an enzyme having DNase activity and at least one perfume compound. The invention further relates to use of the detergent compositions in cleaning processes and/or for deep cleaning of organic stains, to methods of using said compositions for removal or reduction of components of organic matter, and to methods for increasing binding of a perfume to a textile, for boosting the effect of perfumes in detergent compositions, for increasing perfume retention on a textile and for producing perfume-containing compositions with a reduced amount of perfume.

Description of the Related Art

Cleaning compositions comprising one or more perfumes are well-known. Perfumes may be chemical compounds or e.g. natural oils such as essential oils and other natural compounds. The perfumes often have a double function of providing a desired scent and masking undesirable odors, which may stem from the detergent itself or from the fabric.

However, the use of perfume in laundry detergents has faced several challenges. For example, may perfume components are released very quickly, thus the “freshness” effect of perfume often does not last very long. In addition, with the increasing awareness of perfume allergy, there is a trend to use detergents containing less or no perfume. Furthermore, perfume is expensive and can represent a significant factor in the overall cost of e.g. laundry detergents.

Enzymes have been used in cleaning compositions for decades. Usually a cocktail of various enzymes is added to cleaning compositions, wherein each enzyme targets a specific substrate, e.g. amylases are active towards starch stains, proteases on protein stains and so forth. The effectiveness of these commercial enzymes provides cleaning compositions which remove much of the soiling. However, components of organic matters such as biofilm and extracellular polymeric substances (EPS) constitute a challenging type of staining due to the complex nature of such organic matter, and commercially available cleaning compositions are generally not able to effectively remove or reduce EPS and/or biofilm related stains. Textile surfaces and hard surfaces, such as dishes or the inner space of a laundry or dishwashing machine enduring a number of wash/cleaning cycles, become soiled with many different types of

soiling which may be composed of proteins, grease, starch etc. Some types of stain may be associated with organic matter such as biofilm, EPS, etc., which may be composed of different molecules such as polysaccharides, extracellular DNA (eDNA), and proteins. Some organic matter comprises an extracellular polymeric matrix, which may be sticky or gluing, which when present on textiles attracts soils and may cause redeposition or backstaining of soil, resulting in a greying of the textile. Additionally, organic matters such as biofilms often cause malodor issues as various malodor molecules can be adhered by the polysaccharides, extracellular DNA (eDNA) and proteins in the complex extracellular matrix and be slowly released to cause a noticeable malodor.

Cleaning compositions and detergents comprising enzymes having DNase activity have been described e.g. in WO 2014/087011, WO 2015/155350 and WO 2017/060475. WO 2011/163325 describes various perfume raw materials, perfume delivery systems and consumer products comprising such perfume raw materials and/or such perfume delivery systems.

The present invention is based on the surprising discovery that the use of a DNase in a detergent composition results in an enhanced effect of perfume compounds in the composition that allows for the amount of perfume to be reduced while still maintaining a freshness and cleanliness effect of the perfume on a textile washed with the detergent composition.

Summary of the Invention

The present invention relates to detergent compositions comprising an enzyme having DNase activity and at least one perfume compound, as well as to use of a polypeptide having DNase activity in detergent compositions comprising a perfume, e.g. for increasing binding of a perfume to a textile during a laundry process, for increasing perfume retention on a textile after wash, and/or for enhancing the effect of a perfume in a detergent composition.

The invention further relates to a method for increasing binding of a perfume to a textile during a laundry process, and/or for increasing perfume retention on a textile, the method comprising washing the textile with a detergent composition comprising at least one perfume compound and a polypeptide having DNase activity.

The invention further relates to a method for enhancing the effect of a perfume in a laundry detergent composition, and/or for preparing a detergent composition with a reduced perfume content while maintaining perfume effect after wash, the method comprising preparing a detergent composition comprising at least one perfume compound and a polypeptide having DNase activity.

The invention further relates to a method for cleaning an item, wherein the item is preferably a textile, comprising the steps of: a) contacting the item with a detergent composition comprising a polypeptide having DNase activity and at least one perfume compound, and optionally b) rinsing the item.

Overview of sequences

SEQ ID NO: 1 DNase polypeptide obtained from *Aspergillus oryzae*

SEQ ID NO: 2 mature DNase polypeptide obtained from *Aspergillus oryzae*

SEQ ID NO: 3 mature DNase polypeptide obtained from *Aspergillus oryzae*

SEQ ID NO: 4 motif [D/M/L][S/T]GYSR[D/N]

SEQ ID NO: 5 motif ASXNRSKG

SEQ ID NO: 6 mature DNase polypeptide obtained from *Metabacillus indicus* (previously known as *Bacillus cibi*)

SEQ ID NO: 7 mature polypeptide obtained from *Bacillus sp-62451*

SEQ ID NO: 8 mature polypeptide obtained from *Bacillus horikoshii*

SEQ ID NO: 9 mature polypeptide obtained from *Bacillus sp-62520*

SEQ ID NO: 10 mature polypeptide obtained from *Bacillus sp-62520*

SEQ ID NO: 11 mature polypeptide obtained from *Bacillus horikoshii*

SEQ ID NO: 12 mature polypeptide obtained from *Bacillus horikoshii*

SEQ ID NO: 13 mature polypeptide obtained from *Bacillus sp-16840*

SEQ ID NO: 14 mature polypeptide obtained from *Bacillus sp-16840*

SEQ ID NO: 15 mature polypeptide obtained from *Bacillus sp-62668*

SEQ ID NO: 16 mature polypeptide obtained from *Bacillus sp-13395*

SEQ ID NO: 17 mature polypeptide obtained from *Bacillus horneckia*

SEQ ID NO: 18 mature polypeptide obtained from *Bacillus sp-11238*

SEQ ID NO: 19 mature polypeptide obtained from *Bacillus sp-18318*

SEQ ID NO: 20 mature polypeptide obtained from *Bacillus idriensis*

SEQ ID NO: 21 mature polypeptide obtained from *Bacillus algicola*

SEQ ID NO: 22 mature polypeptide obtained from Xanthan alkaline community J

SEQ ID NO: 23 mature polypeptide obtained from *Bacillus vietnamensis*

SEQ ID NO: 24 mature polypeptide obtained from *Bacillus hwajinpoensis*

SEQ ID NO: 25 mature polypeptide obtained from *Paenibacillus mucilaginosus*

SEQ ID NO: 26 mature polypeptide obtained from *Bacillus indicus*

SEQ ID NO: 27 mature polypeptide obtained from *Bacillus marisflavi*

SEQ ID NO: 28 mature polypeptide obtained from *Bacillus luciferensis*

SEQ ID NO: 29 mature polypeptide obtained from *Bacillus marisflavi*

SEQ ID NO: 30 mature polypeptide obtained from *Bacillus sp. SA2-6*

SEQ ID NO: 31 motif [V/I]PL[S/A]NAWK

SEQ ID NO: 32 motif NPQL

SEQ ID NO: 33 mature polypeptide obtained from *Pyrenochaetopsis sp.*

SEQ ID NO: 34 mature polypeptide obtained from *Vibrissea flavovirens*

SEQ ID NO: 35 mature polypeptide obtained from *Setosphaeria rostrate*

SEQ ID NO: 36 mature polypeptide obtained from *Endophragmiella valdina*
SEQ ID NO: 37 mature polypeptide obtained from *Corynespora cassiicola*
SEQ ID NO: 38 mature polypeptide obtained from *Paraphoma sp. XZ1965*
SEQ ID NO: 39 mature polypeptide obtained from *Monilinia fructicola*
SEQ ID NO: 40 mature polypeptide obtained from *Curvularia lunata*
SEQ ID NO: 41 mature polypeptide obtained from *Penicillium reticulisporum*
SEQ ID NO: 42 mature polypeptide obtained from *Penicillium quercetorum*
SEQ ID NO: 43 mature polypeptide obtained from *Setophaeosphaeria sp.*
SEQ ID NO: 44 mature polypeptide obtained from *Alternaria sp. XZ2545*
SEQ ID NO: 45 mature polypeptide obtained from *Alternaria sp.*
SEQ ID NO: 46 motif P[Q/E]L[W/Y]
SEQ ID NO: 47 motif [K/H/E]NAW
SEQ ID NO: 48 mature polypeptide obtained from *Trichoderma reesei*
SEQ ID NO: 49 mature polypeptide obtained from *Chaetomium thermophilum*
SEQ ID NO: 50 mature polypeptide obtained from *Scytalidium thermophilum*
SEQ ID NO: 51 mature polypeptide obtained from *Metapochonia suchlasporia*
SEQ ID NO: 52 mature polypeptide obtained from *Daldinia fissa*
SEQ ID NO: 53 mature polypeptide obtained from *Acremonium sp. XZ2007*
SEQ ID NO: 54 mature polypeptide obtained from *Acremonium dichromosporum*
SEQ ID NO: 55 mature polypeptide obtained from *Sarocladium sp. XZ2014*
SEQ ID NO: 56 mature polypeptide obtained from *Metarhizium sp. HNA15-2*
SEQ ID NO: 57 mature polypeptide obtained from *Acremonium sp. XZ2414*
SEQ ID NO: 58 mature polypeptide obtained from *Isaria tenuipes*
SEQ ID NO: 59 mature polypeptide obtained from *Scytalidium circinatum*
SEQ ID NO: 60 mature polypeptide obtained from *Metarhizium lepidiotae*
SEQ ID NO: 61 mature polypeptide obtained from *Morchella costata*
SEQ ID NO: 62 mature polypeptide obtained from *Rhizoctonia solani*

Definitions

As used herein, the singular forms "a", "an", and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

If not indicated otherwise, all references to percentages in relation to the disclosed compositions relate to wt.% relative to the total weight of the respective composition.

DNase (deoxyribonuclease): The term “DNase” means a polypeptide or an enzyme with DNase activity that catalyzes the hydrolytic cleavage of phosphodiester linkages in the DNA backbone, thus degrading DNA. For purposes of the present invention, DNase activity is determined according to the procedure described in Assay 1 or Assay 2 herein.

Biofilm: A "biofilm" is organic matter produced by any group of microorganisms in which cells stick to each other or stick to a surface, such as a textile, dishware or hard surface or another kind of surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on living or non-living surfaces. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium. Bacteria living in a biofilm usually have significantly different properties from planktonic bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment for the microorganisms is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. The biofilm living bacteria do not lose their ability to live as planktonic cells if the biofilm matrix is compromised. On laundry, biofilm- or EPS-producing bacteria can e.g. be found among the following species: *Acinetobacter sp., Aeromicrobium sp., Brevundimonas sp., Microbacterium sp., Micrococcus luteus, Pseudomonas sp., Staphylococcus epidermidis* and *Stenotrophomonas sp.*

Clade: The term "clade" means a group of polypeptides clustered together on the basis of homologous features traced to a common ancestor. Polypeptide clades can be visualized as phylogenetic trees and a clade is a group of polypeptides that consists of a common ancestor and all its lineal descendants.

Deep cleaning: The term "deep cleaning" refers to disruption or removal of a biofilm or components of a biofilm such as polysaccharides, proteins, DNA, soil or other components present in the biofilm.

Detergent component: The term "detergent component" (or "cleaning component") means a detergent adjunct ingredient that is different from the DNase polypeptide of this invention. The precise nature of these additional cleaning or adjunct components, and levels of incorporation thereof, will depend on the physical form of the composition and the nature of the operation for which it is to be used. Suitable detergent components include, but are not limited to the components described below, such as surfactants, builders and co-builders, flocculating aid, chelating agents, dye transfer inhibitors, enzymes (other than the enzymes of the invention), enzyme stabilizers, enzyme inhibitors, catalytic materials, bleach activators, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, fabric hueing agents, anti-foaming agents, dispersants, processing aids, and/or pigments. Detergent compositions will typically contain at least one surfactant along with additional components such as at least one builder and/or at least one bleach component.

Detergent composition: The term "detergent composition" or "cleaning composition" refers to compositions that find use in the removal of undesired compounds from items to be cleaned, such as textiles. The detergent composition may be used to e.g. clean textiles for both household cleaning and industrial cleaning. The term encompasses any materials/compounds selected for the particular type of detergent composition desired and the form of the product (e.g., liquid, gel, powder, granulate, paste, or spray compositions) and includes, but is not limited to, detergent compositions such as liquid and/or solid laundry detergents and fine fabric detergents; fabric fresheners; fabric softeners; and textile and laundry pre-spotters/pretreatment. In addition to containing the enzyme of the invention, the detergent formulation may contain one or more additional enzymes (such as proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases, mannanases, nucleases or any mixture thereof), and/or detergent adjunct ingredients such as surfactants, builders, chelators or chelating agents, bleach system or bleach components, polymers, fabric conditioners, foam boosters, suds suppressors, dyes, perfume, tannish inhibitors, optical brighteners, bactericides, fungicides, soil suspending agents, anti-corrosion agents, enzyme inhibitors or stabilizers, enzyme activators, transferases, hydrolytic enzymes, oxido reductases, bluing agents and fluorescent dyes, antioxidants, and solubilizers. In one preferred embodiment, the detergent composition is a laundry detergent composition. In another preferred embodiment, the detergent composition is a fabric softener.

Detergent enzyme: The term "detergent enzyme" as used herein refers to enzymes that are not encompassed by the term "DNase" as defined herein. The term "detergent enzyme" includes enzymes traditionally used in detergent compositions, including but not limited to proteases, amylases, lipases, mannanases, pectate lyases and cellulases. Other detergent enzymes may e.g. include carbohydrases, pectinases, arabinases, galactanases, xylanases, oxidases or peroxidases.

Expression: The term "expression" includes any step involved in the production of a polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

Fragment: The term "fragment" means a polypeptide having one or more amino acids absent from the amino and/or carboxyl terminus of a mature polypeptide or domain, where the fragment has DNase activity.

Host cell: The term "host cell" means any cell type that is susceptible to transformation, transfection, transduction, or the like with a nucleic acid construct or expression vector comprising a polynucleotide of the present invention. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication.

Isolated: The term "isolated" means a polypeptide, nucleic acid, cell, or other specified material or component that is separated from at least one other component with which it is

naturally associated, including but not limited to, for example, other proteins, nucleic acids, cells, etc. An isolated polypeptide, nucleic acid, cell or other material is thus in a form that does not occur in nature.

Laundering: The term "laundering" relates to both household laundering and industrial laundering and means the process of treating textiles with a solution containing a cleaning or detergent composition of the present invention. The laundering process can for example be carried out using e.g. a household or an industrial washing machine or can be carried out by hand. A "laundry process" is intended to include any steps related to laundry, whether by hand or by machine, including but not limited to pre-treatment of fabrics, washing steps using a solution containing a laundry detergent composition, and rinsing steps e.g. using a fabric softener.

Malodor: The term "malodor" means an odor which is not desired on clean items. One example of malodor is compounds with an unpleasant smell, which may be produced by microorganisms. Another example is unpleasant smells which can be sweat or body odor adhered to an item which has been in contact with human or animal. Another example of malodor can be the odor from spices which stick to items, for example curry or other spices which smell strongly.

Mature polypeptide: The term "mature polypeptide" means a polypeptide in its mature form following N-terminal processing (e.g., removal of signal peptide). It is known in the art that a host cell may produce a mixture of two of more different mature polypeptides (i.e., with a different C-terminal and/or N-terminal amino acid) expressed by the same polynucleotide. It is also known that different host cells process polypeptides differently, and thus, one host cell expressing a polynucleotide may produce a different mature polypeptide (e.g., having a different C-terminal and/or N-terminal amino acid) as compared to another host cell expressing the same polynucleotide. Mature polypeptides of the invention may therefore have slight differences at the N- and/or C-terminal due to such differentiated expression by the host cell, while still having the same enzyme activity. A mature polypeptide having one or more amino acids absent from the N- and/or C-terminal may be considered to be a "fragment" of the full-length polypeptide. Similarly, a mature polypeptide having one or more additional amino acids at the N- and/or C-terminal due to differentiated expression may be considered to be an "extended" polypeptide.

Perfume effect: The term "perfume effect" in the context of the present invention is related to the human perception of perfume on washed clothes. The perfume effect of a polypeptide of the invention having DNase activity can be analyzed e.g. by means of a sensory evaluation such as that described in Example 1. The perfume effect can alternatively be measured by GC-MS analysis and expressed quantitatively as a "fragrance intensity" e.g. as described in Examples 2 and 3. An increased perfume effect can thus be expressed as an increased fragrance intensity, such that washing an item with a DNase polypeptide according to the invention results in an increase in fragrance intensity compared to washing the item under the same conditions but

without the DNase polypeptide.

Sequence identity: The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity". For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

(Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

Textile: The term "textile" means any textile material including yarns, yarn intermediates, fibers, non-woven materials, natural materials, synthetic materials, and any other textile material, fabrics made of these materials and products made from fabrics (e.g., garments and other articles), and is intended to include the term "fabric" as well. The textile or fabric may be in the form of knits, wovens, denims, non-wovens, felts, yarns, and towelling. The textile may be cellulose based such as natural cellulosics, including cotton, flax/linen, jute, ramie, sisal or coir or manmade cellulosics (e.g. originating from wood pulp) including viscose/rayon, cellulose acetate fibers (tricell), lyocell or blends thereof. The textile or fabric may also be non-cellulose based such as natural polyamides including wool, camel, cashmere, mohair, rabbit and silk or synthetic polymers such as nylon, aramid, polyester, acrylic, polypropylene and spandex/elastane, or blends thereof as well as blends of cellulose based and non-cellulose based fibers. Examples of blends are blends of cotton and/or rayon/viscose with one or more companion material such as wool, synthetic fiber (e.g. polyamide fiber, acrylic fiber, polyester fiber, polyvinyl chloride fiber, polyurethane fiber, polyurea fiber, aramid fiber), and/or cellulose-containing fiber (e.g. rayon/viscose, ramie, flax/linen, jute, cellulose acetate fiber, lyocell). Fabric may be conventional washable laundry, for example stained household laundry. When the term fabric or garment is used it is intended to include the broader term textiles as well.

Variant: The term "variant" means a polypeptide having DNase activity comprising an alteration, *i.e.*, a substitution, insertion, and/or deletion, at one or more positions. A substitution means replacement of the amino acid occupying a position with a different amino acid; a deletion means removal of the amino acid occupying a position; and an insertion means adding an amino acid adjacent to and immediately following the amino acid occupying a position.

Wash cycle: The term "wash cycle" is defined herein as a washing operation wherein textiles are immersed in a wash liquor, mechanical action of some kind is applied to the textile in order to release stains and to facilitate flow of wash liquor in and out of the textile, and finally the

superfluous wash liquor is removed. After one or more wash cycles, the textile is generally rinsed and dried.

Wash liquor: The term "wash liquor" is intended to mean the solution or mixture of water and detergents, in particular a detergent composition of the invention, used for laundering textiles, or for hard surface cleaning or dishwashing. The term is also intended to include e.g. rinse solutions comprising a fabric softener comprising a DNase polypeptide used for rinsing textiles.

Nomenclature

For purposes of the present invention, the nomenclature [E/Q] or [EQ] means that the amino acid at this position may be a glutamic acid (Glu, E) or a glutamine (Gln, Q). Likewise, the nomenclature [V/G/A/I] or [VGAI] means that the amino acid at this position may be a valine (Val, V), glycine (Gly, G), alanine (Ala, A) or isoleucine (Ile, I), and so forth for other combinations as described herein. Unless otherwise limited further, the amino acid X is defined such that it may be any of the 20 natural amino acids.

Substitutions are typically indicated with the original amino acid, the position number, and the replacement amino acid. For example, A226V indicates that the original alanine residue in position 226 has been replaced by a valine residue.

Deletions are indicated with an asterisk (*). For example, G184* indicates that the original glycine residue in position 184 has been deleted.

Insertions are indicated by listing the original amino acid, the position number, the original amino acid and the inserted amino acid. For example, S97SD indicates that an aspartic acid residue has been inserted after the serine residue in position 97.

Detailed Description of the Invention

The inventors have surprisingly found that enzymes having DNase activity act synergistically with volatile perfume compounds, resulting in an enhanced binding of perfume compounds to textiles washed with a detergent composition comprise a DNase and one or more perfume compounds. As a result of the increased retention of perfume compounds by the textiles, an enhanced perfume effect is obtained, allowing the amount of perfume in such detergent compositions to be reduced while still maintaining the desired perfume effect.

Volatile perfume compounds are often added to detergent compositions, e.g. laundry detergents or fabric softeners, to mask malodors and to provide a fresh and clean scent in the washed items, e.g. textiles. These malodors may come from various sources. One example is buildup of organic matter such as sebum, body soils, cell debris, biofilm, EPS etc. It has previously been shown that polypeptides having DNase activity can be used for preventing or removing biofilm on items such as textiles or fabric; see e.g. WO 2015/155350, WO 2017/060475 and Morales-Garcia et al, *J Surfact Deterg* (2020) DOI 10.1002/jsde.12398. Polypeptides having

DNase activity have also been shown to be able to reduce malodor in washed textiles. However, there is no suggestion in the art that there might actually be a synergistic effect between polypeptides having DNase activity and perfume compounds present in detergent compositions. While perfumes and the associated fresh and clean scent are often desired by consumers in detergent compositions such as laundry detergents, there are as noted above disadvantages to the use of perfumes, including the high cost of perfume compounds as well as the risk of allergy.

The present invention addresses these challenges by providing detergent compositions comprising polypeptides with DNase activity that have been found to have synergistic effects with perfume compounds, and which can thereby reduce the need for perfume addition to detergent compositions, in particular laundry detergent compositions.

Thus, one embodiment of the invention relates to a detergent composition comprising a polypeptide having DNase activity and at least one perfume compound, where the amount of perfume is reduced compared to a comparable detergent composition without a DNase. It will be understood that such compositions will have a perfume effect corresponding to that of a comparable detergent composition without the DNase and with a greater amount of perfume. This may allow the amount of perfume to be reduced to e.g. no more than 90%, such as no more than 80% or even lower, e.g. no more than 70% or no more than 60%, relative to the comparable composition without a DNase, with substantially the same perfume effect. This may be assessed e.g. using a sensory evaluation such as that described in Example 1 or quantitatively by GC-MS analysis e.g. as described in Example 2.

The invention thus provides a detergent composition comprising a DNase and at least one perfume compound, wherein the composition has an increased perfume effect relative to a comparable detergent composition without a DNase.

In a preferred embodiment, the detergent composition is a laundry detergent composition, wherein the composition provides an increased perfume retention on a textile.

In this embodiment, the invention thus provides a laundry detergent composition comprising a DNase and at least one perfume compound, wherein the composition has increased perfume retention on a textile after wash relative to a comparable detergent composition without a DNase.

In another preferred embodiment, the detergent composition is a fabric softener composition, wherein the composition provides an increased perfume retention on a textile.

In this embodiment, the invention thus provides a fabric softener composition comprising a DNase and at least one perfume compound, wherein the composition has increased perfume retention on a textile after rinse relative to a comparable fabric softener composition without a DNase.

In another embodiment, the detergent composition may be for cleaning of hard surfaces, for example for dishwashing, or for cleaning hard surfaces such as those found in kitchens and bathrooms.

Enzyme having DNase activity (DNase)

The DNase in the detergent compositions, uses and methods of the present invention is a nuclease polypeptide having DNase activity that catalyzes the hydrolytic cleavage of phosphodiester linkages in a DNA backbone, thus degrading DNA. The terms "DNase" and "polypeptide with/having DNase activity" may be used interchangeably herein.

Preferably, the DNase is selected from any of the enzyme classes E.C. 3.1.21.X, where X = 1, 2, 3, 4, 5, 6, 7, 8 or 9, e.g. Deoxyribonuclease I, Deoxyribonuclease IV, Type I site-specific deoxyribonuclease, Type II site-specific deoxyribonuclease, Type III site-specific deoxyribonuclease, CC-preferring endo-deoxyribonuclease, Deoxyribonuclease V, T(4) deoxyribonuclease II, T(4) deoxyribonuclease IV or E.C. 3.1.22.Y where Y = 1, 2, 4 or 5, e.g. Deoxyribonuclease II, Aspergillus deoxyribonuclease K(1), Crossover junction endo-deoxyribonuclease, or Deoxyribonuclease X.

Preferably, the DNase activity is obtained from a microorganism, and the DNase is a microbial enzyme. The DNase is more preferably of fungal or bacterial origin.

The polypeptide having DNase activity may for example be a fungal DNase obtained from *Aspergillus*, for example from *Aspergillus oryzae*.

Suitable bacterial DNases may, for example, be obtained from species of *Bacillus* and related genera (cf. Patel and Gupta, *Int. J. Syst. Evol. Microbiol.* 2020; 70:406–438, who proposed six new *Bacillaceae* genera from species formerly classified as belonging to the genus *Bacillus*), e.g. from *Bacillus, Cytobacillus, Metabacillus, Alkalihalobacillus, Rossellomorea* or *Mesobacillus*. Examples of species from which DNases may be obtained include *Bacillus licheniformis, Bacillus subtilis, Bacillus horikoshii, Cytobacillus horneckiae, Metabacillus indicus, Alkalihalobacillus algicola, Rossellomorea vietnamensis, Alkalihalobacillus hwajinpoensis, Metabacillus indicus, Mesobacillus campisalis, Bacillus idriensis, Bacillus algicola, Bacillus marisflavi* and *Bacillus luciferensis*. Preferred bacterial DNases include those obtained from *Metabacillus indicus* (previously known as *Bacillus cibi*) and variants thereof.

The DNase may also be obtained from any of the following: *Pyrenochaetopsis sp., Vibrissea flavovirens, Setosphaeria rostrate, Endophragmiella valdina, Corynespora cassiicola, Paraphoma sp., Monilinia fructicola, Curvularia lunata, Penicillium reticulisporum, Penicillium quercetorum, Setophaeosphaeria sp., Alternaria, Alternaria sp., Trichoderma reesei, Chaetomium thermophilum, Scytalidium thermophilum, Metapochonia suchlasporia, Daldinia fissa, Acremonium sp., Acremonium dichromosporum, Sarocladium sp., Metarhizium sp. HNA15-2, Isaria tenuipes Scytalidium circinatum, Metarhizium lepidiotae, Thermobispora*

bispora, Sporormia fimetaria, Pycnidiophora cf. dispera, Clavicipitaceae sp., Westerdykella sp., Humicolopsis cephalosporioides, Neosartorya massa, Roussoella intermedia, Pleosporales, Phaeosphaeria or Didymosphaeria futilis.

In some embodiments, the polypeptides having DNase activity are polypeptides comprising the PFAM domain DUF1524 ((pfam.xfam.org/), "The Pfam protein families database: towards a more sustainable future", R.D. Finn, et.al. Nucleic Acids Research (2016) Database Issue 44:D279-D285"). The DUF1524 domain contains a conserved HXXP sequence (where H is the amino acid histidine, P is the amino acid proline, and X is any amino acid motif) commonly found in nucleases (M.A. Machnicka, et al. Phylogenomics and sequence-structure-function relationships in the GmrSD family of Type IV restriction enzymes, BMC Bioinformatics, 2015, 16, 336). DUF stands for domain of unknown function, and polypeptide families comprising, e.g., DUF have been collected in the Pfam database, which provides sequence alignments and hidden Markov models that define the collected protein domains.

Thus, in some embodiments the polypeptides having DNase activity in the composition of the invention comprise the DUF1524 domain. For further information on DNases comprising the DUF1524 domain, see WO 2017/060475, which is hereby incorporated by reference.

In some embodiments, the DNase is a NUC1 or NUC1_A DNase. A NUC1 DNase is a DNase comprising a domain termed NUC1, and polypeptides with this domain are in addition to having DNase activity characterized by comprising certain motifs. Similarly, a NUC1 sub-domain had been identified, termed the NUC1_A domain, which also is characterized by comprising certain motifs. The NUC1 and NUC1_A DNases are described in WO 2017/060475 and WO 2018/184873, which are hereby incorporated by reference.

The preparation of the polypeptide having DNase activity as described herein can e.g. be performed as described in WO 2017/059802 (incorporated herein by reference), in particular in the sections Nucleic Acid Construct, Expression Vectors, Host Cells, Methods of Production and Fermentation Broth Formulations. See also WO 2015/155350, WO 2017/060475, WO 2017/060505, WO 2017/064269 and WO 2018/177936, the contents of which are incorporated herein by reference.

In one embodiment, the polypeptide having DNase activity is a polypeptide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% sequence identity to the polypeptide of SEQ ID NO: 1, or a fragment thereof having DNase activity.

It is known in the art that a host cell may produce a mixture of two of more different mature polypeptides (i.e., with a different C-terminal and/or N-terminal amino acid) expressed by the same polynucleotide. It is also known that different host cells process polypeptides differently, and thus, one host cell expressing a polynucleotide may produce a different mature polypeptide

(e.g., having a different C-terminal and/or N-terminal amino acid) as compared to another host cell expressing the same polynucleotide.

In the context of the polypeptide having SEQ ID NO: 1, the N-terminal amino acid portion, e.g. the 15 or 17 N-terminal amino acid residues, may be a propeptide sequence. Thus, in one embodiment, the mature polypeptide is a fragment of SEQ ID NO: 1 comprising 206 amino acid residues, corresponding to amino acids 16 to 221 of SEQ ID NO: 1 (shown as SEQ ID NO: 2). In a similar embodiment, the mature polypeptide is a fragment of SEQ ID NO: 1 comprising 204 amino acid residues, corresponding to amino acids 18 to 221 of SEQ ID NO: 1 (shown as SEQ ID NO: 3). The polypeptide having DNase activity may thus be a polypeptide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% sequence identity to the mature polypeptide of SEQ ID NO: 2 or SEQ ID NO: 3.

A polypeptide having DNase activity and at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% sequence identity to the polypeptide of SEQ ID NO: 1, or a fragment thereof having DNase activity, e.g. a polypeptide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% sequence identity to the polypeptide of SEQ ID NO: 2 or SEQ ID NO: 3, may be a variant having one or more alterations, e.g. substitutions, compared to SEQ ID NO: 1. Polypeptide variants may be engineered using protein engineering methods known in the art to produce a polypeptide having one or more improved properties compared to the parent polypeptide, for example improved detergent stability and/or wash performance, e.g. an improved deep cleaning effect.

A variant of the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 may, for example, include any of the alterations disclosed in WO 2017/064269 (incorporated herein by reference). In some embodiments, the polypeptide may thus be a variant of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 having DNase activity and comprising a substitution at one or more positions corresponding to positions 4, 17, 19, 36, 38, 39, 40, 41, 45, 51, 53, 54, 55, 57, 64, 66, 67, 68, 69, 70, 71, 72, 74, 75, 77, 82, 83, 84, 85, 86, 88, 91, 99, 101, 105, 106, 115, 116, 135, 136, 138, 139, 140, 141, 151, 152, 153, 154, 162, 163, 164, 166, 168, 169, 173, 182, 183, 184, 185, 186, 189, 212 and 215 of SEQ ID NO: 1, wherein the variant has a sequence identity to the polypeptide shown in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 of at least 80%, e.g. at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99%.

A DNase variant of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 may e.g. comprise one or more substitutions selected from the group consisting of N4E, L17E, T19A, T19G, T19I, K36P, Q38P, S39V, S39R, A40P, A40H, L41T, L41H, V45H, L51G, K53T, K53P, G54P, A55P, N57H, E64A, E64Q, E64R, E64T, E64I, E64S, T66H, K67A, K67T, N68V, N68P, N68I, N68H, S69A, S69D, S69E, S69K, S69L, S69W, S69Y, S69Q, N70T, N70H, N70G, R71T, D72E, S74H, S74G, G75I, N77T, K82P, K82I, D83T, D83P, D83I, D83H, D83G, P84H, Q85T, Q85P, Q85H, K86T,

K86P, K86H, G88P, G88H, A91P, W99T, A101W, K105E, K105N, K105T, K105D, S106T, S115T, L116I, Q135L, G136L, V138I, V138L, V138P, V138Q, L139A, N140R, N140L, N140A, G141L, F151R, D152Y, D152L, D152I, D152A, P153E, S154R, T162R, W163E, F164R, I166Y, I166R, K168N, F169R, F169E, A173I, A173R, A173T, S182R, N183E, D184I, K185Y, S186I, D189G, D189H, K212G, K212P and K215I, wherein position numbers correspond to the positions of SEQ ID NO 1.

In one aspect, the polypeptide having DNase activity belongs to the GYS-clade and comprises one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5).

In one embodiment of this aspect, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% sequence identity to the polypeptide of SEQ ID NO: 6, or a fragment thereof having DNase activity. In this embodiment, the polypeptide having DNase activity may be a variant having one or more alterations, e.g. substitutions, compared to SEQ ID NO: 6.

A variant of the polypeptide of SEQ ID NO: 6 may, for example, include any of the alterations disclosed in WO 2018/011277 (incorporated herein by reference). In some embodiments, the polypeptide thus may be a variant of SEQ ID NO: 6 having DNase activity and comprising a substitution at one or more positions corresponding to positions 1, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 16, 17, 19, 21, 22, 24, 25, 27, 28, 29, 30, 32, 38, 39, 40, 42, 49, 51, 52, 55, 56, 57, 58, 59, 61, 63, 65, 68, 76, 77, 78, 79, 80, 82, 83, 92, 93, 94, 99, 101, 102, 104, 105, 107, 109, 112, 116, 125, 126, 127, 130, 132, 135, 138, 139, 143, 144, 145, 147, 149, 152, 156, 157, 159, 160, 161, 162, 164, 166, 167, 168, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 181 and 182 of SEQ ID NO: 6, wherein the variant has a sequence identity to the polypeptide shown in SEQ ID NO: 6 of at least 80%, e.g. at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99%.

A DNase variant of SEQ ID NO: 6 may e.g. comprise one or more substitutions selected from the group consisting of T1I, T1L, T1V, T1F, T1Y, T1M, T1E, G4N, T5F, T5C, P6V, P6G, S7D, S7T, K8V, S9K, S9Q, S9V, S9L, S9F, S9P, S9R, A10D, A10M, A10I, A10Q, A10T, A10V, A10L, A10K, Q12S, Q12V, Q12E, S13D, S13Y, S13T, S13Q, S13F, S13R, S13V, S13N, S13H, S13M, S13W, S13K, S13L, S13E, Q14M, Q14R, N16S, A17C, A17V, A17E, A17T, A17S, T19K, T19N, T19L, T19S, T19I, T19V, K21Q, K21E, K21M, T22P, T22A, T22V, T22D, T22R, T22K, T22M, T22E, T22H, T22L, T22W, T22F, T22C, T22S, T22I, G24Y, S25P, S25T, S27N, S27I, S27M, S27D, S27T, S27V, S27F, S27A, S27C, S27L, S27E, G28L, Y29W, S30K, S30D, S30H, S30T, D32Q, I38V, I38M, S39A, S39P, S39Y, S39H, S39E, S39N, S39M, S39D, Q40V, S42G, S42C, S42D, S42L, S42M, S42F, S42N, S42W, V49R, L51I, K52I, K52Q, K52H, A55S, D56I,

D56L, D56T, S57W, S57Y, S57F, S57H, S57C, S57P, S57V, S57R, S57T, Y58A, Y58T, S59C, S59T, S59L, S59Q, S59V, S59K, S59R, S59M, S59I, S59H, N61D, P63A, T65L, T65I, T65V, T65R, T65K, S68V, S68I, S68W, S68K, S68Y, S68H, S68C, S68T, S68L, V76G, V76L, V76C, V76K, V76H, V76E, V76A, V76Y, V76N, V76M, V76R, V76I, V76F, T77N, T77Y, T77W, T77R, F78L, F78I, F78H, F78V, F78Y, F78C, T79G, T79R, N80K, N80S, S82L, S82E, S82K, S82R, S82H, D83C, D83F, D83L, L92T, A93G, E94N, G99S, S101D, S101A, S102M, S102L, S102V, S102A, S102K, S102T, S102R, T104S, T104P, T104A, T105V, T105I, K107L, K107C, K107R, K107H, K107S, K107M, K107E, K107A, K107Q, K107D, Q109K, Q109R, Q109S, A112S, S116D, S116R, S116Q, S116H, S116V, S116A, S116E, S116K, A125K, S126I, S126E, S126A, S126C, T127C, T127V, T127S, S130E, G132R, D135R, T138Q, W139R, R143E, R143K, S144Q, S144H, S144A, S144L, S144P, S144E, S144K, G145V, G145E, G145D, G145A, A147H, A147R, A147K, A147Q, A147W, A147N, A147S, G149S, K152H, K152R, S156C, S156G, S156K, S156R, S156T, S156A, T157S, Y159H, Y159F, K160R, K160V, W161L, W161Y, G162Q, G162N, G162D, G162M, G162R, G162A, G162S, G162E, G162L, G162K, G162V, G162H, S164R, S164H, S164N, S164T, Q166D, S167M, S167L, S167F, S167W, S167E, S167A, S167Y, S167H, S167C, S167I, S167Q, S167V, S167T, S168V, S168E, S168D, S168L, K170S, K170L, K170F, K170R, T171D, T171E, T171N, T171A, T171S, T171C, A172G, A172S, L173T, L173A, L173V, Q174L, G175D, G175E, G175N, G175R, G175S, M176H, L177I, N178D, N178E, N178T, N178S, N178A, S179E, S181R, S181E, S181D, S181I, S181F, S181H, S181W, S181L, S181M, S181Y, S181Q, S181V, S181G, S181A, Y182M, Y182C, Y182K, Y182G, Y182A, Y182S, Y182V, Y182D, Y182Q, Y182F, Y182L, Y182N, Y182I, Y182E, Y182T and Y182W compared to the polypeptide shown in SEQ ID NO: 6.

In a further embodiments, the polypeptide having DNase activity may be a DNase variant which compared to the DNase of SEQ ID NO: 6 comprises two or more substitutions selected from the group consisting of: T1I, T1L, T1V, S13Y, T22P, S25P, S27L, S39P, S42G, S42A, S42T, S57W, S57Y, S57F, S59V, S59I, S59L, V76L, V76I, Q109R, S116D, S116E, T127V, T127I, T127L, S144P, A147H, S167L, S167I, S167V, G175D and G175E, wherein the variant has a sequence identity to the polypeptide shown in SEQ ID NO: 6 of at least 80%, e.g. at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99%. DNase variants comprising such alterations are disclosed in WO 2019/081724 (incorporated herein by reference).

In still further embodiments, the polypeptide having DNase activity may be a DNase variant which compared to a DNase of SEQ ID NO: 6 comprises at least one substitution selected from the group consisting of: G4K, S7G, K8R, S9I, N16G, S27K, S27R, D32F, D32I, D32L, D32R, D32V, L33H ,L33R, L33K, L33V, L33Y, S39C, G41P, S42H, D45E, Q48D, N61E, T65M, T65W, S66R ,S66M, S66W, S66Y, S66V, F78L, P91L, S101N, S106L, S106R, S106H, Q109E, A112E, T127P, S130A, S130Y, T138D, Q140V, Q140G, A147P, C148A, W154Y, T157V, Y159A, Y159R,

G162C, Q174N, L177Y, S179L and C180A, wherein the variant has a sequence identity to the polypeptide shown in SEQ ID NO: 6 of at least 80%, e.g. at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99%. DNase variants comprising such alterations are disclosed in WO 2019/081721 (incorporated herein by reference).

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 7, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 8, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 9, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 10, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 11, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 12, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100%

sequence identity to the polypeptide of SEQ ID NO: 13, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 14, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 135, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 16, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 17, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 18, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 19, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: , or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 21, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 22, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 23, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 24, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 25, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 26, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 27, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID

NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 28, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 29, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 30, or a fragment thereof having DNase activity.

In one aspect, the polypeptide having DNase activity belongs to the NAWK clade and comprises one or both of the motifs [V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32).

In one embodiment of this aspect, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 33, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 34, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 35, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 36, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 37, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 38, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 39, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 40, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 41, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 42, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 43, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 44, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 45, or a fragment thereof having DNase activity.

In one aspect, the polypeptide having DNase activity belongs to the KNAW clade and comprises one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47).

In one embodiment of this aspect, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID

NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 48, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 49, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 50, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 51, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 52, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 53, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 54, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 55, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 56, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47)

and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 57, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 58, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 59, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 60, or a fragment thereof having DNase activity.

In further aspects, the DNase used in the present invention may be any of those that are disclosed in WO 2018/177203, WO 2018/177936 or WO 2018/177938, the contents of which are incorporated herein by reference. For example, the DNase may be the mature polypeptide obtained from *Morchella costata* disclosed as SEQ ID NO: 12 in WO 2018/177203 (SEQ ID NO: 61 herein), or a variant thereof; or a mature polypeptide obtained from *Rhizoctonia solani* disclosed in WO 2018/177938, such as the polypeptide of SEQ ID NO: 33, 36, 39, 42, 45, 48 or 51 therein, or a variant thereof, for example SEQ ID NO: 45 disclosed in WO 2018/177938 (SEQ ID NO: 62 herein), or a variant thereof.

Thus, in another embodiment, the polypeptide having DNase activity is a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 61, or a fragment thereof having DNase activity.

In another embodiment, the polypeptide having DNase activity is a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 62, or a fragment thereof having DNase activity.

The DNase can be included in the detergent composition of the invention at a level of from 0.01 to 1000 ppm, from 1 to 1000 ppm, from 10 to 1000 ppm, from 50 to 1000 ppm, from 100 to 1000 ppm, from 150 to 1000 ppm, from 200 to 1000 ppm, from 250 to 1000 ppm, from 250 to 750 ppm, or from 250 to 500 ppm based on active protein.

The DNase can be included in a wash liquor solution at a level of from 0.00001 to 100 ppm, from 0.00005 to 50 ppm, from 0.0001 to 50 ppm, from 0.0002 to 20 ppm, from 0.001 to 10 ppm, from 0.002 to 10 ppm, from 0.01 to 10 ppm, from 0.02 to 10 ppm, from 0.1 to 10 ppm, from 0.2 to 10 ppm, or from 0.5 to 5 ppm based on active protein.

Perfumes

The term “perfume compound” refers to a volatile fragrance compound that is suitable for use in a perfume. A “perfume compound” may also be referred to by similar terms such as a “perfume component” or a “fragrance compound”.

The term "perfume" includes perfume raw materials and compositions, scents and oils, e.g. essential oils. A wide variety of chemicals are known for fragrance (i.e., perfume) uses, including compounds such as aldehydes, ketones and esters. Also naturally occurring plant and animal oils and exudates comprising complex mixtures of various chemical components are known for use as fragrances.

A perfume is a blend of volatile compounds with different volatilities which can bind to receptors in the nose and therefore has a smell or odor, usually a pleasant one. These compounds are also known as odorants or fragrances. Most perfumes possess molar weights of up to approximately 200 g/mol, in some cases up to about 300 g/mol. Larger molecules are not volatile enough to be perceived by the human nose.

The volatility of a compound describes how readily it vaporizes by way of evaporation or boiling. Perfume compounds vaporize, depending on their volatility, by evaporation at room temperature and atmospheric pressure. Volatility is often described using vapor pressure or boiling point, with a high vapor pressure or low boiling point indicating a high volatility. Although the volatility of a compound is related to its molecular weight, other factors such as structure and polarity also play a role, as does interaction between fragrance compounds.

The most volatile fragrance compounds are referred to as top notes or head notes, whereas increasingly less volatile compounds are referred to as heart notes or middle notes, and the least volatile as base notes or back notes. The top notes are responsible for the first impression of a detergent, and the heart notes represent the characteristic smell. The base notes ensure the more substantial, long-lasting effect of the perfume.

The top, heart (middle) and base notes may be grouped based in different criteria. One such grouping is that of Poucher (Poucher, W. A. (1993). *Poucher's Perfumes, Cosmetics and Soaps*, Vol. 2 (Ninth ed.), Chapman & Hall, page 55). Poucher classified fragrance compounds according to an evaporation coefficient, with top notes having a coefficient of from 1 to 14, middle notes having a coefficient of from 15 to 60, and base notes having a coefficient of from 61 to 100.

Fragrance manufacturers and sellers provide information about their fragrance compounds such as molecular weight, vapor pressure and boiling point, and may also indicate whether individual fragrance compounds are top notes, middle notes or base notes. Such information is e.g. provided at iff.com/portfolio/products/fragrance-ingredients/online-compendium and at shop.perfumersapprentice.com.

For purposes of the present invention, top, heart and base notes may also be defined based on retention time (in minutes) in GC-MS performed using the parameters as defined in

Example 2 herein. Using this definition, top notes are defined as fragrance compounds that have a retention time in GC-MS of less than 8.76 min, heart notes are defined as fragrance compounds that have a retention time in GC-MS in the range of from 8.76 to 16.51 min, and base notes are defined as fragrance compounds that have a retention time in GC-MS of more than 16.51 min.

In one embodiment of the invention, the presence of a polypeptide having DNase activity in a detergent composition, e.g. a laundry detergent composition or a fabric softener, results in an increase in binding and/or retention of top note and/or heart note perfume compounds compared to base note compounds, and thus an increased relative perfume effect for these notes. In one particular embodiment, at least the heart note compounds are increased relative to the base note compounds. In another particular embodiment, the top note and heart note compounds are increased relative to the base note compounds. In a further particular embodiment, at least the top note compounds are increased relative to the base note compounds.

In an embodiment, the presence of a polypeptide having DNase activity in a detergent composition, e.g. a laundry detergent composition or a fabric softener, results in an increase in binding and/or retention of top note and/or heart note perfume compounds compared to base note compounds when measured on a polyester material.

It should be noted that in the context of the invention, the precise classification of any given fragrance compound as being a top, heart or base note is not important. Rather, as it will be apparent from the explanation above, it is a question of how a DNase polypeptide in a detergent composition effects the overall binding and/or retention of the different groups of fragrance compounds, where such compounds are broadly classified into the three categories of top, heart and base notes.

Besides volatility, the odor detection threshold of the fragrance compounds is also important for the perfume functions. The odor detection threshold value is defined as the minimal concentration of a substance that can be detected by a human nose. Thus, compounds with a lower detection odor threshold are more easily detected by humans. Although the threshold is subjective and may vary, it mainly depends on three factors, the vapor pressure, water solubility and the water/organic solvent (octanol) partition coefficient, which together account for 77% of variance in threshold values (Rodriguez et al., 2011, Flavour and Fragrance Journal 26: 421-428).

Just like fragrances, malodors are also volatile compounds that can bind to receptors in the nose. However, in contrast to fragrances used in perfume, malodors are perceived as unpleasant. A perfume can interfere with the perception of malodors by competing with the receptors in the nose, thereby masking the malodors. In another words, fragrances do not interact with malodors, and malodors are not physically removed or chemically eliminated. Rather, perfume compositions are carefully designed to mask the anticipated malodors.

The duration of the “freshness” or “cleanliness” effect provided by a perfume in a detergent composition is influenced by how fragrances and malodors are retained on the washed fabric.

Laundry malodors can come from various sources, including human body odor as well as malodors from the environment such as kitchen odors, cigarettes, food stains, etc. Another important source of malodors is from microbes present in the textile, which can metabolize the substances transferred from the human body (sweat, dead cells, sebum, etc.) and generate malodors during drying, storage or wearing (Bockmuehl et al., 2019, Microbial Cell 6: 299-306).

Fiber type also plays an important role in retaining and release of odor compounds, e.g. malodor compounds may be more effectively removed from cotton than from polyester. This is partly related to the polarity (hydrophilicity) of the odor compounds and that of the textile fibers, with cotton containing mainly highly polar cellulosic fibers, while fibers of polyester and wool are relatively non-polar compared to cotton fibers. In general, the order of compound polarity of perfume compounds from high to low is as follows: Amide > Acid > Alcohol > Ketone ≈ Aldehyde > Ester > Alkane.

When some typical laundry malodor compounds were added to cotton or polyester, they were effectively removed from cotton, while they strongly adhered to the more hydrophobic polyester after wash. The strong association of odorants with the polyester fibers during wash was found to contribute to a more complex odor profile in polyester than in cotton (Munk et al., 2001, Journal of Surfactants and Detergents 4: 385-394).

In addition, “invisible dirt” present on the textile also affects odor retention. Used textiles often contain human secretion (e.g. sebum, keratin) and microbial soil (e.g. biofilm, DNA, carbohydrates), which may not be visible but which contribute to binding of odor compounds on the textile.

Perfume compounds used in laundry detergents may be chemical compounds from any of several different classes or essential oils or other natural compounds. The perfumes that may be used in the context of the present invention are not subject to any restrictions. Thus, in particular synthetic or natural odorant substance compounds of the types esters, ethers, aldehydes (fragrance aldehydes, odorant aldehydes), ketones (fragrance ketones, odorant ketones), alcohols, hydrocarbons, acids, carbonic acid esters, aromatic hydrocarbons, aliphatic hydrocarbons, saturated and/or unsaturated hydrocarbons and mixtures of these may be used as perfume compounds.

Individual perfume compounds, e.g. synthetic products of the ester, ether, aldehyde, ketone, alcohol, and hydrocarbon types, can be used as well as mixtures thereof. It is preferred, however, to use mixtures of different perfume compounds, which together generate an attractive scent note. Such mixtures can also contain natural perfume mixtures such as those accessible from plant sources, e.g. pine, citrus, jasmine, patchouli, rose or ylang-ylang oil.

Non-limiting examples of different types of perfumes are provided below.

Suitable perfumes of the ester type include e.g. benzyl acetate, phenoxy ethyl isobutyrate, p-tert-butyl cyclohexyl acetate, linalyl acetate, dimethyl benzyl carbinyl acetate (DMBCA), phenyl

ethyl acetate, ethyl methyl phenyl glycinate, allyl cyclohexyl propionate, styrallyl propionate, benzyl salicylate, cyclohexyl salicylate, floramate, melusate and jasmacyclate.

Odorant substance compounds of the hydrocarbon type include e.g. terpenes such as limonene and pinene.

Suitable perfumes of the ether type include e.g. benzyl ethyl ether and ambroxan.

Suitable perfume alcohols include e.g. 10-undecen-1-ol, 2,6-dimethyl heptan-2-ol, 2-methyl butanol, 2-methyl pentanol, 2-phenoxy ethanol, 2-phenyl propanol, 2-tert-butyl cyclohexanol, 3,5,5-trimethyl cyclohexanol, 3-hexanol, 3-methyl-5-phenyl pentanol, 3-octanol, 1-octen-3-ol, 3-phenyl propanol, 4-heptenol, 4-isopropyl cyclohexanol, 4-tert-butyl cyclohexanol, 6,8-dimethyl-2-nonanol, 6-nonen-1-ol, 9-decen-1-ol, alpha-methyl benzyl alcohol, alpha-terpineol, amyl salicylate, benzyl alcohol, benzyl salicylate, beta-terpineol, butyl salicylate, citronellol, cyclohexyl salicylate, decanol, dihydro myrcenol, dimethyl benzyl carbinol, dimethyl heptanol, dimethyl octanol, ethyl salicylate, ethyl vanillin, anethol, eugenol, geraniol, heptanol, hexyl salicylate, isoborneol, isoeugenol, isopulegol, linalool, menthol, myrtenol, n-hexanol, nerol, nonanol, octanol, para-menthan-7-ol, phenyl ethyl alcohol, phenol, phenyl salicylate, tetrahydro geraniol, tetrahydro linalool, thymol, trans-2-cis-6-nonadienol, trans-2-nonen-1-ol, trans-2-octenol, undecanol, vanillin, and cinnamic alcohol, wherein when multiple perfume alcohols are present, they may be selected independently of one another.

Suitable perfume ketones can include all ketones that can lend a desired scent or a sensation of freshness. Mixtures of different ketones can also be used. For example the ketone can be selected from the group consisting of buccoxime, iso-jasmone, methyl-beta-naphthyl ketone, Moschus indanone, Tonalid/Moschus plus, alpha-damascone, beta-damascone, delta-damascone, isodamascone, damascenone, damarose, methyl dihydro jasmonate, menthone, carvone, campher, fenchone, alpha-ionene, beta-ionone, dihydro-beta-ionone, gamma-methyl ionone, fleuramone, dihydro jasmone, cis-jasmone, iso-E-Super, methyl cedrenyl ketone or methyl cedrylone, acetophenone, methyl acetophenone, para-methoxy acetophenone, benzyl acetone, benzophenone, para-hydroxy-phenyl butanone, celery ketone or livescone, 6-isopropyl decahydro-2-naphtone, dimethyl octenone, frescomenthe, 4-(1-ethoxyvinyl)-3,3,5,5-tetramethyl cyclohexanone, methyl heptenone, 2-(2-(4-methyl-3-cyclohexen-1-yl)-propyl) cyclopentanone, 1-(para-menthen-6(2)-yl)-1-propanone, 4-(4-hydroxy-3-methoxy phenyl)-2-butanone, 2-acetyl-3,3-dimethyl norbomane, 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone, 4-damascol, dulcinyl or cassion, gelsone, hexalone, isocyclemone E, methyl cyclocitrone, methyl lavender ketone, orivone, para-tert-butyl cyclohexanone, verdone, delphone, muscone, neobutenone, plicatone, veloutone, 2,4,4,7-tetramethyl-oct-6-en-3-one, tetrameran, hedione and mixtures thereof. Preferred ketones may e.g. be selected from alpha-damascone, delta-damascone, isodamascone, carvone, gamma-methyl ionone, iso-E-super, 2,4,4,7-tetramethyl-oct-6-en-3-one,

benzyl acetone, beta-damascone, damascenone, methyl dihydro jasmonate, methyl cedrylone, hedione and mixtures thereof.

Suitable perfume aldehydes can be any aldehydes that produce a desired scent or a sensation of freshness. They may be individual aldehydes or mixtures of aldehydes. Exemplary suitable aldehydes are melonal, triplal, ligustral, adoxal, anis aldehyde, cymal, ethyl vanillin, florhydral, helional, heliotropine, hydroxy citronellal, koavone, laurin aldehyde, lyral, methyl nonyl acetaldehyde, para-tert-bucinal, phenyl acetaldehyde, undecylene aldehyde, vanillin, 2,6,10-trimethyl-9-andecenal, 3-dodecen-1-al, alpha-n-amyl cinnamaldehyde, 4-methoxy benzaldehyde, benzaldehyde, 3-(4-tert-butylphenyl)-propanal, 2-methyl-3-(para-methoxy phenyl propanal), 2-methyl-4-(2,6,6-trimethyl-2(1)-cyclohexen-1-yl)-butanal, 3-phenyl-2-propenal, cis-/trans-3,7-dimethyl-2,6-octadien-1-al, 3,7-dimethyl-6-octen-1-al, [(3,7-dimethyl-6-octenyl)-oxy]-acetaldehyde, 4-isopropyl benzyaldehyde, 1,2,3,4,5,6,7,8-octahydro-8,8-dimethyl-2-naphthaldehyde, 2,4-dimethyl-3-cyclohexen-1-carboxyaldehyde, 2-methyl-3-(isopropyl-phenyl)-propanal, decylaldehyde, 2,6-dimethyl-5-heptenal, 4-(tricyclo-[5.2.10-(2,6)]-decylidene-8)-butanal, octahydro-4,7-methano-1H-indene carboxaldehyde, 3-ethoxy-4-hydroxy benzaldehyde, para-ethyl-alpha-alpha-dimethyl hydro cinnamaldehyde, alpha-methyl-3,4-(methylene dioxy)-hydro cinnamaldehyde, 3,4-methylene dioxy benzaldehyde, alpha-n-hexyl cinnamaldehyde, m-cymene-7-carboxaldehyde, alpha-methyl phenyl acetaldehyde, 7-hydroxy-3,7-dimethyl octanal, undecenal, 2,4,6-trimethyl-3-cyclohexene-1-carboxaldehyde, 4-(3)-(4-methyl-3-pentenyl)-3-cyclohexene carboxaldehyde, 1-dodecanal, 2,4-dimethyl cyclohexene-3-carboxaldehyde, 4-(4-hydroxy-4-methyl pentyl)-3-cyclohexene-1-carboxaldehyde, 7-methoxy-3,7-dimethyl octan-1-al, 2-methyl undecanal, 2-methyl decanal, 1-nonanal, 1-octanal, 2,6,10-trimethyl-5,9-undecadienal, 2-methyl-3-(4-tert-butyl)-propanal, dihydro cinnamaldehyde, 1-methyl-4-(4-methyl-3-pentenyl)-3-cyclohexene-1-carboxaldehyde, 5- or 6-methoxy hexahydro-4,7-methano indane-1 or 2-carboxy aldehyde, 3,7-dimethyl octan-1-al, 1-undecanal, 10-undecen-1-al, 4-hydroxy-3-methoxy benzaldehyde, 1-methyl-3-(4-methyl pentyl)-3-cyclohexene carboxy aldehyde, trans-4-decenal, 2,6-nonadienal, para-tolyl-acetaldehyde, 4-methyl phenyl acetaldehyde, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal, ortho-methoxy cinnamaldehyde, 3,5,6-trimethyl-3-cyclohexene carboxaldehyde, 3,7-dimethyl-2-methylene-6-octenal, phenoxy acetaldehyde, 5,9-dimethyl-4,8-decadienal, peony aldehyde (6,1-dimethyl-3-oxa-5,9-undecadien-1-al), hexahydro-4,7-methanoindane-1-carboxaldehyde, 2-methyloctanal, alpha-methyl-4-(1-methyl ethyl)-benzene acetaldehyde, 6,6-dimethyl-2-norpinene-2-propion aldehyde, para-methyl phenoxy acetaldehyde, 2-methyl-3-phenyl-2-propen-1-al, 3,5,5-trimethyl hexanal, hexahydro-8,8-dimethyl-2-naphthaldehyde, 3-propyl-bicyclo-[2.2.1]-hept-5-ene-2-carbaldehyde, 9-decenal, 3-methyl-5-phenyl-1-pentanal, 1-para-menthene-q-carboxaldehyde, citral or mixtures thereof, lilial citral, 1-decanal, 2,4-dimethyl-3-cyclohexene-1-carboxaldehyde. Preferred aldehydes may e.g. be selected from cis/trans-3,7-dimethyl-2,6-octadien-1-al, heliotropin, 2,4,6-trimethyl-3-

cyclohexene-1-carboxaldehyde, 2,6-nonadienal, alpha-n-amyl cinnamaldehyde, alpha-n-hexyl cinnamaldehyde, para-tert-bucinal, lyral, cymal, methyl nonyl acetaldehyde, trans-2-nonenal, lilial, trans-2-nonenal and mixtures thereof.

Perfume compounds may also be natural odorant mixtures such as those accessible from plant sources, e.g. pine, citrus, jasmine, patchouli, rose or ylang-ylang oil. Also suitable are muscat, sage oil, chamomile oil, clove oil, mint oil, cinnamon leaf oil, lime blossom oil, juniper berry oil, vetiver oil, olibanum (frankincense) oil, galbanum oil and labdanlum oil as well as orange blossom oil, neroli oil, orange peel oil and sandalwood oil. The perfume compounds may also be essential oils, e.g. angelica root oil, anise oil, arnica blossom oil, basal oil, bay oil, champaca blossom oil, silver fir oil, silver fir cone oil, elemi oil, eucalyptus oil, fennel oil, spruce needle oil, geranium oil, gingergrass oil, guaiac wood oil, gurjun balsam oil, helichrysum oil, ho leaf oil, ginger oil, iris oil, cajeput oil, calmus oil, camphor oil, canaga oil, cardamom oil, cassia oil, copaiva balsam oil, coriander oil, spearmint oil, caraway oil, cumen oil, lavender oil, lemongrass oil, lime oil, mandarin oil, lemon balm oil, musk seed oil, myrrh oil, niaouli oil, origanum oil, palmarosa oil, peru balsam oil, petit grain oil, pepper oil, peppermint oil, pimento oil, rosemary oil, celery oil, spike oil, stemanis oil, turpentine oil, thuja oil, thyme oil, verbena oil, vermouth oil, wintergreen oil, ysop oil, cinnamon oil, citronella oil, lemon oil and cypress oil.

Further information about fragrance ingredients may be obtained from The International Fragrance Association (IFRA), which publishes a list of all fragrance ingredients used in consumer goods (ifrafragrance.org/initiatives/transparency/ifra-transparency-list).

In one embodiment a plurality of perfume compounds, e.g. those listed above or on the list maintained by the IFRA, may be included in a detergent composition of the invention. The compositions of the invention may therefore e.g. contain three or more, such as four or more, five or more, six or more or seven or more different perfume components.

The compositions of the invention will typically contain one or more perfume components in a total amount (by weight) of from 0.0001% to 2.5%, such as 0.001-2%, e.g. 0.01-1.5%, for example 0.1-1% percent, based on the total amount of perfume components and the total weight of the composition.

There are no limitations on the type of detergent composition in which perfumes may be incorporated. They may, for example, be included in detergent compositions that are in the form of liquids, gels, powders, granulates, tablets, pods, pouches and soap bars.

Perfume components may be incorporated into detergent compositions in physical forms and using methods known in the art, e.g. adding the perfume components as liquids, solid particles and/or microcapsules.

Cleaning components

The choice of cleaning components may include, for textile care, the consideration of the type of textile to be cleaned, the type and/or degree of soiling, the temperature at which cleaning is to take place, and the formulation of the detergent product. Although components mentioned below are categorized by general header according to a particular functionality, this is not to be construed as a limitation, as a component may comprise additional functionalities as will be appreciated by the skilled artisan.

Surfactants

The cleaning composition may comprise one or more surfactants, which may be anionic and/or cationic and/or non-ionic and/or semi-polar and/or zwitterionic, or a mixture thereof. In a particular embodiment, the detergent composition includes a mixture of one or more nonionic surfactants and one or more anionic surfactants. The surfactant is typically present at a level of from about 1% to 70% by weight, such as about 1 wt% to about 40 wt%, or about 3 wt% to about 20 wt%, or about 3 wt% to about 10 wt%.

The one or more surfactants are chosen based on the desired cleaning application, and may include any conventional surfactants known in the art.

When included therein the detergent will usually contain from about 1% to about 70% by weight of an anionic surfactant, such as from about 5 wt % to about 50 wt %, including from about 5 wt % to about 20 wt %, or from about 15 wt % to about 20 wt %, or from about 20 wt % to about 25 wt % or at least 30 wt%, at least 40 wt% or at least 50 wt% of an anionic surfactant. Non-limiting examples of anionic surfactants include sulfates and sulfonates, in particular, alkylbenzenesulfonates, such as linear alkylbenzenesulfonates (LAS), isomers of LAS, branched alkylbenzenesulfonates (BABS), phenylalkanesulfonates, alpha-olefinsulfonates (AOS), olefin sulfonates, alkene sulfonates, alkane-2,3-diylbis(sulfates), hydroxyalkanesulfonates and disulfonates, alkyl sulfates (AS) such as sodium dodecyl sulfate (SDS), fatty alcohol sulfates (FAS), primary alcohol sulfates (PAS), alcohol ethersulfates (AES or AEOS or FES, also known as alcohol ethoxysulfates or fatty alcohol ether sulfates), secondary alkanesulfonates (SAS), paraffin sulfonates (PS), ester sulfonates, sulfonated fatty acid glycerol esters, alpha-sulfo fatty acid methyl esters (alpha-SFMe or SES) including methyl ester sulfonate (MES), alkyl- or alkenylsuccinic acid, dodecenyl/tetradecenyl succinic acid (DTSA), fatty acid derivatives of amino acids, diesters and monoesters of sulfo-succinic acid or salt of fatty acids (soap), and combinations thereof.

When included therein the detergent will usually contain from about 1% to about 40% by weigh of a cationic surfactant, for example from about 0.5% to about 30%, in particular from about 1% to about 20%, from about 3% to about 10%, such as from about 3% to about 5%, from about 8% to about 12% or from about 10% to about 12%. Non-limiting examples of cationic surfactants include alkyldimethylethanolamine quat (ADMEAQ), cetyltrimethylammonium bromide (CTAB),

dimethyldistearylammonium chloride (DSDMAC), and alkylbenzyldimethylammonium, alkyl quaternary ammonium compounds, alkoxylated quaternary ammonium (AQA) compounds, ester quats, and combinations thereof.

When included therein the detergent will usually contain from about 0.2% to about 40% by weight of a nonionic surfactant, for example from about 0.5 wt% to about 30 wt %, in particular from about 1 wt % to about 20 wt %, from about 3 wt % to about 10 wt %, such as from about 3% wt to about 5 wt %, from about 8 wt % to about 12 wt %, or from about 10 wt % to about 12 wt %. Non-limiting examples of nonionic surfactants include alcohol ethoxylates (AE or AEO), alcohol propoxylates, propoxylated fatty alcohols (PFA), alkoxylated fatty acid alkyl esters, such as ethoxylated and/or propoxylated fatty acid alkyl esters, alkylphenol ethoxylates (APE), nonylphenol ethoxylates (NPE), alkylpolyglycosides (APG), alkoxylated amines, fatty acid monoethanolamides (FAM), fatty acid diethanolamides (FADA), ethoxylated fatty acid monoethanolamides (EFAM), propoxylated fatty acid monoethanolamides (PFAM), polyhydroxyalkyl fatty acid amides, or N-acyl N-alkyl derivatives of glucosamine (glucamides, GA, or fatty acid glucamides, FAGA), as well as products available under the trade names SPAN and TWEEN, and combinations thereof.

When included therein the detergent will usually contain from about 0.01 % to about 10 % by weight of a semipolar surfactant. Non-limiting examples of semipolar surfactants include amine oxides (AO) such as alkyldimethylamineoxide, N-(coco alkyl)-N,N-dimethylamine oxide and N-(tallow-alkyl)-N,N-bis(2-hydroxyethyl)amine oxide, , and combinations thereof.

When included therein the detergent will usually contain from about 0.01 % to about 10 % by weight of a zwitterionic surfactant. Non-limiting examples of zwitterionic surfactants include betaines such as alkyldimethylbetaines, sulfobetaines, and combinations thereof.

Typically, more than one surfactant is present in the cleaning composition, for example at least one anionic and at least one non-ionic surfactant. Preferably, the amount of all surfactant present (total amount) i.e. the amount of anionic, non-ionic, zwitterionic and cationic surfactant present is preferably from about 1 wt% to 80 wt% by weight, such as about 1 wt% to 70 wt%, such as about 1 wt% to 50 wt% such as about 1 wt% to about 40 wt%, or about 5 wt% to about 40 wt%, or about 10 wt% to about 60 wt%. The ratio between the surfactants present depends on the specific composition but the weight ratios may be when an anionic and non-ionic surfactant is included in the composition a weight ratio of the anionic to nonionic surfactant from; 30:1 to 10:1, 20:1 to 1:10, 25: 1 to 1:2, 20:1 to 1:5.

One embodiment relates to a cleaning composition comprising a polypeptide having DNase activity, wherein the cleaning component is at least one surfactant, preferably anionic and/or nonionic, preferably wherein the composition comprises from 1 to 70 wt%, preferably from 5 to 40 wt % surfactant, wherein the surfactant preferably is selected from alkylbenzenesulfonates e.g. LAS, alkyl sulfates (AS) and mixtures thereof, preferably the cleaning composition comprises at least 20 wt % alkylbenzenesulfonate surfactant.

One embodiment relates to a cleaning composition comprising a polypeptide having DNase activity, wherein the cleaning composition comprises at least one anionic surfactant and wherein the cleaning composition additionally comprises a nonionic surfactant, and preferably wherein the weight ratio of the anionic to nonionic surfactant is from 25: 1 to 1:2 or from 1.5:1 to 1:10.

Builders and Co-Builders

The cleaning composition may contain about 0-65% by weight, such as about 5% to about 50%, such as about 0.5% to about 20% of a detergent builder or co-builder, or a mixture thereof. In a dish wash detergent, the level of builder is typically 40-65%, particularly 50-65%. The builder and/or co-builder may particularly be a chelating agent that forms water-soluble complexes with Ca and Mg. Any builder and/or co-builder known in the art for use in cleaning detergents may be utilized. Non-limiting examples of builders include zeolites, diphosphates (pyrophosphates), triphosphates such as sodium triphosphate (STP or STPP), carbonates such as sodium carbonate, soluble silicates such as sodium metasilicate, layered silicates (e.g., SKS-6 from Hoechst), ethanolamines such as 2-aminoethan-1-ol (MEA), diethanolamine (DEA, also known as 2,2'-iminodiethan-1-ol), triethanolamine (TEA, also known as 2,2',2"-nitrilotriethan-1-ol), and (carboxymethyl)inulin (CMI), and combinations thereof.

The detergent composition may also contain 0-50% by weight, such as about 5% to about 30%, of a detergent co-builder. The detergent composition may include a co-builder alone, or in combination with a builder, for example a zeolite builder. Non-limiting examples of co-builders include homopolymers of polyacrylates or copolymers thereof, such as poly(acrylic acid) (PAA) or copoly(acrylic acid/maleic acid) (PAA/PMA). Further non-limiting examples include citrate, chelators such as aminocarboxylates, aminopolycarboxylates and phosphonates, and alkyl- or alkenylsuccinic acid. Additional specific examples include 2,2',2"-nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), iminodisuccinic acid (IDS), ethylenediamine-N,N'-disuccinic acid (EDDS), methylglycinediacetic acid (MGDA), glutamic acid-N,N-diacetic acid (GLDA), 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetra(methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis-(methylenephosphonic acid) (DTMPA or DTPMPA), N-(2-hydroxyethyl)iminodiacetic acid (EDG), aspartic acid-N-monoacetic acid (ASMA), aspartic acid-N,N-diacetic acid (ASDA), aspartic acid-N-monopropionic acid (ASMP), iminodisuccinic acid (IDA), N-(2-sulfomethyl)-aspartic acid (SMAS), N-(2-sulfoethyl)-aspartic acid (SEAS), N-(2-sulfomethyl)-glutamic acid (SMGL), N-(2-sulfoethyl)-glutamic acid (SEGL), N-methyliminodiacetic acid (MIDA), α-alanine-N,N-diacetic acid (α-ALDA), serine-N,N-diacetic acid (SEDA), isoserine-N,N-diacetic acid (ISDA), phenylalanine-N,N-diacetic acid (PHDA), anthranilic acid-N,N-diacetic acid (ANDA), sulfanilic acid-N,N-diacetic acid (SLDA) , taurine-N,N-diacetic acid (TUDA) and sulfomethyl-N,N-diacetic acid (SMDA), N-(2-

hydroxyethyl)ethylenediamine-N,N',N''-triacetic acid (HEDTA), diethanolglycine (DEG), diethylenetriamine penta(methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic acid) (ATMP), and combinations and salts thereof. Further exemplary builders and/or co-builders are described in, e.g., WO 09/102854, US 5977053

Bleaching Systems

The cleaning composition may contain 0-30% by weight, such as about 1% to about 20%, such as about 0.01% to about 10% of a bleaching system. Any bleaching system comprising components known in the art for use in cleaning detergents may be utilized. Suitable bleaching system components include sources of hydrogen peroxide; sources of peracids; and bleach catalysts or boosters.

Suitable sources of hydrogen peroxide are inorganic persalts, including alkali metal salts such as sodium percarbonate and sodium perborates (usually mono- or tetrahydrate), and hydrogen peroxide—urea (1/1).

Peracids may be (a) incorporated directly as preformed peracids or (b) formed in situ in the wash liquor from hydrogen peroxide and a bleach activator (perhydrolysis) or (c) formed in situ in the wash liquor from hydrogen peroxide and a perhydrolase and a suitable substrate for the latter, e.g., an ester.

Suitable preformed peracids include, but are not limited to, peroxycarboxylic acids such as peroxybenzoic acid and its ring-substituted derivatives, peroxy-α-naphthoic acid, peroxyphthalic acid, peroxylauric acid, peroxystearic acid, ε-phthalimidoperoxycaproic acid [phthalimidoperoxyhexanoic acid (PAP)], and o-carboxybenzamidoperoxycaproic acid; aliphatic and aromatic diperoxydicarboxylic acids such as diperoxydodecanedioic acid, diperoxyazelaic acid, diperoxysebacic acid, diperoxybrassylic acid, 2-decyldiperoxybutanedioic acid, and diperoxyphthalic, -isophthalic and -terephthalic acids; perimidic acids; peroxymonosulfuric acid; peroxydisulfuric acid; peroxyphosphoric acid; peroxysilicic acid; and mixtures of said compounds. It is understood that the peracids mentioned may in some cases be best added as suitable salts, such as alkali metal salts (e.g., Oxone®) or alkaline earth-metal salts.

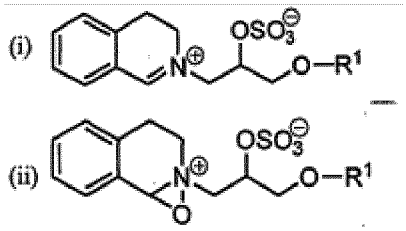
Suitable bleach activators include those belonging to the class of esters, amides, imides, nitriles or anhydrides and, where applicable, salts thereof. Suitable examples are tetraacetylethylenediamine (TAED), sodium 4-[(3,5,5-trimethylhexanoyl)oxy]benzene-1-sulfonate (ISONOBS), sodium 4-(dodecanoyloxy)benzene-1-sulfonate (LOBS), sodium 4-(decanoyloxy)benzene-1-sulfonate, 4-(decanoyloxy)benzoic acid (DOBA), sodium 4-(nonanoyloxy)benzene-1-sulfonate (NOBS), and/or those disclosed in WO98/17767. A particular family of bleach activators of interest was disclosed in EP624154 and particularly preferred in that family is acetyl triethyl citrate (ATC). ATC or a short chain triglyceride like triacetin has the advantage that they are environmentally friendly. Furthermore, acetyl triethyl citrate and triacetin have good

hydrolytical stability in the product upon storage and are efficient bleach activators. Finally, ATC is multifunctional, as the citrate released in the perhydrolysis reaction may function as a builder.

Bleach catalysts and boosters

The bleaching system may also include a bleach catalyst or booster. Some non-limiting examples of bleach catalysts that may be used in the compositions of the present invention include manganese oxalate, manganese acetate, manganese-collagen, cobalt-amine catalysts and manganese triazacyclononane (MnTACN) catalysts; particularly preferred are complexes of manganese with 1,4,7-trimethyl-1,4,7-triazacyclononane (Me3-TACN) or 1,2,4,7-tetramethyl-1,4,7-triazacyclononane (Me4-TACN), in particular Me3-TACN, such as the dinuclear manganese complex [(Me3-TACN)Mn(O)3Mn(Me3-TACN)](PF6)2, and [2,2',2"-nitrilotris(ethane-1,2-diylazanylylidene-κN-methanylylidene)triphenolato-κ3O]manganese(III). The bleach catalysts may also be other metal compounds; such as iron or cobalt complexes.

In some embodiments, where a source of a peracid is included, an organic bleach catalyst or bleach booster may be used having one of the following formulae:



(iii) and mixtures thereof; wherein each R1 is independently a branched alkyl group containing from 9 to 24 carbons or linear alkyl group containing from 11 to 24 carbons, preferably each R1 is independently a branched alkyl group containing from 9 to 18 carbons or linear alkyl group containing from 11 to 18 carbons, more preferably each R1 is independently selected from the group consisting of 2-propylheptyl, 2-butyloctyl, 2-pentylnonyl, 2-hexyldecyl, dodecyl, tetradecyl, hexadecyl, octadecyl, isononyl, isodecyl, isotridecyl and isopentadecyl.

Other exemplary bleaching systems are described e.g. in WO2007/087258, WO2007/087244, WO2007/087259, EP1867708 (Vitamin K) and WO2007/087242. Suitable photobleaches may for example be sulfonated zinc or aluminium phthalocyanines.

Metal care agents

Metal care agents may prevent or reduce the tarnishing, corrosion or oxidation of metals, including aluminium, stainless steel and non-ferrous metals, such as silver and copper. Suitable examples include one or more of the following:

(a) benzatriazoles, including benzotriazole or bis-benzotriazole and substituted

derivatives thereof. Benzotriazole derivatives are those compounds in which the available substitution sites on the aromatic ring are partially or completely substituted. Suitable substituents include linear or branch-chain Ci-C20- alkyl groups (e.g., C1-C20- alkyl groups) and hydroxyl, thio, phenyl or halogen such as fluorine, chlorine, bromine and iodine.

(b) metal salts and complexes chosen from the group consisting of zinc, manganese, titanium, zirconium, hafnium, vanadium, cobalt, gallium and cerium salts and/or complexes, the metals being in one of the oxidation states II, III, IV, V or VI. In one aspect, suitable metal salts and/or metal complexes may be chosen from the group consisting of Mn(II) sulphate, Mn(II) citrate, Mn(II) stearate, Mn(II) acetylacetonate, K^TiF6 (e.g., K2TiF6), K^ZrF6 (e.g., K2ZrF6), CoSO4, Co(NOs)2 and Ce(NOs)3, zinc salts, for example zinc sulphate, hydrozincite or zinc acetate;

(c) silicates, including sodium or potassium silicate, sodium disilicate, sodium metasilicate, crystalline phyllosilicate and mixtures thereof.

Further suitable organic and inorganic redox-active substances that act as silver/copper corrosion inhibitors are disclosed in WO 94/26860 and WO 94/26859. Preferably the composition of the invention comprises from 0.1 to 5% by weight of the composition of a metal care agent, preferably the metal care agent is a zinc salt.

Hydrotropes

The cleaning composition may contain 0-10% by weight, for example 0-5% by weight, such as about 0.5 to about 5%, or about 3% to about 5%, of a hydrotrope. Any hydrotrope known in the art for use in detergents may be utilized. Non-limiting examples of hydrotropes include sodium benzenesulfonate, sodium p-toluene sulfonate (STS), sodium xylene sulfonate (SXS), sodium cumene sulfonate (SCS), sodium cymene sulfonate, amine oxides, alcohols and polyglycolethers, sodium hydroxynaphthoate, sodium hydroxynaphthalene sulfonate, sodium ethylhexyl sulfate, and combinations thereof.

Polymers

The cleaning composition may contain 0-10% by weight, such as 0.5-5%, 2-5%, 0.5-2% or 0.2-1% of a polymer. Any polymer known in the art for use in detergents may be utilized. The polymer may function as a co-builder as mentioned above, or may provide antiredeposition, fiber protection, soil release, dye transfer inhibition, grease cleaning and/or anti-foaming properties. Some polymers may have more than one of the above-mentioned properties and/or more than one of the below-mentioned motifs. Exemplary polymers include (carboxymethyl)cellulose (CMC), poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP), poly(ethyleneglycol) or poly(ethylene oxide) (PEG), ethoxylated poly(ethyleneimine), carboxymethyl inulin (CMI), and polycarboxylates such as PAA, PAA/PMA, poly-aspartic acid, and lauryl methacrylate/acrylic acid copolymers , hydrophobically modified CMC

(HM-CMC) and silicones, copolymers of terephthalic acid and oligomeric glycols, copolymers of poly(ethylene terephthalate) and poly(oxyethene terephthalate) (PET-POET), PVP, poly(vinylimidazole) (PVI), poly(vinylpyridine-N-oxide) (PVPO or PVPNO) and polyvinylpyrrolidone-vinylimidazole (PVPVI). Suitable examples include PVP-K15, PVP-K30, ChromaBond S-400, ChromaBond S- 403E and Chromabond S-100 from Ashland Aqualon, and Sokalan® HP 165, Sokalan® HP 50 (Dispersing agent), Sokalan® HP 53 (Dispersing agent), Sokalan® HP 59 (Dispersing agent), Sokalan® HP 56 (dye transfer inhibitor), Sokalan® HP 66 K (dye transfer inhibitor) from BASF. Further exemplary polymers include sulfonated polycarboxylates, polyethylene oxide and polypropylene oxide (PEO-PPO) and diquaternium ethoxy sulfate. Other exemplary polymers are disclosed in, e.g., WO 2006/130575. Salts of the above-mentioned polymers are also contemplated. Particularly preferred polymer is ethoxylated homopolymer Sokalan® HP 20 from BASF, which helps to prevent redeposition of soil in the wash liquor.

Fabric hueing agents

The cleaning compositions of the present invention may also include fabric hueing agents such as dyes or pigments, which when formulated in detergent compositions can deposit onto a fabric when said fabric is contacted with a wash liquor comprising said detergent compositions and thus altering the tint of said fabric through absorption/reflection of visible light. Fluorescent whitening agents emit at least some visible light. In contrast, fabric hueing agents alter the tint of a surface as they absorb at least a portion of the visible light spectrum. Suitable fabric hueing agents include dyes and dye-clay conjugates, and may also include pigments. Suitable dyes include small molecule dyes and polymeric dyes. Suitable small molecule dyes include small molecule dyes selected from the group consisting of dyes falling into the Colour Index (C.I.) classifications of Direct Blue, Direct Red, Direct Violet, Acid Blue, Acid Red, Acid Violet, Basic Blue, Basic Violet and Basic Red, or mixtures thereof, for example as described in WO2005/03274, WO2005/03275, WO2005/03276 and EP1876226 (hereby incorporated by reference). The detergent composition preferably comprises from about 0.00003 wt% to about 0.2 wt%, from about 0.00008 wt% to about 0.05 wt%, or even from about 0.0001 wt% to about 0.04 wt% fabric hueing agent. The composition may comprise from 0.0001 wt% to 0.2 wt% fabric hueing agent, this may be especially preferred when the composition is in the form of a unit dose pouch. Suitable hueing agents are also disclosed in, e.g. WO 2007/087257 and WO2007/087243.

Dispersants

The cleaning compositions of the present invention can also contain dispersants. In particular, powdered detergents may comprise dispersants. Suitable water-soluble organic materials include the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms. Suitable

dispersants are for example described in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc.

Dye Transfer Inhibiting Agents

The cleaning compositions of the present invention may also include one or more dye transfer inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof. When present in a subject composition, the dye transfer inhibiting agents may be present at levels from about 0.0001 % to about 10%, from about 0.01% to about 5% or even from about 0.1% to about 3% by weight of the composition.

Fluorescent whitening agent

The cleaning compositions of the present invention will preferably also contain additional components that may tint articles being cleaned, such as fluorescent whitening agents or optical brighteners. Where present the brightener is preferably at a level of about 0.01% to about 0.5%. Any fluorescent whitening agent suitable for use in a laundry detergent composition may be used in the composition of the present invention. The most commonly used fluorescent whitening agents are those belonging to the classes of diaminostilbene-sulfonic acid derivatives, diarylpyrazoline derivatives and bisphenyl-distyryl derivatives. Examples of the diaminostilbene-sulfonic acid derivative type of fluorescent whitening agents include the sodium salts of: 4,4'-bis-(2-diethanolamino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(2,4-dianilino-s-triazin-6-ylamino) stilbene-2.2'-disulfonate, 4,4'-bis-(2-anilino-4-(N-methyl-N-2-hydroxy-ethylamino)-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(4-phenyl-1,2,3-triazol-2-yl)stilbene-2,2'-disulfonate and sodium 5-(2H-naphtho[1,2-d][1,2,3]triazol-2-yl)-2-[(E)-2-phenylvinyl]-benzenesulfonate. Preferred fluorescent whitening agents are Tinopal DMS and Tinopal CBS available from Ciba-Geigy AG, Basel, Switzerland. Tinopal DMS is the disodium salt of 4,4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate. Tinopal CBS is the disodium salt of 2,2'-bis-(phenyl-styryl)-disulfonate. Also preferred are fluorescent whitening agents is the commercially available Parawhite KX, supplied by Paramount Minerals and Chemicals, Mumbai, India. Other fluorescers suitable for use in the invention include the 1-3-diaryl pyrazolines and the 7-alkylaminocoumarins. Suitable fluorescent brightener levels include lower levels of from about 0.01, from 0.05, from about 0.1 or even from about 0.2 wt % to upper levels of 0.5 or even 0.75 wt%.

Soil release polymers

The cleaning compositions of the present invention may also include one or more soil release polymers which aid the removal of soils from fabrics such as cotton and polyester based

fabrics, in particular the removal of hydrophobic soils from polyester based fabrics. The soil release polymers may for example be nonionic or anionic terephthalte based polymers, polyvinyl caprolactam and related copolymers, vinyl graft copolymers, polyester polyamides see for example Chapter 7 in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc. Another type of soil release polymers is amphiphilic alkoxylated grease cleaning polymers comprising a core structure and a plurality of alkoxylate groups attached to that core structure. The core structure may comprise a polyalkylenimine structure or a polyalkanolamine structure as described in detail in WO 2009/087523 (hereby incorporated by reference). Furthermore, random graft co-polymers are suitable soil release polymers. Suitable graft co-polymers are described in more detail in WO 2007/138054, WO 2006/108856 and WO 2006/113314 (hereby incorporated by reference). Suitable polyethylene glycol polymers include random graft co-polymers comprising: (i) hydrophilic backbone comprising polyethylene glycol; and (ii) side chain(s) selected from the group consisting of: C4-C25 alkyl group, polypropylene, polybutylene, vinyl ester of a saturated C1-C6 mono-carboxylic acid, Cl-C 6 alkyl ester of acrylic or methacrylic acid, and mixtures thereof. Suitable polyethylene glycol polymers have a polyethylene glycol backbone with random grafted polyvinyl acetate side chains. The average molecular weight of the polyethylene glycol backbone can be in the range of from 2,000 Da to 20,000 Da, or from 4,000 Da to 8,000 Da. The molecular weight ratio of the polyethylene glycol backbone to the polyvinyl acetate side chains can be in the range of from 1: 1 to 1:5, or from 1: 1.2 to 1:2. The average number of graft sites per ethylene oxide units can be less than 1, or less than 0.8, the average number of graft sites per ethylene oxide units can be in the range of from 0.5 to 0.9, or the average number of graft sites per ethylene oxide units can be in the range of from 0.1 to 0.5, or from 0.2 to 0.4. A suitable polyethylene glycol polymer is Sokalan HP22. Other soil release polymers are substituted polysaccharide structures especially substituted cellulosic structures such as modified cellulose deriviatives such as those described in EP 1867808 or WO 2003/040279 (both are hereby incorporated by reference). Suitable cellulosic polymers include cellulose, cellulose ethers, cellulose esters, cellulose amides and mixtures thereof. Suitable cellulosic polymers include anionically modified cellulose, nonionically modified cellulose, cationically modified cellulose, zwitterionically modified cellulose, and mixtures thereof. Suitable cellulosic polymers include methyl cellulose, carboxy methyl cellulose, ethyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose, ester carboxy methyl cellulose, and mixtures thereof.

Anti-redeposition agents

The cleaning compositions of the present invention may also include one or more anti-redeposition agents such as carboxymethylcellulose (CMC), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), polyoxyethylene and/or polyethyleneglycol (PEG), homopolymers of acrylic acid, copolymers of acrylic acid and maleic acid, and ethoxylated polyethyleneimines. The

cellulose based polymers described under soil release polymers above may also function as anti-redeposition agents.

Rheology Modifiers

The cleaning compositions of the present invention may also include one or more rheology modifiers, structurants or thickeners, as distinct from viscosity reducing agents. The rheology modifiers are selected from the group consisting of non-polymeric crystalline, hydroxy-functional materials, polymeric rheology modifiers which impart shear thinning characteristics to the aqueous liquid matrix of a liquid detergent composition. The rheology and viscosity of the detergent can be modified and adjusted by methods known in the art, for example as shown in EP 2169040.

Other suitable cleaning composition components include, but are not limited to, anti-shrink agents, anti-wrinkling agents, bactericides, binders, carriers, dyes, enzyme stabilizers, fabric softeners, fillers, foam regulators, hydrotropes, perfumes, pigments, sod suppressors, solvents, and structurants for liquid detergents and/or structure elasticizing agents.

Polymers

The cleaning composition may contain 0-10% by weight, such as 0.5-5%, 2-5%, 0.5-2% or 0.2-1% of a polymer. Any polymer known in the art for use in detergents may be utilized. The polymer may function as a co-builder as mentioned above, or may provide antiredeposition, fiber protection, soil release, dye transfer inhibition, grease cleaning and/or anti-foaming properties. Some polymers may have more than one of the above-mentioned properties and/or more than one of the below-mentioned motifs. Exemplary polymers include (carboxymethyl)cellulose (CMC), poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP), poly(ethyleneglycol) or poly(ethylene oxide) (PEG), ethoxylated poly(ethyleneimine), carboxymethyl inulin (CMI), and polycarboxylates such as PAA, PAA/PMA, poly-aspartic acid, and lauryl methacrylate/acrylic acid copolymers , hydrophobically modified CMC (HM-CMC) and silicones, copolymers of terephthalic acid and oligomeric glycols, copolymers of poly(ethylene terephthalate) and poly(oxyethene terephthalate) (PET-POET), PVP, poly(vinylimidazole) (PVI), poly(vinylpyridine-N-oxide) (PVPO or PVPNO) and polyvinylpyrrolidone-vinylimidazole (PVPVI). Suitable examples include PVP-K15, PVP-K30, ChromaBond S-400, ChromaBond S- 403E and Chromabond S-100 from Ashland Aqualon, and Sokalan® HP 165, Sokalan® HP 50 (Dispersing agent), Sokalan® HP 53 (Dispersing agent), Sokalan® HP 59 (Dispersing agent), Sokalan® HP 56 (dye transfer inhibitor), Sokalan® HP 66 K (dye transfer inhibitor) from BASF. Further exemplary polymers include sulfonated polycarboxylates, polyethylene oxide and polypropylene oxide (PEO-PPO) and diquaternium ethoxy sulfate. Other exemplary polymers are disclosed in, e.g., WO 2006/130575. Salts of the above-mentioned polymers are also contemplated. Particularly preferred polymer is ethoxylated homopolymer Sokalan® HP 20 from BASF, which helps to prevent redeposition of soil in the wash liquor.

Additional enzymes

The cleaning composition may comprise, in addition to the at least one polypeptide having DNase activity and optionally at least one DNase, one or more additional enzymes such as one or more lipase, cutinase, an amylase, carbohydrase, cellulase, pectinase, mannanase, arabinase, galactanase, xylanase, oxidase, e.g., a laccase, and/or peroxidase. In general, the properties of the selected enzymes should be compatible with the selected detergent, (i.e., pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.), and the enzymes should be present in effective amounts.

Mannanases

Suitable mannanases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. The mannanase may be an alkaline mannanase of Family 5 or 26. It may be a wild-type from *Bacillus* or *Humicola*, particularly *B. agaradhaerens, B. licheniformis, B. halodurans, B. clausii*, or *H. insolens*. Suitable mannanases are described in WO 1999/064619. A commercially available mannanase is Mannaway (Novozymes A/S).

Cellulases

Suitable cellulases include complete cellulases or mono-component endoglucanases of bacterial or fungal origin. Chemically or genetically modified mutants are included. The cellulase may for example be a mono-component or a mixture of mono-component endo-1,4-beta-glucanase often just termed endoglucanases. Suitable cellulases include a fungal cellulase from *Humicola insolens* (US 4,435,307) or from *Trichoderma*, e.g. *T. reesei* or *T. viride*. Examples of cellulases are described in EP 0 495 257. Other suitable cellulases are from *Thielavia e.g. Thielavia terrestris as described in* WO 96/29397 or *Fusarium oxysporum as described in* WO 91/17244 or from *Bacillus* as described in, WO 02/099091 and JP 2000210081. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 Commercially available cellulases include Carezyme®, Celluzyme®, Celluclean®, Celluclast® and Endolase®; Renozyme®; Whitezyme® (Novozymes A/S) Puradax®, Puradax HA, and Puradax EG (available from Genencor).

Proteases

Suitable proteases may be of any origin, but are preferably of bacterial or fungal origin, optionally in the form of protein engineered or chemically modified mutants. The protease may be an alkaline protease, such as a serine protease or a metalloprotease. A serine protease may for example be of the S1 family, such as trypsin, or the S8 family such as a subtilisin. A

metalloprotease may for example be a thermolysin, e.g. from the M4 family, or another metalloprotease such as those from the M5, M7 or M35 families.

The term "subtilases" refers to a sub-group of serine proteases according to Siezen et al., *Protein Eng.* 4 (1991) 719-737 and Siezen et al., *Protein Sci.* 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 six subdivisions, the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family.

Although proteases suitable for detergent use may be obtained from a variety of organisms, including fungi such as *Aspergillus*, detergent proteases have generally been obtained from bacteria and in particular from *Bacillus*. Examples of *Bacillus* species from which subtilases have been derived include *Bacillus lentus, Bacillus alkalophilus, Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus pumilus* and *Bacillus gibsonii*. Particular subtilisins include *subtilisin lentus*, *subtilisin* Novo, *subtilisin* Carlsberg, *subtilisin* BPN', *subtilisin* 309, *subtilisin* 147 and *subtilisin* 168 and e.g. protease PD138 (described in WO 93/18140). Other useful proteases are e.g. those described in WO 01/16285 and WO 02/16547.

Examples of trypsin-like proteases include the *Fusarium* protease described in WO 94/25583 and WO 2005/040372, and the chymotrypsin proteases derived from *Cellumonas* described in WO 2005/052161 and WO 2005/052146.

Examples of metalloproteases include the neutral metalloproteases described in WO 2007/044993 such as those derived from *Bacillus amyloliquefaciens*, as well as e.g. the metalloproteases described in WO 2015/158723 and WO 2016/075078.

Examples of useful proteases are the protease variants described in WO 89/06279 WO 92/19729, WO 96/34946, WO 98/20115, WO 98/20116, WO 99/11768, WO 01/44452, WO 03/006602, WO 2004/003186, WO 2004/041979, WO 2007/006305, WO 2011/036263, WO 2014/207227, WO 2016/087617 and WO 2016/174234. Preferred protease variants may, for example, comprise one or more of the mutations selected from the group consisting of: S3T, V4I, S9R, S9E, A15T, S24G, S24R, K27R, N42R, S55P, G59E, G59D, N60D, N60E, V66A, N74D, S85R, A96S, S97G, S97D, S97A, S97SD, S99E, S99D, S99G, S99M, S99N, S99R, S99H, S101A, V102I, V102Y, V102N, S104A, G116V, G116R, H118D, H118N, A120S, S126L, P127Q, S128A, S154D, A156E, G157D, G157P, S158E, Y161A, R164S, Q176E, N179E, S182E, Q185N, A188P, G189E, V193M, N198D, V199I, Q200L, Y203W, S206G, L211Q, L211D, N212D, N212S, M216S, A226V, K229L, Q230H, Q239R, N246K, S253D, N255W, N255D, N255E, L256E, L256D T268A and R269H, wherein position numbers correspond to positions of the *Bacillus lentus* protease shown in SEQ ID NO: 1 of WO 2016/001449. Protease variants having one or more of these mutations are preferably variants of the *Bacillus lentus* protease (Savinase®, also known as subtilisin 309) shown in SEQ ID NO: 1 of WO 2016/001449 or of the *Bacillus amyloliquefaciens*

protease (BPN') shown in SEQ ID NO: 2 of WO 2016/001449. Such protease variants preferably have at least 80% sequence identity to SEQ ID NO: 1 or to SEQ ID NO: 2 of WO 2016/001449.

Another protease of interest is the alkaline protease from *Bacillus lentus* DSM 5483, as described for example in WO 91/02792, and variants thereof which are described for example in WO 92/21760, WO 95/23221, EP 1921147, EP 1921148 and WO 2016/096711.

The protease may alternatively be a variant of the TY145 protease having SEQ ID NO: 1 of WO 2004/067737, for example a variant comprising a substitution at one or more positions corresponding to positions 27, 109, 111, 171, 173, 174, 175, 180, 182, 184, 198, 199 and 297 of SEQ ID NO: 1 of WO 2004/067737, wherein said protease variant has a sequence identity of at least 75% but less than 100% to SEQ ID NO: 1 of WO 2004/067737. TY145 variants of interest are described in e.g. WO 2015/014790, WO 2015/014803, WO 2015/014804, WO 2016/097350, WO 2016/097352, WO 2016/097357 and WO 2016/097354.

Examples of preferred proteases include:

(a) variants of SEQ ID NO: 1 of WO 2016/001449 comprising two or more substitutions selected from the group consisting of S9E, N43R, N76D, Q206L, Y209W, S259D and L262E, for example a variant with the substitutions S9E, N43R, N76D, V205I, Q206L, Y209W, S259D, N261W and L262E, or with the substitutions S9E, N43R, N76D, N185E, S188E, Q191N, A194P, Q206L, Y209W, S259D and L262E, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449;

(b) a variant of the polypeptide of SEQ ID NO: 1 of WO 2016/001449 with the mutation S99SE, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449;

(c) a variant of the polypeptide of SEQ ID NO: 1 of WO 2016/001449 with the mutation S99AD, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449;

(d) a variant of the polypeptide of SEQ ID NO: 1 of WO 2016/001449 with the substitutions Y167A+R170S+A194P, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449;

(e) a variant of the polypeptide of SEQ ID NO: 1 of WO 2016/001449 with the substitutions S9R+A15T+V68A+N218D+Q245R, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449;

(f) a variant of the polypeptide of SEQ ID NO: 1 of WO 2016/001449 with the substitutions S9R+A15T+G61E+V68A+A194P+V205I+Q245R+N261D, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449;

(g) a variant of the polypeptide of SEQ ID NO: 1 of WO 2016/001449 with the substitutions S99D+S101R/E+S103A+V104I+G160S; for example a variant of SEQ ID NO: 1 of

WO 2016/001449 with the substitutions S3T+V4I+S99D+S101E+S103A+V104I+G160S+V205I, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449;

(h) a variant of the polypeptide of SEQ ID NO: 2 of WO 2016/001449 with the substitutions S24G+S53G+S78N+S101N+G128A/S+Y217Q, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449;

(i) the polypeptide disclosed in GENESEQP under accession number BER84782, corresponding to SEQ ID NO: 302 in WO 2017/210295;

(j) a variant of the polypeptide of SEQ ID NO: 1 of WO 2016/001449 with the substitutions S99D+S101E+S103A+V104I+S156D+G160S+L262E, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449;

(k) a variant of the polypeptide of SEQ ID NO: 1 of WO 2016/001449 with the substitutions S9R+A15T+G61E+V68A+N76D+S99G+N218D+Q245R, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449;

(l) a variant of the polypeptide of SEQ ID NO: 1 of WO 2016/001449 with the substitutions V68A+S106A, wherein position numbers are based on the numbering of SEQ ID NO: 2 of WO 2016/001449; and

(m) a variant of the polypeptide of SEQ ID NO: 1 of WO 2004/067737 with the substitutions S27K+N109K+S111E+S171E+S173P+G174K+S175P+F180Y+G182A+L184F+Q198E+N199+T297P, wherein position numbers are based on the numbering of SEQ ID NO: 1 of WO 2004/067737.

Suitable commercially available protease enzymes include those sold under the trade names Alcalase®, Duralase™, Durazym™, Relase®, Relase® Ultra, Savinase®, Savinase® Ultra, Primase™, Polarzyme®, Kannase®, Liquanase®, Liquanase® Ultra, Ovozyme®, Coronase®, Coronase® Ultra, Blaze®, Blaze Evity® 100T, Blaze Evity® 125T, Blaze Evity® 150T, Blaze Evity® 200T, Neutrase®, Everlase®, Esperase®, Progress® Uno, Progress® In and Progress® Excel (Novozymes A/S), those sold under the tradename Maxatase™, Maxacal™, Maxapem®, Purafect® Ox, Purafect® OxP, Puramax®, FN2™, FN3™, FN4^{ex}™, Excellase®, Excellenz™ P1000, Excellenz™ P1250, Eraser™, Preferenz® P100, Purafect Prime, Preferenz® P110, Preferenz® P300, Effectenz P1000™, Purafect®, Effectenz P1050™, Purafect® Ox, Effectenz ™ P2000, Purafast™, Properase®, Opticlean™ and Optimase® (Danisco/DuPont), BLAP (sequence shown in Figure 29 of US 5352604) and variants hereof (Henkel AG), and KAP (*Bacillus alkalophilus* subtilisin) from Kao.

Lipases and Cutinases

Suitable lipases and cutinases include those of bacterial or fungal origin. Chemically modified or protein engineered mutant enzymes are included. Examples include lipase from *Thermomyces*, e.g. from *T. lanuginosus* (previously named *Humicola lanuginosa*) as described

in EP258068 and EP305216, cutinase from *Humicola*, e.g. *H. insolens* (WO96/13580), lipase from strains of *Pseudomonas* (some of these now renamed to *Burkholderia*), e.g. *P. alcaligenes* or *P. pseudoalcaligenes* (EP218272), *P. cepacia* (EP331376), *P. sp.* strain SD705 (WO95/06720 & WO96/27002), *P. wisconsinensis* (WO96/12012), GDSL-type *Streptomyces* lipases (WO10/065455), cutinase from *Magnaporthe grisea* (WO10/107560), cutinase from *Pseudomonas mendocina* (US5,389,536), lipase from *Thermobifida fusca* (WO11/084412), *Geobacillus stearothermophilus* lipase (WO11/084417), lipase from *Bacillus subtilis* (WO11/084599), and lipase from *Streptomyces griseus* (WO11/150157) and *S. pristinaespiralis* (WO12/137147).

Other examples are lipase variants such as those described in EP407225, WO92/05249, WO94/01541, WO94/25578, WO95/14783, WO95/30744, WO95/35381, WO95/22615, WO96/00292, WO97/04079, WO97/07202, WO00/34450, WO00/60063, WO01/92502, WO07/87508 and WO09/109500.

Preferred commercial lipase products include Lipolase™, Lipex™; Lipolex™ and Lipoclean™ (Novozymes A/S), Lumafast (originally from Genencor) and Lipomax (originally from Gist-Brocades). Still other examples are lipases sometimes referred to as acyltransferases or perhydrolases, e.g. acyltransferases with homology to *Candida antarctica* lipase A (WO10/111143), acyltransferase from *Mycobacterium smegmatis* (WO05/56782), perhydrolases from the CE 7 family (WO09/67279), and variants of the *M. smegmatis* perhydrolase in particular the S54V variant used in the commercial product Gentle Power Bleach from Huntsman Textile Effects Pte Ltd (WO10/100028).

Amylases

Suitable amylases may be an alpha-amylase or a glucoamylase and may be 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 *Bacillus licheniformis*, described in more detail in GB 1,296,839.

Suitable amylases include amylases having SEQ ID NO: 2 in WO 95/10603 or variants having 90% sequence identity to SEQ ID NO: 3 thereof. Preferred variants are described in WO 94/02597, WO 94/18314, WO 97/43424 and SEQ ID NO: 4 of WO 99/019467, such as variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 178, 179, 181, 188, 190, 197, 201, 202, 207, 208, 209, 211, 243, 264, 304, 305, 391, 408, and 444.

Different suitable amylases include amylases having SEQ ID NO: 6 in WO 02/010355 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a deletion in positions 181 and 182 and a substitution in position 193.

Other amylases which are suitable are hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from *B. amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of the *B. licheniformis* alpha-amylase shown in SEQ ID NO: 4 of WO 2006/066594 or variants having 90% sequence identity thereof. Preferred variants of this hybrid alpha-amylase are those having a substitution, a deletion or an insertion in one of more of the following positions: G48, T49, G107, H156, A181, N190, M197, I201, A209 and Q264. Most preferred variants of the hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from *B. amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 are those having the substitutions:

M197T;

H156Y+A181T+N190F+A209V+Q264S; or

G48A+T49I+G107A+H156Y+A181T+N190F+I201F+A209V+Q264S.

Further amylases which are suitable are amylases having SEQ ID NO: 6 in WO 99/019467 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a substitution, a deletion or an insertion in one or more of the following positions: R181, G182, H183, G184, N195, I206, E212, E216 and K269. Particularly preferred amylases are those having deletion in positions R181 and G182, or positions H183 and G184.

Additional amylases which can be used are those having SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873 or variants thereof having 90% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7. Preferred variants of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7 are those having a substitution, a deletion or an insertion in one or more of the following positions: 140, 181, 182, 183, 184, 195, 206, 212, 243, 260, 269, 304 and 476, using SEQ ID 2 of WO 96/023873 for numbering. More preferred variants are those having a deletion in two positions selected from 181, 182, 183 and 184, such as 181 and 182, 182 and 183, or positions 183 and 184. Most preferred amylase variants of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 are those having a deletion in positions 183 and 184 and a substitution in one or more of positions 140, 195, 206, 243, 260, 304 and 476.

Other amylases which can be used are amylases having SEQ ID NO: 2 of WO 08/153815, SEQ ID NO: 10 in WO 01/66712 or variants thereof having 90% sequence identity to SEQ ID NO: 2 of WO 08/153815 or 90% sequence identity to SEQ ID NO: 10 in WO 01/66712. Preferred variants of SEQ ID NO: 10 in WO 01/66712 are those having a substitution, a deletion or an insertion in one of more of the following positions: 176, 177, 178, 179, 190, 201, 207, 211 and 264.

Further suitable amylases are amylases having SEQ ID NO: 2 of WO 09/061380 or variants having 90% sequence identity to SEQ ID NO: 2 thereof. Preferred variants of SEQ ID NO: 2 are those having a truncation of the C-terminus and/or a substitution, a deletion or an

insertion in one of more of the following positions: Q87, Q98, S125, N128, T131, T165, K178, R180, S181, T182, G183, M201, F202, N225, S243, N272, N282, Y305, R309, D319, Q320, Q359, K444 and G475. More preferred variants of SEQ ID NO: 2 are those having the substitution in one of more of the following positions: Q87E,R, Q98R, S125A, N128C, T131I, T165I, K178L, T182G, M201L, F202Y, N225E,R, N272E,R, S243Q,A,E,D, Y305R, R309A, Q320R, Q359E, K444E and G475K and/or deletion in position R180 and/or S181 or of T182 and/or G183. Most preferred amylase variants of SEQ ID NO: 2 are those having the substitutions:

N128C+K178L+T182G+Y305R+G475K;

N128C+K178L+T182G+F202Y+Y305R+D319T+G475K;

S125A+N128C+K178L+T182G+Y305R+G475K; or

S125A+N128C+T131I+T165I+K178L+T182G+Y305R+G475K wherein the variants are C-terminally truncated and optionally further comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181.

Further suitable amylases are amylases having SEQ ID NO: 1 of WO13184577 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants of SEQ ID NO: 1 are those having a substitution, a deletion or an insertion in one of more of the following positions: K176, R178, G179, T180, G181, E187, N192, M199, I203, S241, R458, T459, D460, G476 and G477. More preferred variants of SEQ ID NO: 1 are those having the substitution in one of more of the following positions: K176L, E187P, N192FYH, M199L, I203YF, S241QADN, R458N, T459S, D460T, G476K and G477K and/or deletion in position R178 and/or S179 or of T180 and/or G181. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:

E187P+I203Y+G476K

E187P+I203Y+R458N+T459S+D460T+G476K

wherein the variants optionally further comprise a substitution at position 241 and/or a deletion at position 178 and/or position 179.

Further suitable amylases are amylases having SEQ ID NO: 1 of WO10104675 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants of SEQ ID NO: 1 are those having a substitution, a deletion or an insertion in one of more of the following positions: N21, D97, V128 K177, R179, S180, I181, G182, M200, L204, E242, G477 and G478. More preferred variants of SEQ ID NO: 1 are those having the substitution in one of more of the following positions: N21D, D97N, V128I K177L, M200L, L204YF, E242QA, G477K and G478K and/or deletion in position R179 and/or S180 or of I181 and/or G182. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:

N21D+D97N+V128I,

wherein the variants optionally further comprise a substitution at position 200 and/or a deletion at position 180 and/or position 181.

Other suitable amylases are the alpha-amylase having SEQ ID NO: 12 in WO01/66712 or a variant having at least 90% sequence identity to SEQ ID NO: 12. Preferred amylase variants are those having a substitution, a deletion or an insertion in one of more of the following positions of SEQ ID NO: 12 in WO01/66712: R28, R118, N174; R181, G182, D183, G184, G186, W189, N195, M202, Y298, N299, K302, S303, N306, R310, N314; R320, H324, E345, Y396, R400, W439, R444, N445, K446, Q449, R458, N471, N484. Particular preferred amylases include variants having a deletion of D183 and G184 and having the substitutions R118K, N195F, R320K and R458K, and a variant additionally having substitutions in one or more position selected from the group: M9, G149, G182, G186, M202, T257, Y295, N299, M323, E345 and A339, most preferred a variant that additionally has substitutions in all these positions.

Other examples are amylase variants such as those described in WO2011/098531, WO2013/001078 and WO2013/001087.

Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™, Stainzyme™, Stainzyme Plus™, Natalase™, Liquozyme X and BAN™ (from Novozymes A/S), and Rapidase™, Purastar™/Effectenz™, Powerase, Preferenz® S1000, Preferenz® S100, Preferenz® S110 and Preferenz® S210 (from Genencor International Inc./DuPont).

Peroxidases/Oxidases

A peroxidase may be an enzyme comprised by the enzyme classification EC 1.11.1.7, as set out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), or any fragment derived therefrom, exhibiting peroxidase activity. Suitable peroxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinopsis*, *e.g.*, from *C. cinerea* (EP 179,486), and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257. A peroxidase may also include a haloperoxidase enzyme, such as chloroperoxidase, bromoperoxidase and compounds exhibiting chloroperoxidase or bromoperoxidase activity. Haloperoxidases are classified according to their specificity for halide ions. Chloroperoxidases (E.C. 1.11.1.10) catalyze formation of hypochlorite from chloride ions. The haloperoxidase may be a chloroperoxidase. Preferably, the haloperoxidase is a vanadium haloperoxidase, *i.e.*, a vanadate-containing haloperoxidase. In a preferred method the vanadate-containing haloperoxidase is combined with a source of chloride ion. Haloperoxidases have been isolated from many different fungi, in particular from the fungus group dematiaceous hyphomycetes, such as *Caldariomyces*, *e.g.*, *C. fumago*, *Alternaria*, *Curvularia*, *e.g.*, *C. verruculosa* and *C. inaequalis*, *Drechslera*, *Ulocladium* and *Botrytis*. Haloperoxidases have also been isolated from bacteria such as *Pseudomonas*, *e.g.*, *P. pyrrocinia* and *Streptomyces*, *e.g.*, *S. aureofaciens*. The haloperoxidase may be derivable from *Curvularia* sp., in particular *Curvularia verruculosa* or *Curvularia inaequalis*, such as *C. inaequalis* CBS

102.42 as described in WO 95/27046; or *C. verruculosa* CBS 147.63 or *C. verruculosa* CBS 444.70 as described in WO 97/04102; or from *Drechslera hartlebii* as described in WO 01/79459, *Dendryphiella salina* as described in WO 01/79458, *Phaeotrichoconis crotalarie* as described in WO 01/79461, or *Geniculosporium* sp. as described in WO 01/79460.

Oxidases include any laccase enzyme comprised by the enzyme classification EC 1.10.3.2, or any fragment derived therefrom exhibiting laccase activity, or a compound exhibiting a similar activity, such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5). Preferred laccase enzymes are enzymes of microbial origin. The enzymes may be derived from plants, bacteria or fungi (including filamentous fungi and yeasts). Suitable examples from fungi include a laccase derivable from a strain of *Aspergillus, Neurospora*, *e.g.*, *N. crassa*, *Podospora*, *Botrytis*, *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*, *Trametes*, *e.g.*, *T. villosa* and *T. versicolor*, *Rhizoctonia*, *e.g.*, *R. solani*, *Coprinopsis*, *e.g.*, *C. cinerea*, *C. comatus*, *C. friesii*, and *C. plicatilis*, *Psathyrella*, *e.g.*, *P. condelleana*, *Panaeolus*, *e.g.*, *P. papilionaceus*, *Myceliophthora*, *e.g.*, *M. thermophila*, *Schytalidium*, *e.g.*, *S. thermophilum*, *Polyporus*, *e.g.*, *P. pinsitus*, *Phlebia*, *e.g.*, *P. radiata* (WO 92/01046), or *Coriolus*, *e.g.*, *C. hirsutus* (JP 2238885). Suitable examples from bacteria include a laccase derivable from a strain of *Bacillus*. A laccase derived from *Coprinopsis* or *Myceliophthora* is preferred; in particular a laccase derived from *Coprinopsis cinerea*, as disclosed in WO 97/08325; or from *Myceliophthora thermophila*, as disclosed in WO 95/33836.

Microorganisms

The detergent additive as well as the detergent composition may also comprise one or more microorganisms, such as one or more fungi, yeast, or bacteria. In an embodiment, the one or more microorganisms are dehydrated (for example by lyophilization) bacteria or yeast, such as a strain of *Lactobacillus*. In another embodiment, the microorganisms are one or more microbial spores (as opposed to vegetative cells), such as bacterial spores; or fungal spores, conidia, hypha. Preferably, the one or more spores are *Bacillus* endospores; even more preferably the one or more spores are endospores of *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, or *Bacillus megaterium*. The microorganisms may be included in the detergent composition or additive in the same way as enzymes (see above).

Formulation of detergent products

The cleaning composition of the present invention may be formulated, for example, as a hand or machine laundry detergent composition including a laundry additive composition suitable for pre-treatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations, or be formulated for hand or machine dishwashing operations. In a specific aspect, the present invention

provides a detergent additive comprising one or more enzymes as described herein. The cleaning composition of the invention may be in any convenient form, e.g., a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid.

Pouches can be configured as single or multicompartments. It can be of any form, shape and material which is suitable for hold the composition, e.g. without allowing the release of the composition to release of the composition from the pouch prior to water contact. The pouch is made from water soluble film which encloses an inner volume. Said inner volume can be divided into compartments of the pouch. Preferred films are polymeric materials preferably polymers which are formed into a film or sheet. Preferred polymers, copolymers or derivates thereof are selected polyacrylates, and water-soluble acrylate copolymers, methyl cellulose, carboxy methyl cellulose, sodium dextrin, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, malto dextrin, poly methacrylates, most preferably polyvinyl alcohol copolymers and, hydroxypropyl methyl cellulose (HPMC). Preferably the level of polymer in the film for example PVA is at least about 60%. Preferred average molecular weight will typically be about 20,000 to about 150,000. Films can also be of blended compositions comprising hydrolytically degradable and water-soluble polymer blends such as polylactide and polyvinyl alcohol (known under the Trade reference M8630 as sold by MonoSol LLC, Indiana, USA) plus plasticisers like glycerol, ethylene glycerol, propylene glycol, sorbitol and mixtures thereof. The pouches can comprise a solid laundry cleaning composition or part components and/or a liquid cleaning composition or part components separated by the water-soluble film. The compartment for liquid components can be different in composition than compartments containing solids: US2009/0011970 A1.

Detergent ingredients can be separated physically from each other by compartments in water dissolvable pouches or in different layers of tablets. Thereby negative storage interaction between components can be avoided. Different dissolution profiles of each of the compartments can also give rise to delayed dissolution of selected components in the wash solution.

A liquid or gel detergent which is not unit dosed may be aqueous, typically containing at least 20% by weight and up to 95% water, such as up to about 70% water, up to about 65% water, up to about 55% water, up to about 45% water, up to about 35% water. Other types of liquids, including without limitation, alkanols, amines, diols, ethers and polyols may be included in an aqueous liquid or gel. An aqueous liquid or gel detergent may contain from 0-30% organic solvent. A liquid or gel detergent may also be non-aqueous.

Formulation of enzyme in granules

Non-dusting granulates may be produced e.g. as disclosed in US 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1000 to 20000;

ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono-, di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

The compositions of the invention may be formulated as a granule, for example as a co-granule that combines one or more enzymes. Each enzyme will then be present in more granules, securing a more uniform distribution of enzymes in the detergent. This also reduces the physical segregation of different enzymes due to different particle sizes. Methods for producing multi-enzyme co-granulates for the detergent industry are disclosed in the IP.com disclosure IPCOM000200739D.

Another example of formulation of enzymes by the use of co-granulates is disclosed in WO 2013/188331, which relates to a detergent composition comprising (a) a multi-enzyme co- granule; (b) less than 10 wt% zeolite (anhydrous basis); and (c) less than 10 wt% phosphate salt (anhydrous basis), wherein said enzyme co-granule comprises from 10 to 98 wt% moisture sink component and the composition additionally comprises from 20 to 80 wt% detergent moisture sink component. WO 2013/188331 also relates to a method of treating and/or cleaning a surface, preferably a fabric surface comprising the steps of (i) contacting said surface with the detergent composition in aqueous wash liquor, (ii) rinsing and/or drying the surface.

A multi-enzyme co-granule may comprise an enzyme of the invention and one or more enzymes selected from the group consisting of proteases, lipases, cellulases, xyloglucanases, perhydrolases, peroxidases, lipoxygenases, laccases, hemicellulases, proteases, cellulases, cellobiose dehydrogenases, xylanases, phospho lipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, tannases, pentosanases, lichenases glucanases, arabinosidases, hyaluronidase, chondroitinase, amylases, nucleases, hexosaminidases and mixtures thereof.

An embodiment of the invention relates to an enzyme granule/particle comprising the enzyme of the invention. The granule is composed of a core, and optionally one or more coatings (outer layers) surrounding the core. Typically, the granule/particle size, measured as equivalent spherical diameter (volume based average particle size), of the granule is 20-2000 µm, particularly 50-1500 µm, 100-1500 µm or 250-1200 µm.

The core may include additional materials such as fillers, fibre materials (cellulose or synthetic fibres), stabilizing agents, solubilising agents, suspension agents, viscosity regulating agents, light spheres, plasticizers, salts, lubricants and fragrances.

The core may include binders, such as synthetic polymer, wax, fat, or carbohydrate.

The core may comprise a salt of a multivalent cation, a reducing agent, an antioxidant, a peroxide decomposing catalyst and/or an acidic buffer component, typically as a homogenous blend.

The core may consist of an inert particle with the enzyme absorbed into it, or applied onto the surface, *e.g.*, by fluid bed coating.

The core may have a diameter of 20-2000 µm, particularly 50-1500 µm, 100-1500 µm or 250-1200 µm.

The core can be prepared by granulating a blend of the ingredients, *e.g.*, by a method comprising granulation techniques such as crystallization, precipitation, pan-coating, fluid bed coating, fluid bed agglomeration, rotary atomization, extrusion, prilling, spheronization, size reduction methods, drum granulation, and/or high shear granulation.

Methods for preparing the core can be found in Handbook of Powder Technology; Particle size enlargement by C. E. Capes; Volume 1; 1980; Elsevier.

The core of the enzyme granule/particle may be surrounded by at least one coating, *e.g.*, to improve the storage stability, to reduce dust formation during handling, or for coloring the granule. The optional coating(s) may include a salt coating, or other suitable coating materials, such as polyethylene glycol (PEG), methyl hydroxy-propyl cellulose (MHPC) and polyvinyl alcohol (PVA). Examples of enzyme granules with multiple coatings are shown in WO 93/07263 and WO 97/23606.

The coating may be applied in an amount of at least 0.1% by weight of the core, *e.g.*, at least 0.5%, 1% or 5%. The amount may be at most 100%, 70%, 50%, 40% or 30%.

The coating is preferably at least 0.1 µm thick, particularly at least 0.5 µm, at least 1 µm or at least 5 µm. In a particular embodiment, the thickness of the coating is below 100 µm. In a more particular embodiment the thickness of the coating is below 60 µm. In an even more particular embodiment the total thickness of the coating is below 40 µm.

The coating should encapsulate the core unit by forming a substantially continuous layer. A substantially continuous layer is to be understood as a coating having few or no holes, so that the core unit it is encapsulating/enclosing has few or none uncoated areas. The layer or coating should be homogeneous in thickness.

The coating can further contain other materials as known in the art, *e.g.*, fillers, antisticking agents, pigments, dyes, plasticizers and/or binders, such as titanium dioxide, kaolin, calcium carbonate or talc.

A salt coating may comprise at least 60% by weight w/w of a salt, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% by weight w/w.

The salt may be added from a salt solution where the salt is completely dissolved or from a salt suspension wherein the fine particles is less than 50 µm, such as less than 10 µm or less than 5 µm.

The salt coating may comprise a single salt or a mixture of two or more salts. The salt may be water soluble, in particular having a solubility at least 0.1 grams in 100 g of water at 20°C, preferably at least 0.5 g per 100 g water, *e.g.*, at least 1 g per 100 g water, *e.g.*, at least 5 g per 100 g water.

The salt may be an inorganic salt, *e.g.*, salts of sulfate, sulfite, phosphate, phosphonate, nitrate, chloride or carbonate or salts of simple organic acids (less than 10 carbon atoms, *e.g.*, 6 or less carbon atoms) such as citrate, malonate or acetate. Examples of cations in these salts are alkali or earth alkali metal ions, the ammonium ion or metal ions of the first transition series, such as sodium, potassium, magnesium, calcium, zinc or aluminium. Examples of anions include chloride, bromide, iodide, sulfate, sulfite, bisulfite, thiosulfate, phosphate, monobasic phosphate, dibasic phosphate, hypophosphite, dihydrogen pyrophosphate, tetraborate, borate, carbonate, bicarbonate, metasilicate, citrate, malate, maleate, malonate, succinate, lactate, formate, acetate, butyrate, propionate, benzoate, tartrate, ascorbate or gluconate. In particular alkali- or earth alkali metal salts of sulfate, sulfite, phosphate, phosphonate, nitrate, chloride or carbonate or salts of simple organic acids such as citrate, malonate or acetate may be used.

The salt in the coating may have a constant humidity at 20°C above 60%, particularly above 70%, above 80% or above 85%, or it may be another hydrate form of such a salt (*e.g.*, anhydrate). The salt coating may be as described in WO 00/01793 or WO 2006/034710.

Specific examples of suitable salts are NaCl ($CH_{20°C}$=76%), Na_2CO_3 ($CH_{20°C}$=92%), $NaNO_3$ ($CH_{20°C}$=73%), Na_2HPO_4 ($CH_{20°C}$=95%), Na_3PO_4 ($CH_{25°C}$=92%), NH_4Cl ($CH_{20°C}$ = 79.5%), $(NH_4)_2HPO_4$ ($CH_{20°C}$ = 93,0%), $NH_4H_2PO_4$ ($CH_{20°C}$ = 93.1%), $(NH_4)_2SO_4$ ($CH_{20°C}$=81.1%), KCl ($CH_{20°C}$=85%), K_2HPO_4 ($CH_{20°C}$=92%), KH_2PO_4 ($CH_{20°C}$=96.5%), KNO_3 ($CH_{20°C}$=93.5%), Na_2SO_4 ($CH_{20°C}$=93%), K_2SO_4 ($CH_{20°C}$=98%), $KHSO_4$ ($CH_{20°C}$=86%), $MgSO_4$ ($CH_{20°C}$=90%), $ZnSO_4$ ($CH_{20°C}$=90%) and sodium citrate ($CH_{25°C}$=86%). Other examples include NaH_2PO_4, $(NH_4)H_2PO_4$, $CuSO_4$, $Mg(NO_3)_2$ and magnesium acetate.

The salt may be in anhydrous form, or it may be a hydrated salt, i.e. a crystalline salt hydrate with bound water of crystallization, such as described in WO 99/32595. Specific examples include anhydrous sodium sulfate (Na_2SO_4), anhydrous magnesium sulfate ($MgSO_4$), magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$), sodium phosphate dibasic heptahydrate ($Na_2HPO_4{\cdot}7H_2O$), magnesium nitrate hexahydrate ($Mg(NO_3)_2(6H_2O)$), sodium citrate dihydrate and magnesium acetate tetrahydrate. Preferably the salt is applied as a solution of the salt, *e.g.*, using a fluid bed.

Thus, in a further aspect, the present invention provides a granule comprising:

(a) a core comprising an enzyme according to the invention,

(b) optionally, a coating consisting of one or more layers surrounding the core; and

(c) wherein the granule preferably is a co-granulate comprising one or more additional enzymes, preferably wherein at least one additional enzyme is selected from proteases, amylases and cellulases.

In one embodiment, the present invention provides a granule comprising:

(a) a core comprising a polypeptide having DNase degrading activity,

(b) optionally, a coating consisting of one or more layers surrounding the core; and

(c) wherein the granule preferably is a co-granulate comprising one or more additional enzymes, preferably wherein at least one additional enzyme is selected from proteases, amylases and cellulases.

Fabric softener compositions

Fabric softener compositions are well-known in the art. Such compositions include at least one fabric softener component, typically selected from the group consisting of cationic softener components, silicone softener components, paraffins, waxes, dispersible polyolefins and mixtures thereof. Preferred softener components include cationic surfactants, more preferably cationic surfactants of the quaternary ammonium type, for example in the form of a quaternary ammonium ester.

Uses

The present invention is directed to methods for using the compositions comprising a DNase as defined herein and at least one perfume compound, in particular for cleaning of laundry/textiles/fabrics, including household laundry and industrial laundry, and for hard surface cleaning, including automatic dishwashing (ADW), car washing and industrial surface cleaning.

Use of cleaning compositions

The detergent composition of the present invention may be formulated, for example, for use as a hand or machine laundry detergent composition, including as a laundry additive composition suitable for pre-treatment of stained fabrics or a rinse added to a fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations, or be formulated for hand or machine dishwashing. Also provided herein is a detergent additive comprising a DNase and at least one perfume compound.

In one embodiment, the invention relates to use of a DNase in a detergent composition for increasing binding of a perfume to a textile during a laundry process, wherein the detergent composition comprises at least one perfume compound.

In one embodiment, the invention relates to use of a DNase in a detergent composition for increasing perfume retention on a textile after wash, wherein the detergent composition comprises at least one perfume compound.

In one embodiment, the invention relates to use of a DNase for enhancing the effect of a perfume in a laundry detergent composition, wherein the detergent composition comprises at least one perfume compound.

Methods

In one embodiment, the invention provides a method for increasing binding of a perfume to a textile during a laundry process, the method comprising washing the textile with a detergent composition comprising at least one perfume compound and a DNase.

In one embodiment, the invention provides a method for increasing perfume retention on a textile after wash, the method comprising washing the textile with a detergent composition comprising at least one perfume compound and a DNase.

In one embodiment, the invention provides a method for enhancing the effect of a perfume in a laundry detergent composition, the method comprising preparing a detergent composition comprising at least one perfume compound and a DNase.

In one embodiment, the invention provides a method for preparing a detergent composition with reduced perfume content but with maintained perfume effect after wash relative to a comparable composition without DNase, the method comprising mixing at least one perfume compound, a polypeptide having DNase activity and at least one detergent component.

In one embodiment, the invention provides a method for cleaning an item, wherein the item is preferably a textile, comprising the steps of:

a) contacting the item with a wash liquor comprising a detergent composition as defined herein comprising an enzyme having DNase activity and at least one perfume compound, and optionally

b) rinsing the item.

The pH of the liquid wash liquor solution is typically in the range of from about 5.5 to about 12, such as from about 7 to about 11, such as from about 7 to about 10, e.g. from about 7 to about 9.

The wash liquor may have a temperature in the range of 5ºC to 95ºC, such as in the range of 10ºC to 80ºC, in the range of 10ºC to 70ºC, in the range of 10ºC to 60ºC, in the range of 10ºC to 50ºC, in the range of 15ºC to 40ºC or in the range of 20ºC to 30ºC.

The present invention is further described by the following examples that should not be construed as limiting the scope of the invention.

Examples

Assay 1: Determination of DNase activity

DNase activity may be determined on DNase Test Agar with Methyl Green (BD, Franklin Lakes, NJ, USA), which is prepared according to the manual from the supplier. Briefly, 21 g of agar is dissolved in 500 ml water and then autoclaved for 15 min at 121°C. Autoclaved agar is tempered to 48°C in water bath, and 20 ml of agar is poured into petri dishes and allowed to solidify by incubation overnight at room temperature. On solidified agar plates, 5 µl of enzyme solutions are added and DNase activity is observed as colorless zones around the spotted enzyme solutions.

Assay 2: Determination of DNase activity

DNase activity may be determined using the DNaseAlert™ Kit (11-02-01-04, IDT Intergrated DNA Technologies) according to the supplier's manual. Briefly, 95 µl DNase sample is mixed with 5 µl substrate in a microtiter plate, and fluorescence is immediately measured using a Clariostar microtiter reader from BMG Labtech (536 nm excitation, 556 nm emission).

Example 1: Sensory evaluation of perfume retention after wash in a liquid detergent with DNase

The evaluation of this example included the following steps:

Step 1. Preparation of test items

Step 2. Wash test in detergent with and without DNase

Step 3. Human panel evaluation of washed items

Subsequent to the sensory evaluation, GC-MS analysis of washed items was performed. This is described in Example 2.

1. Preparation of test items

The test items were 100% polyester T-shirts weighing 130 g/m^2. The T-shirts were pre-aged before being handed out to be used for exercise by test persons.

Pre-aging procedure: T-shirts weighing a total of 4 kg were added to a Wascator FOM 71 CLS washing machine from Electrolux together with 75 g of IEC* A detergent. The detergent was mixed with 50 g tap water plus 28 g of ballast soil with the following composition:

Ingredient	Weight (g)
Olive oil	180
Bey Sebum (CFT/WFK)	180
NaCl	60
Urea	20

Corn starch	70
Kaolin	90
Lanolin	70
$CaCl_2$	20
Egg white powder	80
Glycerol, 87%	23
Tryptone soy broth	20
Total weight (g)	813

The above ingredients were mixed into a homogenous material by mixing melted lanolin and sebum (melted by heating at 50°C) with the mixed dry ingredients, followed by addition of glycerol and tryptone soy broth and stirring the mixture with a magnetic stirrer at 50°C for about two hours. The mixture was then cooled to room temperature and stored at 5°C.

The Wascator was programmed to wash for 12 hours without a final rinse. 75 g of new detergent IEC* A was then added, and a one-hour wash program with two rinses was performed, after which the T-shirts were tumble dried.

The T-shirts were then handed out for use by members of either a soccer team or a running team. They were worn for at least one hour of intensive training during two separate training sessions, drying the T-shirts in between. They were then returned to the laboratory and incubated in a safety cupboard for safety reasons for three days, after which they were moved to a climate chamber for a 24h incubation at 30°C and 95% relative humidity, followed by collecting all the T-shirts in a box with a lid and letting them incubate at 30°C for another 24h. All T-shirts were sorted by smell. Those without smell were discarded, while those with intense smell could be used as test material.

2. Wash test in detergent with and without DNase

Since left and right armpits can smell differently, cutouts from each were made using a 6x8 cm oval template. Cutout swatches from the left and right armpits of each T-shirt were cut into 4 pieces, resulting in a total of 8 pieces from each T-shirt. These were divided among 4 nylon stockings that were used as washing bags, such that pieces from the left and right armpit and from the front and back side of the armpit were divided into different stockings. Cutouts from 4 T-shirts were divided in this manner among 4 nylon stockings, i.e. each stocking contained a total of 8 pieces from 4 different T-shirts. The nylon stockings were sealed with a knot or a rubber band before wash and were discarded after wash.

The rest of the T-shirts were cut into two pieces each, with one half T-shirt being washed with DNase and the other half without DNase. The nylon stockings containing the armpit samples were washed together with the half T-shirts as well as ballast consisting of 1 kg of discarded

household garments (each cut in two and divided equally between the two washing machines) and 1.5 kg of clean ballast items in the form of 5 cotton T-shirts, 1 towel and 4-6 tea towels.

Three washes were performed in each round:

1st wash: A clean T-shirt was washed using the same detergent as that used in the test. This functioned as a clean background sample to give a "pure fragrance impression".

2nd wash: This was a blank test wash with detergent alone.

3rd wash: Test wash using detergent and DNase.

Washing was performed in a standard US top loader washing machine with the armpit samples, half T-shirts and ballast as described above, using the following wash program:

- Load Size: Medium
- Wash temp.: 30°C
- Extra Rinse: On
- Program: Normal, Regular
- Duration: approx. 55 min

The main wash cycle water volume was approx. 67 liters of water with a hardness of water 6º dH (2:1:1.5 Ca:Mg:$NaHCO_3$). The detergent was Arm & Hammer Oxiclean (Church & Dwight, USA), added directly to the machine in a dose of 54.8 g/wash. For washes with DNase, the DNase of SEQ ID NO: 1 was added to the wash water at a concentration of 2 ppm. For the 1st wash with a clean T-shirt, a single T-shirt was washed in the same manner as the 2nd wash with detergent alone, but without ballast. All items were line dried overnight after wash.

3. Human panel evaluation of washed items

Swatches were transferred from the dried stockings to 60L Nalophan bags sealed at the bottom and filled with filtered air (swatches from one stocking per bag). Background samples from the 1st wash were cut out with a similar weight as the test samples and added to 2 bags. The bags are then stored at 30°C for 2 hours, after which they are ready for the sensory evaluation.

The sensory evaluation was performed using a PureSniff III device from Olfasense GmbH, Germany. The device has three chambers for simultaneous evaluation. The first chamber contained the background sample, while the other two chambers contained the test samples washed with or without DNase in a blinded order.

Each test panel member senses (smells) first the background sample and then the two test samples. The test is rated as a simple preference test in which the test person chooses the sample that he/she prefers.

The test is repeated with the remaining 6 test samples from each wash, each time evaluating a sample washed without DNase and a sample washed with DNase in blinded order.

Each test was repeated 3 times using 8 T-shirts (2 subgroups of 4), i.e. testing a total of 24 T-shirts in 12 pools. Seven test persons participated in the evaluation, thus providing a total of

84 different individual evaluations. The results are provided in Table 1 below.

Table 1. Sensory results for 3 wash tests in liquid detergent with and without DNase (SEQ ID NO: 1)

	Number of panelists preferring T-shirts washed:		Preference for DNase in %
	With DNase	Without DNase	
Replicate 1 Pool 1	4	3	57
Replicate 1 Pool 2	4	3	57
Replicate 1 Pool 3	3	4	43
Replicate 1 Pool 4	6	1	86
Replicate 2 Pool 1	3	4	43
Replicate 2 Pool 2	7	0	100
Replicate 2 Pool 3	6	1	86
Replicate 2 Pool 4	5	2	71
Replicate 3 Pool 1	5	2	71
Replicate 3 Pool 2	5	2	71
Replicate 3 Pool 3	4	3	57
Replicate 3 Pool 4	4	3	57
Total	**56**	**28**	**67%**

The results in Table 1 show that overall, there was a clear preference among the panelists for the T-shirt samples that were washed using a DNase.

Example 2: Perfume retention on textile after wash in liquid detergent with a DNase

The T-shirt samples which were evaluated by the panelists as described in Example 1 were further used for GC-MS analysis. Headspace SPME-GC-MS analysis of fragrances from the commercial detergent on polyester T-shirts was performed using an Agilent 7890 gas chromatograph coupled to an Agilent 5977 mass spectrometry GC-MS system.

- GCMS Agilent 7890 GC with split/splitless injector and 5977 MS with extractor ion source coupled to a Gerstel MPS2 sampler with HS/SPME, SPME needle heater.
- The method used was: GC Oven Temperature: Initial 40°C; hold 2 min; Rate 5°C/min until 150°C; Rate 35°C/min until 240°C; Hold 3 min. Front SS Inlet He: Mode Split; Tª 230°C, Split Ratio 10:1; Split Flow 15 mL/min. Column: Agilent 19091F-433: FFAP-01 HP-FFAP 30 m x 250 µm x 0.25 µm.
- Gerstel MPS SPME Incubator/Agitator. Incubation Temperature: 60ºC. Incubation Time: 10.00 min. Agitator Speed: 250 rpm. Sample parameters: Extraction Time: 2.00 min; Inj. Desorption Time: 120 s.

- Fiber type: Carboxen/Polydimethylsiloxane (CAR/PDMS)
- MS Information: Acquisition Mode: Scan. Solvent Delay (minutes): 1. Scan Parameters: Start Time: 1. Low Mass: 35. High Mass: 350. Threshold: 100. A/D Samples: 4. MSZones: MS Source: 230ºC. MS Quad: 150ºC

Table 2. Fragrance intensity on polyester T-shirts washed in a liquid detergent with or without DNase (SEQ ID NO: 1)

Fragrance name	**CAS No.**	**Chemical group**	**Without DNase (relative intensity)**	**With DNase (relative intensity)**	**Fragrance increase with DNase (%)**
β-Ocimene	13877-91-3	hydrocarbon	14315	14881	4.0
β-Myrcene	123-35-3	hydrocarbon	10257	12116	18.1
D-Limonene	5989-27-5	hydrocarbon	368496	423565	14.9
p-Cymene	99-87-6	hydrocarbon	21976	24590	11.9
α-Pinene	80-56-8	hydrocarbon	6412	5636	-12.1
Hexyl acetate	142-92-7	ester	5009	6375	27.3
Terpinolene	586-62-9	hydrocarbon	7051	7695	9.1
Octanal	124-13-0	aldehyde	26081	30956	18.7
6-Methyl-5-hepten-2-one	110-93-0	ketone	64401	67507	4.8
Nonanal	124-19-6	aldehyde	106550	119938	12.6
Decanal	112-31-2	aldehyde	98551	103405	4.9
4-tert-Butylcyclohexanol	21862-63-5	alcohol	26543	28993	9.2
Isobornyl formate	125-12-2	ester	5102	5458	7.0
cis-4-tert-Butylcyclohexyl acetate	10411-92-4	ester	5744	6302	9.7
Diphenyl ether	101-84-8	ether	25227	27065	7.3
Lilial	80-54-6	aldehyde	16279	16816	3.3
α-Methyl ionone	127-42-4	ketone	86252	108465	25.8
6-Methyl-β-ionone	79-70-9	ketone	14903	18346	23.1
Naphthalene, 2-methoxy-	93-04-9	ether	28828	31414	9.0
Naphthalene, 2-ethoxy-	93-18-5	ether	43233	47972	11.0
Total			**981211**	**1107497**	**12.9**

Example 3: Sensory evaluation of perfume retention after wash in a powder detergent with DNase

Polyester T-shirts were prepared in the same manner as described above in Example 1. For this example, they were washed with a commercial powder detergent, Gut & Günstig (McBride, Germany), in EU front loader machines (Miele Novotronic, Model W 1935 WTL) using tap water from Bagsværd, Denmark with a water hardness of about 20°dH. The wash program, was Cotton, Short (1h 49 min, 1600 rpm) with a wash temperature of 30°C. For washes with

DNase, 2 ppm of the DNase (SEQ ID NO: 1) was added directly to the washing machine. After wash, all items were line dried overnight, and then stored refrigerated before analysis.

T-shirt armpit samples and remaining half T-shirts (weight 530g) prepared as described in Example 1 were washed together with 1400g of ballast consisting of 6 used half polyester T-shirts, 5 dirty half cotton pillowcases, 3 frotté wash cloths (20x20 cm), 3 half shirts (polyester), and 2 dirty half cotton T-shirts.

Three washes were performed as described in Example 1, i.e. a blank wash with a clean T-shirt and detergent to provide a clean background sample, and washes with and without DNase. The sensory evaluation was performed as in Example 1. The results of the sensory evaluation are provided in Table 3 below.

Table 3. Sensory results for 3 wash tests in powder detergent with and without DNase (SEQ ID NO: 1)

	Number of panelists preferring T-shirts washed:		Preference for DNase in %
	With DNase	Without DNase	
Replicate 1 Pool 1	2	3	40
Replicate 1 Pool 2	3	2	60
Replicate 1 Pool 3	3	2	60
Replicate 1 Pool 4	3	2	60
Replicate 2 Pool 1	6	0	100
Replicate 2 Pool 2	5	1	83
Replicate 2 Pool 3	5	1	83
Replicate 2 Pool 4	5	1	83
Replicate 3 Pool 1	3	3	50
Replicate 3 Pool 2	2	4	33
Replicate 3 Pool 3	6	0	100
Replicate 3 Pool 4	2	4	33
Total	**45**	**23**	**66%**

Example 4: Perfume retention on textile after wash in powder detergent with a DNase

T-shirt samples that had been subjected to a sensory evaluated by test persons as described in Example 3 were further used for GC-MS analysis, which was performed as described in Example 2. The results are provided in Table 4 below.

Table 4. Fragrance intensity on the polyester T-shirts washed with or without DNase in a powder detergent.

Fragrance name	**CAS No.**	**Chemical group**	**Without DNase (relative intensity)**	**With DNase (relative intensity)**	**Fragrance increase with DNase (%)**
Citronellyl formate	105-85-1	ester	1464	1509	3.1
trans-4-tert-Butylcyclohexyl acetate	1900-69-2	ester	642	712	10.9
cis-4-tert-Butylcyclohexyl acetate	10411-92-4	ester	1601	1789	11.8
Indan-1,3-diol monopropionate	132075*	ester	1277	1552	21.6
Diphenyl ether	101-84-8	ether	283	359	26.5
Total			**5267**	**5921**	**12.4**

The sensory evaluation data provided above in Tables 1 and 3 demonstrates that test persons clearly preferred the fragrance of the polyester T-shirt samples that had been washed according to the invention with a detergent composition containing a DNase. This was the case for samples washed with both the liquid detergent under North American wash conditions and the powder detergent under European conditions.

Similarly, the GC-MS data in Tables 2 and 4 shows that that the fragrance intensity on polyester T-shirts was increased for a variety of different perfume compounds, and that this was the case using different detergents and different washing conditions, when the samples were washed according to the invention with a composition contained a DNase.

Example 5: Perfume retention on textile after rinse in fabric softeners containing a DNase

The evaluation of this example included the following steps:

Step 1. Preparation of test items

Step 2. Rinse test in softeners with and without DNase

Step 3. GC-MS analysis of the rinsed items

Step 1. Preparation of test items

Prewash

New polyester T-shirts as described above were first prewashed.

Prewash of textiles was done primarily to remove starch, carboxymethyl cellulose (CMC) and other additives from the textiles. Protease (Savinase® 16L, Novozymes A/S, Denmark), amylase (Stainzyme® 12L, Novozymes A/S) and cellulase (Celluclean® ST, Novozymes A/S) were added to the prewashes to remove these additives.

The textiles were washed three times in 86.1 g/wash detergent W-ECE-2 (wfk Testgewebe GmbH, Germany) using water with 15°dH water hardness (3.00 mL of 0.713 mol/L $CaCl_2$, 1.50 mL of 0.357 mol/L $MgCl_2$ and 0.3371 g of $NaHCO_3$ in 1 L deionized water) and containing the following enzymes:

Enzyme	Dosage in prewash 1 (g/wash)	Dosage in prewash 2 (g/wash)	Dosage in prewash 3 (g/wash)
Savinase® 16L	0.39	0	0
Stainzyme® 12L	2.6	2.6	0
Celluclean® ST	3.26	0	0

Adding DNA to the swatches

The prewashed T-shirts were then cut into small round swatches with a diameter of 2 cm. To each swatch, 47 µl of 1% (w/v) deoxyribonucleic acid (DNA) solution was added at the center of the swatch. The DNA solution was made by dissolving 100 mg deoxyribonucleic acid sodium salt from salmon testes (Sigma Aldrich) in 10 ml MilliQ water in a 25 ml beaker with constant stirring for 1 h at room temperature.

The swatches containing the DNA solution were then dried overnight (16 h) at room temperature in a fume hood with constant air flow.

Step 2. Rinse test in softeners with and without DNase

Detergent

Model detergent A2 was used in the main wash of this experiment. A wash solution of liquid model detergent A2 was prepared by weighing out the detergent and dissolving in water with a hardness of 15°dH, with the model detergent A2 dosed at 3.33 g/L.

Model detergent A2 contains the following ingredients:

12% Sodium salt of linear alkylbenzene sulphonates (Na-LAS), 12% alcohol ethoxylate (AEO) Biosoft N25-7 (NI), 4% alkyl ethoxysulfate / sodium laureth sulfate (AEOS/SLES), 2% MPG (monopropylene glycol), 3.1% ethanol, 2% triethanolamine (TEA), 3% palm kernel oil soap, 2% sodium hydroxide, 3.9% sodium citrate, 1.5% diethylenetriaminepenta(methylenephosphonic acid) (DTMPA) and 0.5% phenoxyethanol; remainder water (all percentages are w/w).

Softeners

Three representative commercial European fabric softeners were tested in this experiment: Dun-let Dream of Freshness (Colgate), Lenor Weichspüler Aprilfrisch (Procter & Gamble) and Bamseline Dugfrisk (Unilever). Lenor Weichspüler Aprilfrisch was purchased from amazon.de, and the other two softeners were purchased from a supermarket in Denmark.

Rinse solutions of the above three softeners were prepared by weighing out softeners and

dissolving in water with 15°dH hardness. Dosing of the softeners was calculated according to the suggested dosage for each at standard conditions for a 4-5 kg wash load, which was 25 ml, 27 ml and 27 ml for Lenor Weichspüler Aprilfrisch, Dun-let Dream of Freshness and Bamseline Dugfrisk, respectively. Considering that the average volume of water used in one rinse step in a common EU washing machine (machine size 3.5-5 kg laundry load) is 13.5 L, the dosing of each softener used in this experiment was 1.85 ml/L, 2.0 ml/L and 2.0 ml/L for Lenor Weichspüler Aprilfrisch, Dun-let Dream of Freshness and Bamseline Dugfrisk, respectively.

Main wash

The dried swatches from Step 1 were washed in a Mini Launder-O-Meter (MiniLOM) wash.

The miniLOM assay is a small-scale version of the Launder-OMeter® (LOM). A miniLOM basically consists of closed test tubes being rotated in a heating cabinet for a given time and at a given temperature. Each test tube represents one small washing machine. It is mainly used in testing of detergents and enzymes at European wash conditions.

In this experiment, three swatches were placed in a 50-ml glass tube and 20 ml of wash solution (15º dH water with 3.15 g/L Model A2). To better mimic the wash performance of commercial detergents in Europe, an enzyme blend, Medley® Brilliant (Novozymes A/S, Denmark), was added to all glass tubes at a dosage of 2.5% of the detergent. The enzyme blend contains a variety of commonly used enzymes, including protease, amylase, lipase, mannanase, pectate lyase and two cellulases.

The glass tubes were then mounted in a Mini-Launder-O-Meter (a Stuart Tube Rotator SB3) and rotated at 30°C for 60 minutes at 30 rpm.

First rinse

After the main wash, the rotator was placed at room temperature, and the wash liquor was carefully poured out of each tube. 30 ml of hard water (15º dH water) was then added to each tube. The swatches were then rinsed in the miniLOM at room temperature for 30 min at 30 rpm.

Rinse test in softeners (second rinse)

After the first rinse, the rinse liquor was carefully poured out. 30 ml of rinse solution (softener dissolved in 15° dH water) was then added to each tube. Three replicates (one replicate/tube) were used for each rinse condition (softener/DNase combination). 1.0 ppm DNase was added to the tubes, and tubes without DNase were included as controls. The swatches were then rinsed in the tubes in the miniLOM at room temperature for 30 min at 30 rpm.

Step 3. GC-MS analysis of the rinsed items

After washing and rinsing, each swatch was centrifuged in a falcon tube containing a filter

(HOZELOCK K3 kaldness filters, Aquacadabra, eBay) at 4000g for 5 min to remove excess water. This plastic filter separated the excess water from the swatch after centrifuging.

The three swatches from the same glass tube were then placed in one 20 mL GC-MS vial (Mikrolab Aarhus A/S, Aarhus, Denmark) and capped with silicone screw top lids (Mikrolab Aarhus A/S, Aarhus, Denmark). All vials were then analyzed by GC-MS, as described in Example 2.

Results

This example showed that the fragrance intensity on polyester T-shirts was increased for a variety of different perfume compounds in all tested softeners when the samples were rinsed according to the invention with a composition contained a DNase (Tables 5, 6 and 7).

Table 5. Fragrance intensity on polyester T-shirts rinsed in Dun-let Dream of Freshness softener with or without DNase (SEQ ID NO: 1)

Fragrance name	CAS no.	Chemical group	Without DNase (relative intensity)	With DNase (relative intensity)	Fragrance increase with DNase (%)
α-Pinene	7785-70-8	Hydrocarbon	626	595	-5
Butanoic acid, 2-methyl-, ethyl ester	7452-79-1	Ester	22903	14920	-35
Camphene	79-92-5	Hydrocarbon	8743	10450	20
Levomenthol	2216-51-5	Alcohol	2881	5233	82
Pentanoic acid, 2-methyl-, ethyl ester	39255-32-8	Ester	21597	11016	-49
3-Heptanone, 5-methyl-	541-85-5	Ketone	512	363	-29
D-Limonene	5989-27-5	Hydrocarbon	20053	18681	-7
Eucalyptol	470-82-6	Ether	7281	7748	6
Dicyclopentadiene	77-73-6	Hydrocarbon	2866	2899	1
4-Terpinenyl acetate	4821-04-9	Ester	537	370	-31
o-Cymene	527-84-4	Hydrocarbon	4711	2945	-37
Acetic acid, hexyl ester	142-92-7	Ester	2815	2478	-12
α-terpiene	99-86-5	Hydrocarbon	2544	1523	-40
4-Hexen-1-ol, acetate	72237-36-6	Ester	15321	12704	-17
3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-		Aldehyde	8897	4674	-47
Benzene, 1-methoxy-4-methyl-	104-93-8	Ether	46107	42904	-7
Longifolene	475-20-7	Hydrocarbon	1562	3248	108
2,7-Dimethyl-2,7-octanediol	19781-07-8	Alcohol	121303	50454	-58
3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	18022-66-7	Aldehyde	12431	5893	-53
Longicyclene	1137-12-8	Hydrocarbon	3705	3895	5
β-Chamigrene		Hydrocarbon	1780	3861	117

2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	1135-66-6	Hydrocarbon	255776	537021	110
Ethanol, 2-(3,3-dimethylcyclohexylidene)-, (Z)-	26532-23-0	Alcohol	298	658	121
Benzaldehyde	100-52-7	Aldehyde	5245	5376	2
Linalyl formate	115-99-1	Ester	1871	4270	128
4-tert-Butylcyclohexanol, trans-	21862-63-5	Ester	82049	161963	97
D,L-Isobornyl acetate	92618-89-8	Ester	34409	45899	33
α-Guaiene	3691-12-1	Hydrocarbon	24217	53928	123
Caryophyllene	87-44-5	Hydrocarbon	27021	57333	112
4-tert-Butylcyclohexanol, cis-	98-52-2	Ester	14554	25299	74
2-Undecanone	112-12-9	Ketone	4124	5217	26
Undecanal	112-44-7	Aldehyde	6328	16909	167
Undecanal, 2-methyl-	110-41-8	Aldehyde	113436	173640	53
Benzoic acid, methyl ester	93-58-3	Ester	22702	40023	76
4-tert-Butylcyclohexyl acetate, cis-	10411-92-4	Ester	24666	43319	76
Patchoulene	1405-16-9	Hydrocarbon	2248	4409	96
Acetophenone	98-86-2	Ketone	11664	12559	8
4-tert-Butylcyclohexyl acetate, trans-	1900-69-2	Ester	836	589	-30
Aciphyllene	87745-31-1	Hydrocarbon	3770	6993	85
α-Terpineol	98-55-5	Alcohol	435	995	129
Azulene	3691-11-0	Hydrocarbon	30275	52296	73
Dodecanal	112-54-9	Aldehyde	40846	88261	116
Cyclohexanone, 2-methyl-5-(1-methylethenyl)-	7764-50-3	Ketone	1420	1691	19
Acetic acid, phenylmethyl ester	140-11-4	Ester	7500	4548	-39
Isopulegol	89-79-2	Alcohol	1669	956	-43
Cyclodecanol	1502-05-2	Alcohol	5703	3360	-41
Cyclohexanepropanoic acid, 2-propenyl ester	2705-87-5	Ester	2328	3065	32
Citronellol	106-22-9	Alcohol	2844	1366	-52
4,7-Methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydrodimethyl-	94248-21-2	Alcohol	2928	2287	-22
4,7-Methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydrodimethyl-	79771-15-6	Alcohol	13527	9924	-27
4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate	5413-60-5	Ester	52680	40033	-24
4,7-methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate	2500-83-6	Ester	1887	1369	-27
Benzyl alcohol	100-51-6	Alcohol	882	367	-58
β-ionone	14901-07-6	Ketone	15559	14892	-4

Indan-1,3-diol monopropionate		Ester	1943	2376	22
Indan-1,3-diol monopropionate isomer		Ester	6585	7990	21
Phenylethyl Alcohol	60-12-8	Alcohol	955	314	-67
2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl		Furan	803	905	13
Benzaldehyde, 4-methoxy-	123-11-5	Aldehyde	1310	1486	13
5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate		Ester	31300	37438	20
β-Ionone, methyl- (E)	79-77-6	Ketone	703591	715944	2
β-Ionone, methyl- (Z)	35031-06-2	Ketone	161053	161679	0
2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-naphthalen-2-yl)-propionaldehyde		Aldehyde	12850	12810	0
β-Ionone, methyl- isomer	127-43-5	Ketone	39060	38837	-1
Eugenol	97-53-0	Alcohol	2383	2117	-11
β-Methoxynaphthalene	93-04-9	Ether	78356	75478	-4
Benzenemethanol	90-17-5	Alcohol	5596	6851	22
2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	62924-17-8	Alcohol	5730	6356	11
Naphthalene, 6,7-diethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-	55741-10-1	Hydrocarbon	45365	50432	11
Naphthalene, 6,7-diethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl- isomer	55741-10-1	Hydrocarbon	777	842	8
Methyl anthranilate	134-20-3	Ester	913	964	6
γ-undecalactone	104-67-6	Ester	9547	10074	6
Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	24851-98-7	Ester	649	678	4
Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	1222-05-5	Ether	1482	1446	-2
Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl- isomer	1222-05-5	Ether	593972	645309	9
Total			**2829097**	**3407996**	**20**

Table 6. Fragrance intensity on polyester T-shirts rinsed in Lenor Weichspüler Aprilfrisch softener with or without DNase (SEQ ID NO: 1)

Fragrance name	**CAS no.**	**Chemical group**	**Without DNase (relative intensity)**	**With DNase (relative intensity)**	**Fragrance increase with DNase (%)**
2-Decanol	1120-06-5	Alcohol	379	240	-37
2-Hexanol, 3-methyl-	2313-65-7	Alcohol	929	785	-16
Linalyl formate	115-99-1	Ester	1708	1367	-20

(-)-β-Citronellene	10281-56-8	Hydrocarbon	1316	1307	-1
Isopulegol	89-79-2	Alcohol	145	295	103
Butanoic acid, 2-methyl-, ethyl ester	7452-79-1	Ester	58249	38020	-35
Camphene	79-92-5	Hydrocarbon	15012	15999	7
α-Phellandrene	99-83-2	Hydrocarbon	25	459	1762
β-Myrcene	123-35-3	Hydrocarbon	6046	7180	19
α-Terpinene	99-86-5	Hydrocarbon	1397	1823	31
D-Limonene	5989-27-5	Hydrocarbon	7919	9516	20
Eucalyptol	470-82-6	Ether	52710	56120	6
trans-β-Ocimene	3779-61-1	Hydrocarbon	1639	1825	11
γ-Terpinene	99-85-4	Hydrocarbon	695	846	22
β-cis-Ocimene	3338-55-4	Hydrocarbon	2168	2432	12
Butanoic acid, 3-methylbutyl ester	106-27-4	Ester	1379	2738	99
p-Cymene	99-87-6	Hydrocarbon	7215	8544	18
Acetic acid, hexyl ester	142-92-7	Ester	134647	232412	73
Terpinolene	586-62-9	Hydrocarbon	3643	4378	20
Carane, 4,5-epoxy-, trans	6909-20-2	Epoxy	10777	12985	20
3-Decyn-2-ol	69668-93-5	Alcohol	2077	1674	-19
Benzene, 4-ethenyl-1,2-dimethyl- isomer	27831-13-6	Hydrocarbon	8115	10310	27
Rose oxide isomer	16409-43-1	Ether	2657	3559	34
Rose oxide isomer	16409-43-1	Ether	604	743	23
Benzene, 4-ethenyl-1,2-dimethyl- isomer	27831-13-6	Hydrocarbon	4158	5260	27
Linalool tetrahydride	78-69-3	Alcohol	34553	36985	7
Benzene, 1-methoxy-4-methyl-	104-93-8	Ether	5724	7521	31
cis-p-mentha-1(7),8-dien-2-ol		Alcohol	5373	4011	-25
4-(2-Methoxypropan-2-yl)-1-methylcyclohex-1-ene		Ether	2615	5087	95
2,7-Dimethyl-2,7-octanediol	19781-07-8	Alcohol	28287	30198	7
3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-		Alcohol	8352	5812	-30
Decanal	112-31-2	Aldehyde	5662	5684	0
Grandlure II	26532-23-0	Aldehyde	1864	2141	15
Benzaldehyde	100-52-7	Aldehyde	2300	2374	3
(-)-cis-Carane	2778-68-9	Hydrocarbon	982	880	-10
Linalyl formate	115-99-1	Ester	40171	47564	18
trans-Sesquisabinene hydrate	145512-84-1	Alcohol	13768	13852	1
Camphene	79-92-5	Hydrocarbon	19459	22608	16
α-Guaiene	3691-12-1	Hydrocarbon	117183	170163	45
Isocaryophillene		Hydrocarbon	1342	2094	56
Undecanal, 2-methyl-	110-41-8	Aldehyde	868827	1197633	38

1H-3a,7-Methanoazulene, 2,3,6,7,8,8a-hexahydro-1,4,9,9-tetramethyl-, (1α,3aα,7α,8aβ)-	560-32-7	Hydrocarbon	60903	79169	30
Seychellene	20085-93-2	Hydrocarbon	2889	4210	46
Patchoulene	1405-16-9	Hydrocarbon	5551	7997	44
Acetophenone	98-86-2	Ketone	11633	13354	15
10-Undecenal	112-45-8	Aldehyde	57398	103356	80
4-tert-Butylcyclohexyl acetate	32210-23-4	Ester	40567	58919	45
Aciphyllene	87745-31-1	Hydrocarbon	9511	13329	40
α-Terpineol	98-55-5	Alcohol	3438	3941	15
Dodecanal	112-54-9	Aldehyde	193090	307283	59
Acetic acid, phenylmethyl ester	140-11-4	Ester	19133	30886	61
trans-α-Damascone	24720-09-0	Ketone	6879	7858	14
Benzene, 4-ethenyl-1,2-dimethyl-	27831-13-6	Ketone	9712	13049	34
9-Undecenal, 2,6,10-trimethyl-	141-13-9	Aldehyde	27712	29092	5
Citronellol	106-22-9	Alcohol	1633	1643	1
α Isomethyl ionone	127-51-5	Ketone	9827	11610	18
α-Ionone	127-41-3	Ketone	3588	4149	16
2-Butanone, 4-phenyl-	2550-26-7	Ketone	1694	2566	51
Tricyclo[4.2.1.1(2,5)]dec-3-en-9-ol, stereoisomer	70220-93-8	Alcohol	1674	1638	-2
4,7-methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate isomer		Ester	7913	7167	-9
4,5-Heptadien-2-one, 3,3,6-trimethyl-	81250-41-1	Ketone	4905	5329	9
4,7-methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate isomer		Ester	1769	1481	-16
α-N-Methyl ionone	7779-30-8	Ketone	871	1030	18
3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	14901-07-6	Ketone	2153	1563	-27
Tricyclo[4.2.1.1(2,5)]dec-3-en-9-ol, stereoisomer	70220-93-8	Alcohol	646	657	2
4-Butyl-indan-5-ol		Alcohol	1908	1991	4
Indan-1,3-diol monopropionate isomer		Ester	2544	2712	7
Indan-1,3-diol monopropionate isomer		Ester	13229	14225	8
Indan-1,3-diol monopropionate isomer		Ester	429	452	5
Cyclamen aldehyde	103-95-7	Aldehyde	48142	51594	7

Benzenemethanol, 2-hydroxy-5-methyl-	4383-07-7	Alcohol	3356	3790	13
2H-2,4a-Methanonaphthalen-8(5H)-one, 1,3,4,6,7,8a-hexahydro-1,1,5,5-tetramethyl-	23787-90-8	Ketone	820	836	2
β-Iraldeine isomer		Ketone	26681	26285	-1
β-Iraldeine isomer		Ketone	4657	4495	-3
Benzoic acid, 2-hydroxy-, 2-methylbutyl ester	51115-63-0	Ester	4958	5631	14
4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	930090-10-1	Ketone	1399	1315	-6
γ-Decalactone	706-14-9	Ketone	569	647	14
2-((4aS,8R,8aR)-4a,8-Dimethyl-3,4,4a,5,6,7,8,8a-octahydronaphthalen-2-yl)propan-2-ol	194607-96-0	Alcohol	5926	5686	-4
Naphthalene, 2-methoxy-	93-04-9	Ether	6503	4751	-27
Linoleic acid isomer	60-33-3	Carboxylic acid	15441	15114	-2
Linoleic acid isomer	60-33-3	Carboxylic acid	711	685	-4
Linoleic acid ethyl ester	544-35-4	Ester	11044	11074	0
Total			**2115468**	**2837975**	**34**

Table 7. Fragrance intensity on polyester T-shirts rinsed in Bamseline Dugfrisk softener with or without DNase (SEQ ID NO: 1)

Fragrance name	**CAS no.**	**Chemical group**	**Without DNase (relative intensity)**	**With DNase (relative intensity)**	**Fragrance increase with DNase (%)**
2-Octene, 2,6-dimethyl-	4057-42-5	Hydrocarbon	4839	5533	14
3-Undecyne	60212-30-8	Hydrocarbon	4139	5850	41
(-)-β-Citronellene isomer	10281-56-8	Hydrocarbon	2515	3531	40
1,6-Octadiene, 2,6-dimethyl-	31222-43-2	Hydrocarbon	289	527	83
Camphene	79-92-5	Hydrocarbon	10717	14684	37
Cyclopentane, 1,3-dimethyl-2-(1-methylethylidene)-, trans-)	61142-30-1	Hydrocarbon	2158	2723	26
Pentanoic acid, 2-methyl-, ethyl ester	39255-32-8	Ester	3006	3906	30
β-Myrcene	123-35-3	Hydrocarbon	6112	8613	41
Isocineole	470-67-7	Ether	11561	9618	-17

Terpinolene	586-62-9	Hydrocarbon	4400	5831	33
3-Octanone	106-68-3	Ketone	436	597	37
D-Limonene isomer	5989-27-5	Hydrocarbon	154504	203385	32
Eucalyptol	470-82-6	Ether	2422	2167	-11
Pseudolimonen	499-97-8	Hydrocarbon	2559	3508	37
trans-β-Ocimene	3779-61-1	Hydrocarbon	1085	1636	51
γ-Terpinene	99-85-4	Hydrocarbon	2982	4243	42
Cyclofenchene	488-97-1	Hydrocarbon	1669	2402	44
Styrene	100-42-5	Hydrocarbon	2587	2987	15
o-Cymene	527-84-4	Hydrocarbon	41599	52727	27
Acetic acid, hexyl ester	142-92-7	Ester	49136	98737	101
Terpinolene	586-62-9	Hydrocarbon	35760	49480	38
Carane, 4,5-epoxy-, trans	6909-20-2	Epoxy	2184	2511	15
Octanal	124-13-0	Aldehyde	1014	1550	53
cis-Decalin, 2-syn-methyl-		Hydrocarbon	3909	4483	15
Benzene, 4-ethenyl-1,2-dimethyl- isomer	27831-13-6	Hydrocarbon	3409	3904	15
Rose oxide	16409-43-1	Ether	394	511	30
2-Octene, 3,7-dimethyl-, (Z)-	6874-32-4	Hydrocarbon	6942	9048	30
Benzene, 4-ethenyl-1,2-dimethyl- isomer	27831-13-6	Hydrocarbon	2069	2319	12
3-Octanol, 3,7-dimethyl-	78-69-3	Alcohol	32654	28239	-14
3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-		Aldehyde	22573	24103	7
Benzene, 4-ethenyl-1,2-dimethyl- isomer	27831-13-6	Hydrocarbon	7681	9002	17
(-)-β-Citronellene isomer	10281-56-8	Hydrocarbon	6151	8973	46
1,5,5-Trimethyl-6-methylene-cyclohexene	514-95-4	Hydrocarbon	1159	1288	11
2-Methyl-2-nonanol	10297-57-1	Alcohol	69265	67378	-3
humulene oxide II	19888-34-7	Epoxy	53585	57686	8
p-Menthone	89-80-5	Ketone	2589	3182	23
Fenchyl acetate	13851-11-1	Ester	507	802	58
7-Octen-2-ol, 2,6-dimethyl-	18479-58-8	Alcohol	32752	34673	6
Maaliol	527-90-2	Alcohol	25090	41349	65
3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-		Aldehyde	9069	9278	2
Benzene, (2-methoxyethyl)-	3558-60-9	Ether	2322	4639	100
4-tert-Butylphenyl)acetaldehyde	109347-45-7	Aldehyde	3745	5841	56
Decanal	112-31-2	Aldehyde	7099	10810	52
Camphor	76-22-2	Ketone	422	428	1
Grandlure II	26532-23-0	Alcohol	3759	5352	42
Benzaldehyde	100-52-7	Aldehyde	3309	3563	8

Linalyl acetate	115-95-7	Ester	4271	4440	4
(-)-cis-Carane	2778-68-9	Hydrocarbon	2045	1246	-39
4-tert-Butylcyclohexyl acetate	32210-23-4	Ester	184272	264247	43
4-Terpinenyl acetate	4821-04-9	Ester	518	854	65
Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	92618-89-8	Ester	146676	191031	30
α-Guaiene	3691-12-1	Hydrocarbon	27502	46440	69
D-Limonene isomer	5989-27-5	Hydrocarbon	873	1221	40
4-tert-Butylcyclohexanol, cis-	937-05-3	Alcohol	19452	27680	42
2-Undecanone	112-12-9	Ketone	20865	34507	65
Undecanal	112-44-7	Aldehyde	12389	21776	76
Undecanal, 2-methyl	110-41-8	Aldehyde	185041	262617	42
1H-3a,7-Methanoazulene, 2,3,6,7,8,8a-hexahydro-1,4,9,9-tetramethyl-, (1α,3aα,7α,8aβ)-	560-32-7	Hydrocarbon	16962	26073	54
Seychellene	20085-93-2	Hydrocarbon	47945	74267	55
Patchoulene	1405-16-9	Hydrocarbon	1700	2684	58
1,5,5-Trimethyl-6-methylene-cyclohexene	514-95-4	Hydrocarbon	1372	1754	28
Isopropyl-4a-methyloctahydro-2(1H)-naphthalenone)	54594-42-2	Ketone	424	720	70
p-Menth-1(7)-en-9-ol	29548-16-1	Alcohol	276	441	59
2-Dodecenal, (E)-	20407-84-5	Aldehyde	1986	3236	63
4-tert-Butylcyclohexyl acetate	32210-23-4	Ester	4429	5731	29
2-Carene	554-61-0	Hydrocarbon	8977	12224	36
D-Limonene isomer	5989-27-5	Hydrocarbon	90185	116174	29
α-Phenylethyl acetate	93-92-5	Ester	11271	13566	20
δ-Guaiene	3691-11-0	Hydrocarbon	15312	24174	58
Pseudolimonene	499-97-8	Hydrocarbon	1394	1414	1
Acetic acid, phenylmethyl ester	140-11-4	Ester	4914	3935	-20
(-)-β-Pinene	18172-67-3	Hydrocarbon	1417	3692	161
α-(E)-Damascone	24720-09-0	Ketone	3015	3944	31
Benzeneethanol, α,α-dimethyl-, acetate	151-05-3	Ester	3192	3799	19
Methyl salicylate	119-36-8	Ester	3670	5814	58
Dill ether	70786-44-6	Furan	2683	2505	-7
Citronellol	106-22-9	Alcohol	4160	3779	-9
α Isomethyl ionone	127-51-5	Ketone	44804	44615	0
3-Cyclohexen-1-ol, 5-methylene-6-(1-methylethenyl)-, acetate	54832-23-4	Ester	960	901	-6

2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	3155-71-3	Aldehyde	1731	1662	-4
Cedrene	11028-42-5	Hydrocarbon	21441	22127	3
4,7-methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate		Ester	9116	7594	-17
Dill ether isomer	74410-10-9	Ether	13438	11079	-18
4,7-methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate		Ester	379	263	-31
(-)-β-Pinene	18172-67-3	Hydrocarbon	4752	5164	9
Butylated Hydroxytoluene	128-37-0	Alcohol	118187	125346	6
Cyclohexene, 1,4,6,6-tetramethyl-	70092-37-4	Hydrocarbon	7462	7276	-3
trans-β-Ionone	79-77-6	Ketone	8640	8217	-5
Indan-1,3-diol monopropionate		Ester	2726	1977	-27
Propanoic acid, 2-methyl-, 2,3-dihydro-2-hydroxy-1H-indenyl ester		Ester	5942	5588	-6
Indan-1,3-diol monopropionate		Ester	46596	45541	-2
Indan-1,3-diol monopropionate		Ester	3061	2890	-6
Diphenyl ether	101-84-8	Ether	37198	49076	32
(4-tert-Butylphenyl)propan-2-one	81561-77-5	Ketone	71046	62513	-12
Lilial	80-54-6	Aldehyde	815185	768382	-6
β-methyl ionone	127-43-5	Ketone	1366785	1308739	-4
β-methyl ionone isomer	127-43-5	Ketone	285333	272554	-4
Benzoic acid, 2-(methylamino)-, methyl ester	85-91-6	Ester	3434	3390	-1
Benzoic acid, 2-hydroxy-, pentyl ester	2050-08-0	Ester	106398	136615	28
1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-, trans-	54832-81-4	Hydrocarbon	115973	108320	-7
β-ylangene		Hydrocarbon	569	1045	84
Eugenol	97-53-0	Alcohol	3515	3803	8
Naphthalene, 2-methoxy-	93-04-9	Ether	8777	10586	21
Rosacetol	90-17-5	Ester	67976	83421	23
4a-Methyl-1-methylene-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	53084-68-7		23084	31358	36
γ-Undecanolide	104-67-6	Furanone	13173	13283	1
Cyclopentadecanone	502-72-7	Ketone	2942	2956	0
Kharismal	24851-98-7	Ester	3950	3487	-12

4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane	79718-83-5	Hydrocarbon	4447	4390	-1
Total			**4696320**	**5143730**	**10**

The above fragrances can be grouped into different perfume notes, i.e. top, heart or base notes based on their volatility as explained above in the description. It was found that the intensity of the heart note fragrances on polyester T-shirts was increased most in two of the three softeners, compared to the top notes and base notes, when the samples were rinsed according to the invention with a softener composition contained a DNase (Tables 8 and 9). As shown in Table, 9 however, this softener also resulted in a substantial increase in the top notes that was close to the increase observed for the heart notes. The third softener (Table 10) showed the largest increase in intensities in the top notes, compared to the heart notes and base notes, when the samples were rinsed in the softener containing a DNase.

Table 8. Fragrance intensity of different notes on polyester T-shirts rinsed in Dun-let Dream of Freshness softener with or without DNase (SEQ ID NO: 1)

Perfume note	**Without DNase (relative intensity)**	**With DNase (relative intensity)**	**Fragrance increase with DNase (%)**
Top note	92710	75220	-19
Heart note	833162	1302489	56
Base note	1903226	2030286	7
Total	**2829097**	**3407996**	**20**

Table 9. Fragrance intensity of different notes on polyester T-shirts rinsed in Lenor Weichspüler Aprilfrisch softener with or without DNase (SEQ ID NO: 1)

Perfume note	**Without DNase (relative intensity)**	**With DNase (relative intensity)**	**Fragrance increase with DNase (%)**
Top note	347070	452786	30.5
Heart note	1581039	2167011	37.1
Base note	187359	218178	16.4
Total	**2115468**	**2837975**	**34**

Table 10. Fragrance intensity of different notes on polyester T-shirts rinsed in Bamseline Dugfrisk softener with or without DNase (SEQ ID NO: 1)

Perfume note	**Without DNase (relative intensity)**	**With DNase (relative intensity)**	**Fragrance increase with DNase (%)**
Top note	472933	646856	36.8
Heart note	3350673	3488255	4.1
Base note	872714	1008619	15.6
Total	**4696320**	**5143730**	**10**

5

Claims

1. Use of a polypeptide having DNase activity in a detergent composition for increasing binding of a perfume to a textile during a laundry process, for increasing perfume retention on a textile after wash, and/or for enhancing the effect of a perfume in a detergent composition, wherein the detergent composition comprises at least one perfume compound.

2. Use according claim 1, wherein the polypeptide having DNase activity is selected from the group consisting of:
   a) a polypeptide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% sequence identity to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3;
   b) a polypeptide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% sequence identity to the polypeptide of SEQ ID NO: 6;
   c) a polypeptide comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 4) and/or ASXNRSKG (SEQ ID NO: 5) and having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% sequence identity to any of the polypeptides of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 or SEQ ID NO: 30;
   d) a polypeptide comprising one or both of the motifs [V/I]PL[S/A]NAWK (SEQ ID NO: 31) and/or NPQL (SEQ ID NO: 32) and having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% sequence identity to any of the polypeptides of SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44 or SEQ ID NO: 45;
   e) a polypeptide comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 46) and/or [K/H/E]NAW (SEQ ID NO: 47) and having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% sequence identity to any of the polypeptides of SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ

ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60;

f) a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 61; and

g) a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the polypeptide of SEQ ID NO: 62.

3. Use according to claim 2, wherein the polypeptide having DNase activity has at least 80%, at least 85%, at least 90% or at least 95% sequence identity to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3; or at least 80%, at least 85%, at least 90% or at least 95% sequence identity to the polypeptide of SEQ ID NO: 6.

4. Use according to claim 3, wherein the polypeptide having DNase activity comprises a substitution at one or more positions corresponding to positions 4, 17, 19, 36, 38, 39, 40, 41, 45, 51, 53, 54, 55, 57, 64, 66, 67, 68, 69, 70, 71, 72, 74, 75, 77, 82, 83, 84, 85, 86, 88, 91, 99, 101, 105, 106, 115, 116, 135, 136, 138, 139, 140, 141, 151, 152, 153, 154, 162, 163, 164, 166, 168, 169, 173, 182, 183, 184, 185, 186, 189, 212 and 215 of SEQ ID NO: 1, and wherein the polypeptide has at least 80%, at least 85%, at least 90% or at least 95% sequence identity to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

5. Use according to claim 4, wherein the polypeptide having DNase activity comprises one or more substitutions selected from the group consisting of N4E, L17E, T19A, T19G, T19I, K36P, Q38P, S39V, S39R, A40P, A40H, L41T, L41H, V45H, L51G, K53T, K53P, G54P, A55P, N57H, E64A, E64Q, E64R, E64T, E64I, E64S, T66H, K67A, K67T, N68V, N68P, N68I, N68H, S69A, S69D, S69E, S69K, S69L, S69W, S69Y, S69Q, N70T, N70H, N70G, R71T, D72E, S74H, S74G, G75I, N77T, K82P, K82I, D83T, D83P, D83I, D83H, D83G, P84H, Q85T, Q85P, Q85H, K86T, K86P, K86H, G88P, G88H, A91P, W99T, A101W, K105E, K105N, K105T, K105D, S106T, S115T, L116I, Q135L, G136L, V138I, V138L, V138P, V138Q, L139A, N140R, N140L, N140A, G141L, F151R, D152Y, D152L, D152I, D152A, P153E, S154R, T162R, W163E, F164R, I166Y, I166R, K168N, F169R, F169E, A173I, A173R, A173T, S182R, N183E, D184I, K185Y, S186I, D189G, D189H, K212G, K212P and K215I, wherein numbering is based on SEQ ID NO: 1, and wherein the polypeptide has at least 80%, at least 85%, at least 90% or at least 95% sequence identity to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

6. Use according to any of the preceding claims, wherein the detergent composition is a laundry detergent composition or a fabric softener.

7. Use according to any of the preceding claims, wherein the polypeptide having DNase activity results in an increase in binding and/or retention of top note and/or heart note perfume compounds on a textile compared to base note compounds.

8. A method for increasing binding of a perfume to a textile during a laundry process and/or for increasing perfume retention on a textile in a laundry process after washing, the method comprising washing the textile with a detergent composition comprising at least one perfume compound and a polypeptide having DNase activity, wherein the polypeptide is as defined in any of claims 2-5.

9. A method for enhancing the effect of a perfume in a detergent composition, the method comprising preparing a detergent composition comprising at least one perfume compound and a polypeptide having DNase activity, wherein the polypeptide is as defined in any of claims 2-5.

10. A method for preparing a detergent composition with reduced perfume content but with maintained perfume effect after wash relative to a comparable composition without DNase, the method comprising mixing at least one perfume compound, a polypeptide having DNase activity and at least one detergent component, wherein the polypeptide is as defined in any of claims 2-5.

11. The method of any of claims 8-10, wherein the detergent composition is a laundry detergent composition or a fabric softener.

12. The method of any of claims 8-11, wherein the polypeptide having DNase activity results in an increase in binding and/or retention of top note and/or heart note perfume compounds compared to base note compounds.

13. A detergent composition comprising a polypeptide having DNase activity and at least one perfume compound, wherein the polypeptide is as defined in any of claims 2-5, and wherein the composition has an increased perfume effect relative to a comparable detergent composition without a polypeptide having DNase activity.

14. The detergent composition of claim 13, wherein the composition is a laundry detergent composition, and wherein the composition has increased perfume retention on a textile after wash relative to a comparable detergent composition without a polypeptide having DNase

activity; or wherein the composition is a fabric softener, and wherein the composition has increased perfume retention on a textile after rinse relative to a comparable fabric softener without a polypeptide having DNase activity.

15. A method for cleaning an item, wherein the item is preferably a textile, comprising the steps of:

a) contacting the item with a wash liquor comprising a detergent composition comprising a polypeptide having DNase activity and at least one perfume compound, wherein the polypeptide is as defined in any of claims 2-5, and optionally

b) rinsing the item.