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(54) Title: METHOD OF IMPROVING PROTEASE ACTIVITY

(57) Abstract: The invention provides a method of improving protease activity in a detergent composition, said method involving incorporation of from 0.1 to 40 wt.% of a saponin into said composition, wherein the detergent composition comprises from 0.0005 to 2.5 wt.% of a protease enzyme; wherein the saponin has a triterpenoid backbone, and one or more sugar moieties attached to the triterpenoid backbone; and to the use of saponin to improve protease activity in a detergent composition.

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METHOD OF IMPROVING PROTEASE ACTIVITY

Field of Invention

The invention concerns a method, in particular a method relating to a detergent composition comprising a protease.

Background of the Invention

Protease enzymes are a useful ingredient for detergent formulations, particularly for laundry detergents. As with any ingredient, especially expensive ingredients, improvement of activity of the ingredient is a problem to be solved.

This problem is particularly pronounced in laundry detergent formulations, especially liquid laundry detergent formulations.

Summary of the Invention

We have found that the incorporation of saponins in detergent compositions promotes protease activity and improves cleaning performance.

In one aspect the present invention provides a method of improving protease activity in a detergent composition, said method involving incorporation of from 0.1 to 40 wt.%, preferably from 0.5 to 25 wt.%, more preferably from 0.5 to 20 wt.%, most preferably from 0.5 to 15 wt.%, of a saponin into said composition,
wherein the detergent composition comprises from 0.0005 to 2.5 wt.%, preferably from 0.001 to 2 wt.%, more preferably from 0.005 to 1 wt.% of a protease enzyme;
wherein the saponin has a triterpenoid backbone, and one or more sugar moieties attached to the triterpenoid backbone.

The method involves improving protease activity in a detergent composition, preferably in a home care detergent composition, more preferably a laundry detergent composition.

In another aspect the invention provides the use of saponin to improve protease activity in a detergent composition preferably in a home care detergent composition, more preferably a laundry detergent composition, wherein the saponin has a triterpenoid backbone, and one or more sugar moieties attached to the triterpenoid backbone.

Preferably the protease is bacterial, fungal or mammalian in origin, more preferably bacterial or fungal in origin, most preferably bacterial in origin.

Preferably the protease is selected from the following group, serine, acidic, metallo- and cysteine proteases. More preferably the protease is a serine and/or acidic protease.

Preferably the protease is a serine protease. More preferably the serine protease is subtilisin type serine protease.

Preferably the saponin has at least two sugar moieties attached to the triterpenoid backbone.

Preferably the detergent composition comprises anionic and/or nonionic surfactant, more preferably the detergent composition comprises both anionic and nonionic surfactant.

A preferred detergent composition is a laundry detergent composition. Preferably the laundry detergent composition is a liquid, gel or a powder, more preferably the detergent is a liquid detergent.

The laundry detergent preferably comprises from 0.1 to 8 wt.% of an alkoxylated polyamine. Preferably the alkoxylated polyamine comprises an alkoxylated polyethylenimine, and/or alkoxylated polypropylenimine, more preferably the alkoxylation is ethoxylation or propoxylation or a mixture of both.

The laundry detergent composition preferably comprises from 0.1 to 8 wt.% of a soil release polymer, preferably a polyester soil release polymer.

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

Brief description of the figures

Figure 1 graph showing the improvement effect of different saponins on protease activity and the beneficial effect of the sugar moieties present on the triterpenoid backbone.

Figure 2 graph showing that the increased protease activity using saponin is also seen in scaled-up mini-bottle wash studies.

Figure 3 graph showing that the increased protease activity using saponin is also seen across many different proteases.

Figure 4 graph showing that the increased protease activity using saponin is also seen using two different saponins in wash conditions (100ml).

Detailed Description of the Invention

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

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

It is understood that any reference to a preferred ingredient of the detergent composition used in the method/use is envisaged to be combinable subject matter with any other preferred ingredient of the detergent composition disclosed herein.

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

The detergent composition can be applied to any suitable substrate, including but not limited to any substrate to which a home care composition would be applied, for example, textiles, crockery and cutlery. Particularly preferred substrates are textiles. Particularly preferred detergent compositions are laundry detergent compositions. Preferably the laundry

detergent composition is a liquid, gel or a powder, more preferably the detergent is a liquid detergent.

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

Protease

The detergent composition used in the method or use comprises from 0.0005 to 2.5 wt.%, preferably from 0.001 to 2 wt.%, more preferably from 0.005 to 1 wt.% of a protease enzyme.

The protease can be derived from fungal, bacterial or mammalian sources.

Preferably the protease is bacterial, fungal or mammalian in origin, more preferably bacterial or fungal in origin, most preferably bacterial in origin.

Preferably the protease is selected from the following group, serine, acidic, metallo- and cysteine proteases. More preferably the protease is a serine and/or acidic protease.

Preferably the protease is a serine protease. More preferably the serine protease is subtilisin type serine protease.

Protease enzymes hydrolyse bonds within peptides and proteins, in the cleaning context this leads to enhanced removal of protein or peptide containing stains. Serine proteases are preferred. Subtilase type serine proteases are more preferred. The term "subtilases" refers to a sub-group of serine protease according to Siezen et al., Protein Engng. 4 (1991) 719-737 and Siezen et al. Protein Science 6 (1997) 501 -523. Serine proteases are a subgroup of proteases characterized by having a serine in the active site, which forms a covalent adduct with the substrate. The subtilases may be divided into 6 sub-divisions, i.e. the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family.

Examples of subtilases are those derived from Bacillus species such as Bacillus lentus, B. licheniformis, B. alkalophilus, B. subtilis, B. amyloliquefaciens, B. pumilus and B. gibsonii described in; US7262042 and WO09/021867, and subtilisin lentus, subtilisin Novo, subtilisin

Carlsberg, subtilisin BPN', subtilisin 309, subtilisin 147 and subtilisin 168 described in WO 89/06279 and protease PD138 described in (WO 93/18140). Other useful proteases may be those described in WO 92/175177, WO 01/016285, WO 02/026024 and WO 02/016547. Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the Fusarium protease described in WO 89/06270, WO 94/25583 and WO 05/040372, and the chymotrypsin proteases derived from Cellumonas described in WO 05/052161 and WO 05/052146.

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

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

Suitable commercially available protease enzymes include those sold under the trade names names Carnival®, Relase®, Relase® Ultra, Savinase®, Savinase® Ultra, Coronase®, Coronase® Ultra, Kannase®, Liquanase®, Liquanase® Ultra, all could be sold as Ultra® or Evity® (Novozymes A/S).

Saponin

The detergent composition used in the method or use comprises from 0.1 to 40 wt.%, preferably from 0.25 to 30 wt.%, more preferably from 0.5 to 25 wt.%, more preferably from 0.5 to 20 wt.%, most preferably from 0.5 to 15 wt.%, of a saponin.

Saponins are natural compounds which contain sugar moieties bound to a fused system of non-aromatic 4, 5 and 6 membered rings. The ring system are preferably selected from the groups of triterpenoids, for example lanostane, dammarane, lupane, oleanane, ursane and hopane. An overview of these ring systems is provided in Natural Product Reports **27** (2010), 79-132 by R.A. Hill *et al.*

Saponins are discussed in “Saponins Used in Food and Agriculture” Plenum Press, New York 1996, G. R. Waller and K. Yamasaki (eds).

Saponins are preferably extracted from the seed, root, leaf, bulb, fruit, stem, pericarp, bark, tuber or flower of a plant. Saponin extraction and quantification is discussed in Food Research International **59** (2014) 16-40 by R. Sulaiman ey al. Extraction of saponins from agricultural products is discussed in WO2017/019599 and WO1999/053933.

Saponins may also be produced by bacteria (for example glycosylated hopanoids such as ribosylhopane) and marine organisms including sea cucumbers, starfish and sponges (Bahrami, Y., Zhang, W. & Franco, C.M. (2018) Marine Drugs 16: 423-453). Saponins may also be produced through biotechnology, either through enzymatic biosynthesis in vitro or by the engineering of microbial cell factories (Moses, T. et al. (2014) PNAS 28:1634-1639).

The saponin is preferably a Tea saponin (for example preferably derived from Camellia species), Soapnut saponin (for example preferably derived from Sapindus species), Quillaja Bark saponin or Escin (for example preferably derived from Aesculus species).

Preferably the saponin has a structure comprising a triterpenoid backbone and one or more sugar moieties attached to the triterpenoid backbone.

Saponins are listed in the Chemical Entities of Biological Interest (ChEBI) database, (Hastings, J., de Matos, P., Dekker, A., Ennis, M., Harsha, B., Kale, N., Muthukrishnan, V., Owen, G., Turner, S., Williams, M., and Steinbeck, C. (2013) The ChEBI reference database and ontology for biologically relevant chemistry: enhancements for 2013. *Nucleic Acids Res.*). For example, CHEBI:61778 - triterpenoid saponin.

Agricultural residues remaining after harvest may be suitable for extraction and supply of saponins. For example, sugarbeet leaves and skins may be further extracted to derive useful quantities of saponin. In China, the production and supply of tea saponin derived from the seed cake remaining after extraction of Camellia oleifera seeds for tea seed oil is well established. Alternatively, saponins may be extracted from parts of the plant collected from the wild (for example, protodioscin from Tribulus terrestris) or through managed plantations (for example from the bark of Quillaja saponaria).

Preferred Ingredients

Surfactant

The detergent composition used in the method or use preferably comprises surfactant (which includes a mixture of two or more surfactants). The composition comprises from 1 to 60 wt.%, preferably from 2.5 to 50 wt.%, more preferably from 4 to 40 wt.% of surfactant. Even more preferred levels of surfactant are from 6 to 40 wt.%, more preferably from 8 to 35 wt.%.

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

Examples of suitable anionic detergent compounds are rhamnolipids, sodium and potassium alkyl sulphates, especially those obtained by sulphating higher C_8 to C_{18} alcohols, produced for example from tallow or coconut oil, sodium and potassium alkyl C_9 to C_{20} benzene sulphonates, particularly sodium linear secondary alkyl C_{10} to C_{15} benzene sulphonates; and sodium alkyl glyceryl ether sulphates, especially those ethers of the higher alcohols derived from tallow or coconut oil and synthetic alcohols derived from petroleum.

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

The most preferred anionic surfactants are selected from: rhamnolipids, linear alkyl benzene sulphonate; alkyl sulphates; alkyl ether sulphates and mixtures thereof. Preferably the alkyl ether sulphate is a C_{12}-C_{14} n-alkyl ether sulphate with an average of 1 to 3EO (ethoxylate) units.

Sodium lauryl ether sulphate is particularly preferred (SLES). Preferably the linear alkyl benzene sulphonate is a sodium C_{11} to C_{15} alkyl benzene sulphonates. Preferably the alkyl sulphates is a linear or branched sodium C_{12} to C_{18} alkyl sulphates. Sodium dodecyl sulphate is particularly preferred, (SDS, also known as primary alkyl sulphate).

Rhamnolipids may preferably be mono-rhamnolipid rich (over 60%), di-rhamnolipid rich (over 60%), or a 40/60 to 60-40 mixture of mono- and di-rhamnolipid.

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

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

Suitable nonionic detergent compounds which may be used include, in particular, the reaction products of compounds having an aliphatic hydrophobic group and a reactive hydrogen atom, for example, aliphatic alcohols, acids or amides, especially ethylene oxide either alone or with propylene oxide. Preferred nonionic detergent compounds are the condensation products of aliphatic C_8 to C_{18} primary or secondary linear or branched alcohols with ethylene oxide. Alkyl polyglycosides (APG) are also preferred.

Most preferably the nonionic detergent compound is the alkyl ethoxylated non-ionic surfactant is a C_8 to C_{18} primary alcohol with an average ethoxylation of 7EO to 9EO units.

Preferably the surfactants used are saturated.

Soil release polymer

It is preferred that a soil release polymer is included.

The laundry detergent composition preferably comprises from 0.1 to 8 wt.% of a soil release polymer.

Preferred levels of soil release polymer range from 0.2 to 6 wt.%, more preferably from 0.5 to 5 wt.%, most preferably from 1 to 5 wt.%.

Preferably the soil release polymer is a polyester soil release polymer.

More preferably the polyester soil release polymer is a polyethylene and/or polypropylene terephthalate based soil release polymer, most preferably a polypropylene terephthalate based soil release polymer.

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

Alkoxylated polyamine

When the detergent composition is in the form of a laundry composition, it is preferred that an alkoxylated polyamine is included.

The laundry detergent preferably comprises from 0.1 to 8 wt.% of an alkoxylated polyamine.

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

The alkoxylated polyamine may be linear or branched. It may be branched to the extent that it is a dendrimer. The alkoxylation may typically be ethoxylation or propoxylation, or a mixture of both. Preferably the alkoxylated polyamine comprises an alkoxylated polyethylenimine, and/or alkoxylated polypropylenimine, more preferably the alkoxylation is ethoxylation or propoxylation or a mixture of both. Where a nitrogen atom is alkoxylated, a preferred average degree of alkoxylation is from 10 to 30, preferably from 15 to 25.

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

Additional Enzymes

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

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

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

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

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

Suitable lipases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful lipases include lipases from *Humicola* (synonym *Thermomyces*), e.g. from *H. lanuginosa* (*T. lanuginosus*) as described in EP 258 068 and EP 305 216 or from *H. insolens* as described in WO 96/13580, a *Pseudomonas* lipase, e.g. from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), *P. cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012), a *Bacillus* lipase, e.g. from *B. subtilis* (Dartois et al. (1993), Biochemica et Biophysica Acta, 1131, 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422).
Other examples are lipase variants such as those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079 and WO 97/07202, WO 00/60063.

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

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

Phospholipids, such as lecithin or phosphatidylcholine, consist of glycerol esterified with two fatty acids in an outer (sn-1) and the middle (sn-2) positions and esterified with phosphoric acid in the third position; the phosphoric acid, in turn, may be esterified to an amino-alcohol. Phospholipases are enzymes which participate in the hydrolysis of phospholipids. Several types of phospholipase activity can be distinguished, including phospholipases A_1 and A_2

which hydrolyze one fatty acyl group (in the sn-1 and sn-2 position, respectively) to form lysophospholipid; and lysophospholipase (or phospholipase B) which can hydrolyze the remaining fatty acyl group in lysophospholipid. Phospholipase C and phospholipase D (phosphodiesterases) release diacyl glycerol or phosphatidic acid respectively.

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

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

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

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

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

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

Enzyme Stabilizers

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

Further materials

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

Builders or Complexing Agents

The composition may comprise a builder.

Builder materials may be selected from 1) calcium sequestrant materials, 2) precipitating materials, 3) calcium ion-exchange materials and 4) mixtures thereof.

Examples of calcium sequestrant builder materials include alkali metal polyphosphates, such as sodium tripolyphosphate and organic sequestrants, such as ethylene diamine tetra-acetic acid.

Examples of precipitating builder materials include sodium orthophosphate and sodium carbonate.

Examples of calcium ion-exchange builder materials include the various types of water-insoluble crystalline or amorphous aluminosilicates, of which zeolites are well known representatives thereof, e.g. zeolite A, zeolite B (also known as zeolite P), zeolite C, zeolite X, zeolite Y and also the zeolite P-type as described in EP-A-0,384,070.

The composition may also contain 0-65 wt.% of a builder or complexing agent such as ethylenediaminetetraacetic acid, diethylenetriamine-pentaacetic acid, alkyl- or alkenylsuccinic acid, nitrilotriacetic acid or the other builders mentioned below. Many builders are also bleach-stabilising agents by virtue of their ability to complex metal ions.

Zeolite and carbonate (carbonate (including bicarbonate and sesquicarbonate) are preferred builders, with carbonates being particularly preferred.

The composition may contain as builder a crystalline aluminosilicate, preferably an alkali metal aluminosilicate, more preferably a sodium aluminosilicate. This is typically present at a level of less than 15 wt.%.

Aluminosilicates are materials having the general formula:

$$0.8\text{-}1.5\ M_2O.\ Al_2O_3.\ 0.8\text{-}6\ SiO_2,$$

where M is a monovalent cation, preferably sodium.

These materials contain some bound water and are required to have a calcium ion exchange capacity of at least 50 mg CaO/g. The preferred sodium aluminosilicates contain 1.5-3.5 SiO_2 units in the formula above. They can be prepared readily by reaction between sodium silicate and sodium aluminate, as amply described in the literature. The ratio of surfactants to alumuminosilicate (where present) is preferably greater than 5:2, more preferably greater than 3:1.

Alternatively, or additionally to the aluminosilicate builders, phosphate builders may be used. In this art the term 'phosphate' embraces diphosphate, triphosphate, and phosphonate species. Other forms of builder include silicates, such as soluble silicates, metasilicates, layered silicates (e.g. SKS-6 from Hoechst).

If a laundry detergent, then preferably the laundry detergent formulation is a non-phosphate built laundry detergent formulation, i.e., contains less than 1 wt.% of phosphate. Most preferably the laundry detergent formulation is not built i.e. contain less than 1 wt.% of builder.

Chelating Agent

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

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

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

Fluorescent Agent

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

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

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

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

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

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

Perfume

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

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

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

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

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

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

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

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

Some or all of the perfume may be encapsulated, typical perfume components which it is advantageous to encapsulate, include those with a relatively low boiling point, preferably those with a boiling point of less than 300, preferably 100-250 Celsius. It is also advantageous to encapsulate perfume components which have a low CLog P (ie. those which will have a greater tendency to be partitioned into water), preferably with a CLog P of less than 3.0. These materials, of relatively low boiling point and relatively low CLog P have been called the "delayed blooming" perfume ingredients and include one or more of the following materials: allyl caproate, amyl acetate, amyl propionate, anisic aldehyde, anisole, benzaldehyde, benzyl acetate, benzyl acetone, benzyl alcohol, benzyl formate, benzyl iso valerate, benzyl propionate, beta gamma hexenol, camphor gum, laevo-carvone, d-carvone, cinnamic alcohol, cinamyl formate, cis-jasmone, cis-3-hexenyl acetate, cuminic alcohol, cyclal c, dimethyl benzyl carbinol, dimethyl benzyl carbinol acetate, ethyl acetate, ethyl aceto acetate, ethyl amyl ketone, ethyl benzoate, ethyl butyrate, ethyl hexyl ketone, ethyl phenyl acetate, eucalyptol, eugenol, fenchyl acetate, flor acetate (tricyclo decenyl acetate) , frutene (tricyclco decenyl propionate) , geraniol, hexenol, hexenyl acetate, hexyl acetate, hexyl formate, hydratropic alcohol, hydroxycitronellal, indone, isoamyl alcohol, iso menthone, isopulegyl acetate, isoquinolone, ligustral, linalool, linalool oxide, linalyl formate, menthone, menthyl acetphenone, methyl amyl ketone, methyl anthranilate, methyl benzoate, methyl benyl acetate, methyl eugenol, methyl heptenone, methyl heptine carbonate, methyl heptyl ketone, methyl hexyl ketone, methyl phenyl carbinyl acetate, methyl salicylate, methyl-n-methyl anthranilate, nerol, octalactone, octyl alcohol, p-cresol, p-cresol methyl ether, p-methoxy acetophenone, p-methyl acetophenone, phenoxy ethanol, phenyl acetaldehyde, phenyl ethyl acetate, phenyl ethyl alcohol, phenyl ethyl dimethyl carbinol, prenyl acetate, propyl bornate, pulegone, rose oxide, safrole, 4-terpinenol, alpha-terpinenol, and /or viridine. It is commonplace for a plurality of perfume components to be present in a formulation. In the compositions of the present invention it is envisaged that there will be four or more, preferably five or more, more preferably six or more or even seven or more different perfume components from the list given of delayed blooming perfumes given above present in the perfume.

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

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

Shading Dye

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

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

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

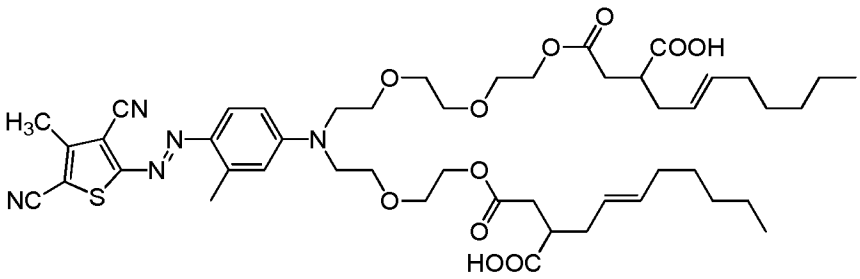
Preferred shading dye chromophores are azo, azine, anthraquinone, and triphenylmethane.

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

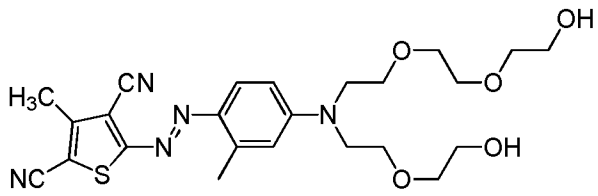
Shading dyes are discussed in WO 2005/003274, WO 2006/032327(Unilever), WO 2006/032397(Unilever), WO 2006/045275(Unilever), WO 2006/027086(Unilever),

WO 2008/017570(Unilever), WO 2008/141880 (Unilever), WO 2009/132870(Unilever), WO 2009/141173 (Unilever), WO 2010/099997(Unilever), WO 2010/102861(Unilever), WO 2010/148624(Unilever), WO 2008/087497 (P&G), WO 2011/011799 (P&G), WO 2012/054820 (P&G), WO 2013/142495 (P&G) and WO 2013/151970 (P&G).

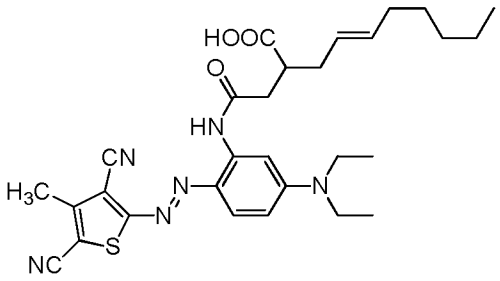
Mono-azo dyes preferably contain a heterocyclic ring and are most preferably thiophene dyes. The mono-azo dyes are preferably alkoxylated and are preferably uncharged or anionically charged at pH=7. Alkoxylated thiophene dyes are discussed in WO/2013/142495 and WO/2008/087497. Preferred examples of thiophene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



;



; and,



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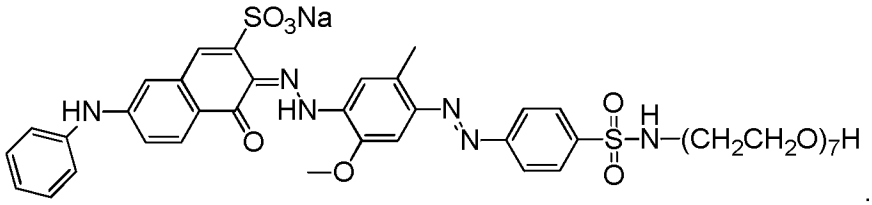
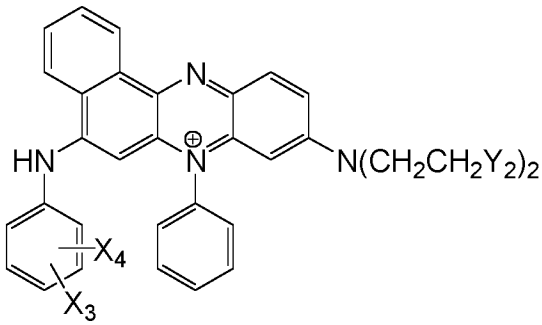
Bis-azo dyes are preferably sulphonated bis-azo dyes. Preferred examples of sulphonated bis-azo compounds are direct violet 7, direct violet 9, direct violet 11, direct violet 26, direct violet 31, direct violet 35, direct violet 40, direct violet 41, direct violet 51, Direct Violet 66, direct violet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

An example of an alkoxylated bis-azo dye is :



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

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



,

wherein:

X_3 is selected from: -H; -F; $-CH_3$; $-C_2H_5$; $-OCH_3$; and, $-OC_2H_5$;

X_4 is selected from: -H; $-CH_3$; $-C_2H_5$; $-OCH_3$; and, $-OC_2H_5$;

Y_2 is selected from: –OH; $-OCH_2CH_2OH$; $-CH(OH)CH_2OH$; $-OC(O)CH_3$; and, $C(O)OCH_3$.

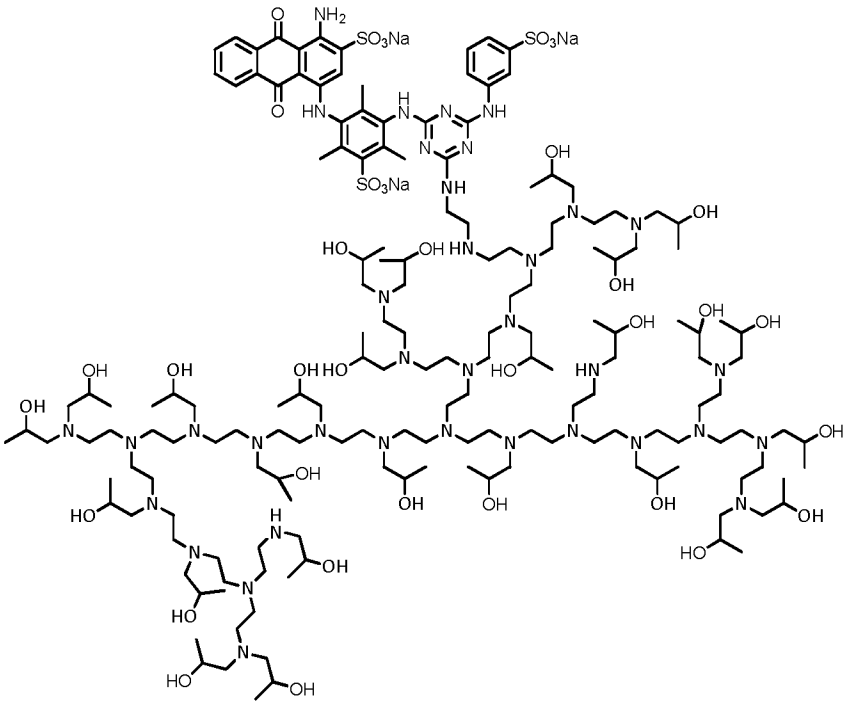
The shading dye is present is present in the composition in range from 0.0001 to 0.5 wt %, preferably 0.001 to 0.1 wt%. Depending upon the nature of the shading dye there are preferred ranges depending upon the efficacy of the shading dye which is dependent on class and particular efficacy within any particular class. As stated above the shading dye is a blue or violet shading dye.

A mixture of shading dyes may be used.

The shading dye is most preferably a reactive blue anthraquinone dye covalently linked to an alkoxylated polyethyleneimine. The alkoxylation is preferably selected from ethoxylation and

propoxylation, most preferably propoxylation. Preferably 80 to 95 mol% of the N-H groups in the polyethylene imine are replaced with iso-propyl alcohol groups by propoxylation. Preferably the polyethylene imine before reaction with the dye and the propoxylation has a molecular weight of 600 to 1800.

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



(Structure I).

Polymers

The composition may comprise one or more further polymers. A preferred detergent composition comprises from 0.1 to 20 wt.%, preferably from 0.5 to 15 wt.% or one or more polymers. Examples are carboxymethylcellulose, poly (ethylene glycol), poly(vinyl alcohol), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

Examples

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

Experimental methods:

Biochemical determination of protease activity

Substrate (N-Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, was dissolved in methanol to give a stock solution of 20mM. In Tris-HCl (pH 8.5, 50mM), a working stock solution 1mM of substrate was prepared. In a 96 well plate, 140µL of Tris-HCl (pH 8.5, 50mM), 20µL of stock solution of enzyme (to give a final concentration between 10-100ng/mL), 20 µL of corresponding saponin working-stock solution, and 20µL of substrate (final concentration of 100uM) were added, before incubating the plate in a Tecan Infinite plate reader at ambient temperature to monitor the release of p-nitroanilide for 15min at 405nm.

Wash studies in microtitre plates (MTP)

Cotton stained with aged blood CS01 (Centre for Testmaterials - Netherlands) was cut into empty 96-well microtitre plates and pre-wash readings taken for stain intensity. Using FH32 water, the stored formulation samples were diluted to give a final protease concentration of 5mg/L in FH32 water containing (1g/L) of a laundry detergent formulation containing 15 wt.% surfactant (anionic/nonionic mix) also comprising EPEI and polyester soil release polymer plus dosed saponin, and subsequently transferred (200µL) to the stains using a multi-channel pipette just prior to incubation at 40°C, with shaking at 200rpm for 1h. Following washing, the wash liquor was immediately removed using a multi-channel pipette, and the stain discs washed 3x with 200µL dH2O, before leaving overnight in a cupboard to dry. After drying, the stain plates were digitally scanned and the deltaE measured. This value is used to express cleaning effect and is defined as the colour difference between a white cloth and that of the stained cloth after being washed.

Mathematically, the definition of deltaE is:

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

wherein ΔL is a measure of the difference in darkness between the washed and white cloth; Δa and Δb are measures for the difference in redness and yellowness respectively between both cloths.

From this equation, it is clear that the lower the value of deltaE, the whiter the cloth will be. With regard to this colour measurement technique, reference is made to Commission International de l'Eclairage (CIE); Recommendation on Uniform Colour Spaces, colour difference equations, psychometric colour terms, supplement no. 2 to CIE Publication, no. 15, Colorimetry, Bureau Central de la CIE, Paris 1978.

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

SRI = 100 - deltaE.

The higher the SRI the cleaner the cloth, and it follows that SRI = 100 is white.

Wash studies in Mini-bottles

Cotton swatches stained with blood/milk/ink on cotton E116 (Centre for Testmaterials - Netherlands) together with cotton ballast were used for the mini-bottle washes. Using either Prenton water (FH26) or water prepared to FH32, containing 1g/L EU liquid formulation (15% surfactancy mixture of anionic/non-ionic, comprising EPEI and a polyester soil release polymer), protease was added to a concentration of 5mg/L in a total volume of 63mL which also contained the dosed saponin. Washes were carried out at 40°C, with shaking at 200rpm for 1h. Following washing, the stains were separated from the wash liquor and rinsed 3x in Prenton water, before leaving to dry overnight. The washed stains were then measured for stain intensity and the dSRI calculated as a measure of cleaning effectiveness.

Example 1 – showing the use of saponin to improve protease activity

Biochemical assays for protease activity were initially used in high-throughput microtitre plate format to enable different saponins at different doses to be screened for their effect on protease activity. The protease used was Carnival Evity protease – supplied by Novozymes. The saponin was tested at the concentrations listed in table 1 without protease, and no effect on the assay was seen by the saponin alone.

Table 1 shows that when using a concentration of 100ng/mL protease, a positive effect on (increasing) protease activity is observed from addition of saponin (in this instance Escin). In this case, a step-up in activity corresponding to 30% was noted from Escin concentrations >0.5μM, in particular at concentrations ≥10μM.

	Protease activity (Δabs/min @405nm)
Saponin conc. (μM)	Saponin + Protease
0	0.126 (±0.001)
0.01	0.125 (±0.001)
0.1	0.121 (±0.002)
0.5	0.126 (±0.005)
10	0.170 (±0.003)
25	0.160 (±0.0004)
50	0.159 (±0.001)
125	0.154 (±0.006)
250	0.158 (±0.001)
500	0.156 (±0.0006)

Table 1 Biochemical measurement of Carnival Evity protease (100ng/mL) activity in the presence of different doses of Escin saponin.

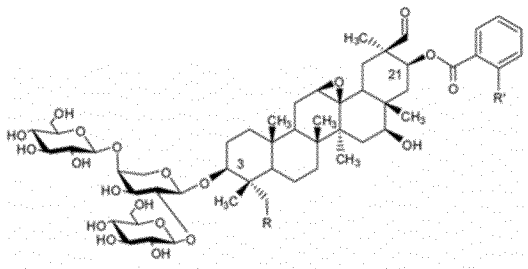
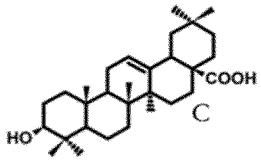
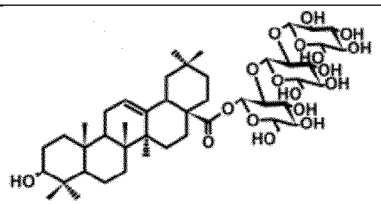
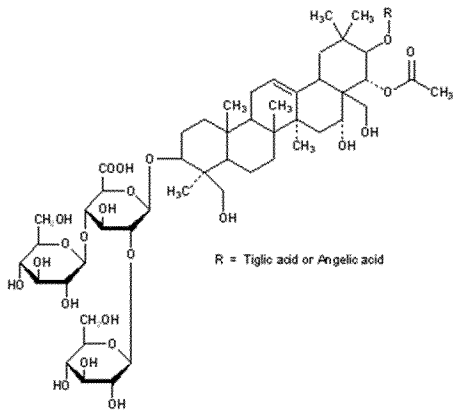
Example 2 – showing the use of different saponins to improve protease activity

Further examples of saponin molecules (in addition to Escin, Tea saponin and Quillaja Bark saponin) which are able to activate a laundry protease were subsequently found.

The effect of glycosylation appears key in conferring the activation benefits to molecules derived from oleanolic acid. The non-glycosylated triterpenoid oleanolic acid does not activate protease, the 3x and 4x glycosylated variants (OA-28 x 3Gly and OA-28 x4Gly) do clearly show these benefits (figure 1). Another saponin, Avenacin, was also shown to be highly activating of protease (figure 1).

The graph in figure 1 shows the effect of saponin at various levels for: a control (without protease); oleanolic acid (a triterpenoid backbone without sugar moieties); and saponins within the definition of the invention: the 3x and 4x glycosylated variants of oleanolic acid (OA-28 x 3Gly and OA-28 x4Gly), escin and avenacin. The level of saponin at 0μm corresponds to the protease only activity. The saponins within the scope of the invention improved the protease activity by a statistically significant amount at a saponin level of 5μM and above.

Similarity between structures of effective saponin activators of protease are observed (figure 1); with common structural features of a triterpenoid backbone to which are linked sugar moieties.

Avenacin (showing major molecular structures in the Avenacin extract)	
Oleanolic acid	
Oleanolic acid-28-x 3 Gly	
Oleanolic acid-28-x 4 Gly	Structure is similar to Oleanolic acid-28-x 3 Gly (above) but with glycosylation to a 4 sugar chain – the material used was as Oleanolic acid-28-x 3 Gly with an extra identical sugar added to make Oleanolic acid-28-x 4 Gly
Escin (showing major molecular structures in the Escin extract)	

Example 3 – showing the activity of saponin on cleaning performance

A similar type of dose response experiment to the biochemical testing is displayed in table 2, this time showing the effect of Tea saponin on cleaning performance of Carnival Evity protease towards an aged blood stain (on cotton fabric). This experiment provides a link between the previously shown biochemical activity measurements for protease (table 1) and transfers them to a cleaning performance measurement with wash relevant levels of protease – also in microtitre plates. The activity boosting effect (improved cleaning) appears when increasing the concentration of saponin from 1mM to 5mM. Typical wash levels of protease (5mg/L) were used in this experiment.

Table 2 also shows that increasing the dose of saponin does not result in improved cleaning from just the saponin alone. A relatively constant cleaning performance due to saponin (~5 dSRI) is observed at concentrations of up to 25mM (table 2). The 'add-on' effect of boosted protease activity is noted when using Tea saponin >1mM, which leads to increased values for dSRI (~18).

	Cleaning performance (dSRI)	
Saponin conc. (mM)	**Saponin only**	**Saponin + Protease**
0	4.95 (±0.94)	6.00 (±1.06)
0.01	5.06 (±0.83)	5.85 (±0.97)
0.1	4.62 (±0.95)	6.95 (±1.87)
0.5	5.78 (±1.71)	6.16 (±1.04)
1	5.50 (±0.63)	6.29 (±1.57)
5	4.99 (±1.70)	18.49 (±3.13)
25	6.88 (±1.09)	18.33 (±2.69)

Table 2 MTP wash study using wash-levels of Carnival Evity protease (5mg/L) towards aged blood stain (CS01), with varying dose of Tea saponin added. A 5mM dose of saponin corresponds to 0.6 wt.% addition in a formulation.

Example 4 – showing the activity of saponin on scaled-up cleaning performance

The same effect of increased protease activity is observed when scaling-up the wash study from MTP to mini-bottles (figure 2).

Figure 2 shows that the saponin effect on increased protease activity is also seen in scaled-up mini-bottle wash studies (63ml). The figure is a graph showing the increased protease activity when 5mM of saponin is used. A statistically significant improvement in protease activity is demonstrated at this saponin level.

The effects of this improved cleaning can be visualised in de-colouration of the stain E116 (blood/milk/ink). With a liquid:cloth ratio of 53:1 used, this highlights the fact that saponins could be used to increase the cleaning performance of proteases in dilute solution, which further extends the potential application of this technology.

The reproducibility of this work in mini-bottle washes was also confirmed by protease activity boosting using Escin. For Escin, an even lower concentration of 1mM was found to give the same improved effect.

Example 5 – showing the activity of saponin on different proteases

This example shows that many proteases are found to be activated by saponins. This includes a range of commercial bacterial laundry proteases, as well as other bacterial derived protease (Streptomyces) and proteases of fungal origin (Aspergillus and Rhizopus). An example of a mammalian protease activated by escin is also shown (Bovine pancreas). Interestingly proteases of different active site configuration/mechanism can also be activated by saponin (examples shown are subtilisin-type alkaline proteases with a catalytic serine residue, as well as an acidic protease) (figure 3).

Figure 3 shows that the increased protease activity using saponin is also seen across many different proteases, both from origin and from protease type.

Example 6 – showing the activity using different saponins at scaled-up mini-bottle (100ml) wash performance across different time points of the wash

With the improvements in protease-catalysed cleaning shown for the addition of Escin and Tea saponin, a follow-up study looked into these cleaning improvements over the time-course of a mini-bottle wash (100mL volume). In this study, the previously optimised levels of saponin used for the activity boosting effect on protease were applied (Escin = 1mM, Tea saponin = 5mM) – see example 4. Multiple swatches of the same E116 stain were included within the 100mL wash, with extraction of 1 piece of stained fabric every 10 minutes. The results (figure 4) show very clearly the reproducible benefit of saponin addition with approximately +5 SRI units observed for Escin and +10 SRI units observed for Tea saponin addition at concentrations used.

In summary the experiments included herein support the technical effect of improved protease activity across the many different proteases and saponins tested.

CLAIMS

1. A method of improving protease activity in a detergent composition, said method involving incorporation of from 0.1 to 40 wt.%, preferably from 0.5 to 25 wt.%, more preferably from 0.5 to 20 wt.%, most preferably from 0.5 to 15 wt.%, of a saponin into said composition,
wherein the detergent composition comprises from 0.0005 to 2.5 wt.%, preferably from 0.001 to 2 wt.%, more preferably from 0.005 to 1 wt.% of a protease enzyme;
wherein the saponin has a triterpenoid backbone, and one or more sugar moieties attached to the triterpenoid backbone.

2. A method according to claim 1, wherein the detergent composition, is a home care detergent composition, preferably a laundry detergent composition.

3. Use of saponin to improve protease activity in a detergent composition preferably in a home care detergent composition, more preferably a laundry detergent composition, wherein the saponin has a triterpenoid backbone, and one or more sugar moieties attached to the triterpenoid backbone.

4. A method or use according to any one of claims 1 to 3, wherein the protease is bacterial, fungal or mammalian in origin, preferably bacterial or fungal in origin, most preferably bacterial in origin.

5. A method or use according to any one of claims 1 to 4, wherein the protease is selected from the following group: serine, acidic, metallo- and cysteine proteases, preferably the protease is a serine and/or acidic protease.

6. A method or use according to any one of claims 1 to 5, wherein the protease is a serine protease, preferably the serine protease is subtilisin type serine protease.

7. A method or use according to any one of claims 1 to 6, wherein the saponin has at least two sugar moieties attached to the triterpenoid backbone.

8. A method or use according to any one of claims 1 to 7, wherein the detergent composition comprises from 1 to 60 wt.%, preferably from 2.5 to 50 wt.%, more preferably from 4 to 40 wt.%, most preferably from 8 to 35 wt.% of a surfactant, said surfactant not including saponin.

9. A method or use according to any one of claims 1 to 8, wherein the detergent composition comprises anionic and/or nonionic surfactant, preferably comprising both anionic and nonionic surfactant.

10. A method or use according to any one of claims 1 to 9, wherein the composition is laundry detergent composition, preferably the laundry detergent composition is a liquid, gel or a powder, more preferably the laundry detergent is a liquid detergent.

11. A method or use according to any one of claims 1 to 10, wherein the laundry detergent composition comprises an alkoxylated polyamine, preferably at a level of from 0.1 to 8 wt.%, more preferably from 0.2 to 6 wt.%, most preferably from 0.5 to 5 wt.%, preferably the alkoxylated polyamine is an alkoxylated polyethylenimine, and/or alkoxylated polypropylenimine, more preferably the alkoxylation is ethoxylation or propoxylation or a mixture of both.

12. A method or use according to any one of claims 1 to 11, wherein the laundry detergent composition comprises a soil release polymer, preferably at a level of from 0.1 to 8 wt.%, more preferably from 0.2 to 6 wt.%, most preferably from 0.5 to 5 wt.%, preferably the soil release polymer is a polyester soil release polymer.

13. A method or use according to any one of claims 1 to 12, additionally comprising a further enzyme selected from the group consisting of: lipases, cellulases, alpha-amylases, peroxidases/oxidases, pectate lyases, and/or mannanases.

Figure 1

Graph showing the improvement effect of different saponins on protease activity and the beneficial effect of the sugar moieties present on the triterpenoid backbone

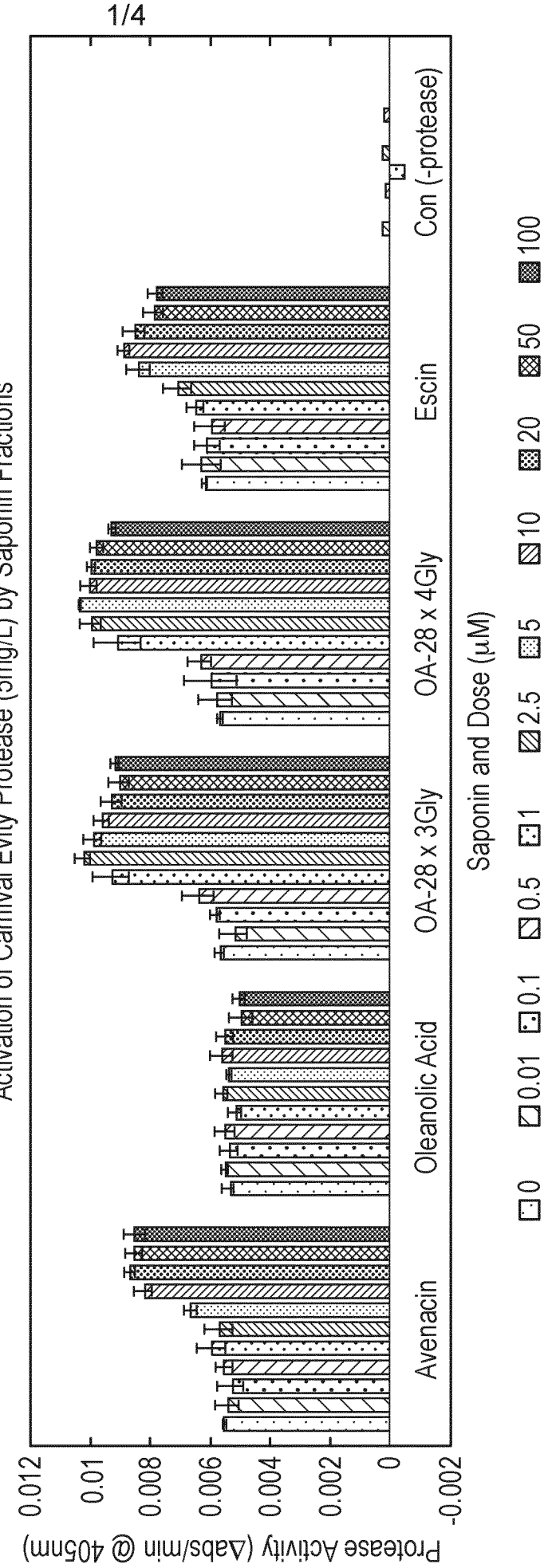


Figure 2

Graph showing that the increased protease activity using saponin is also seen in scaled-up mini-bottle wash studies

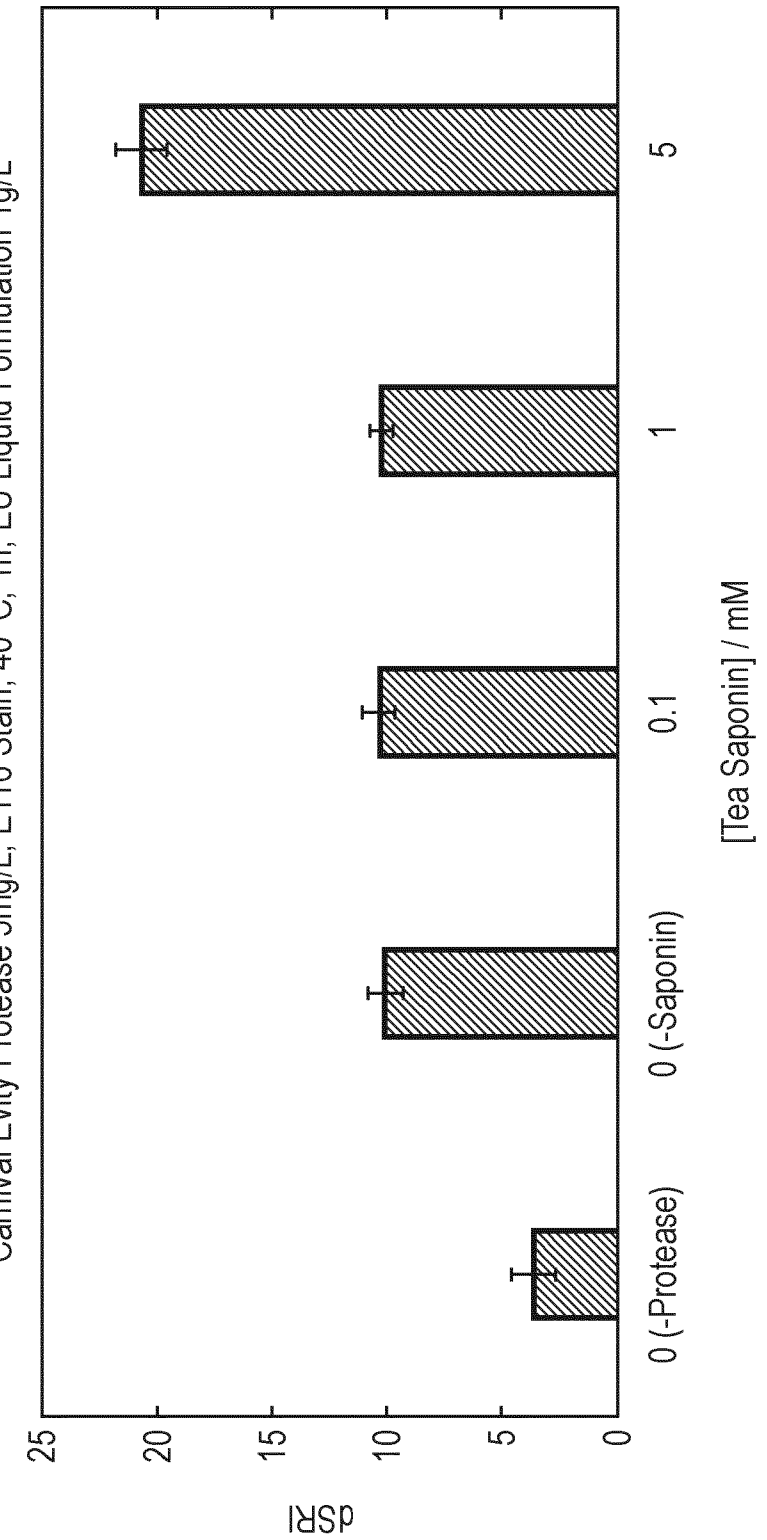


Figure 3

Graph showing that the increased protease activity using saponin is also seen across many different proteases

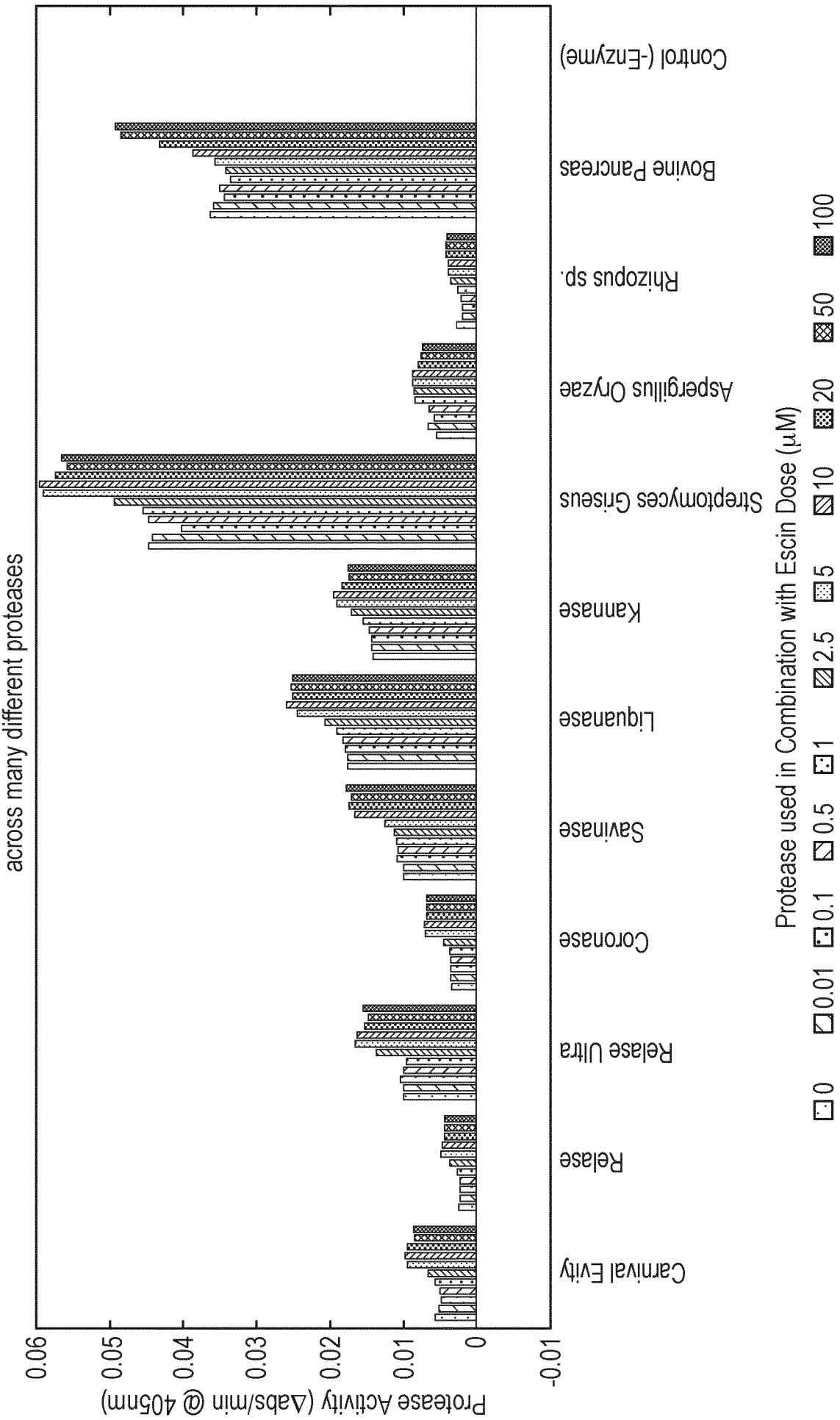
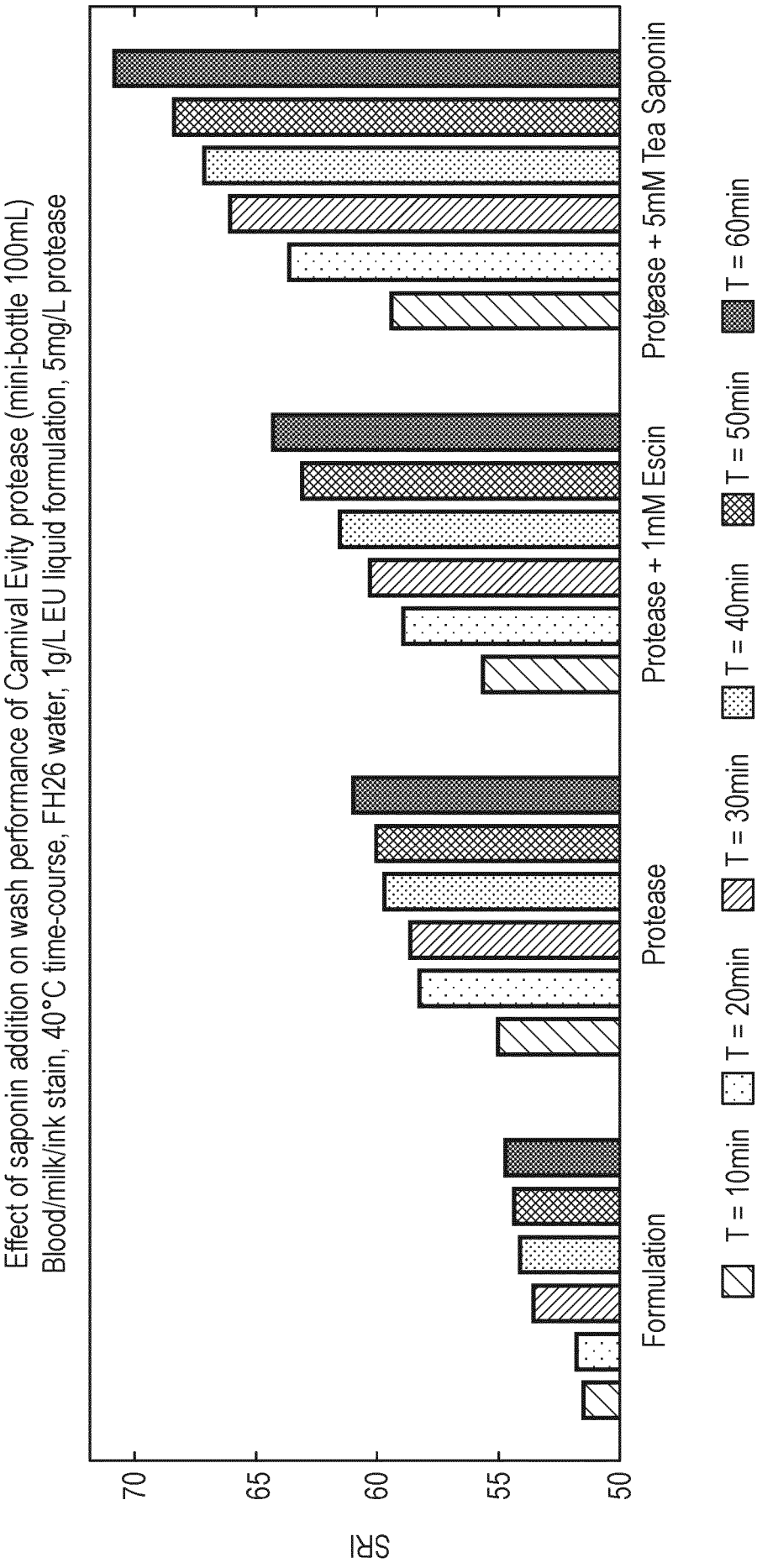


Figure 4

Graph showing that the increased protease activity using saponin is also seen using two different saponins in wash conditions (100mL)



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/065145

A. CLASSIFICATION OF SUBJECT MATTER
INV. C11D3/386 C11D3/382
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99/57236 A1 (PROCTER & GAMBLE [US]; MITRA ASHOKE KUMAR [US] ET AL.) 11 November 1999 (1999-11-11) examples page 6, paragraph 3 - page 7, paragraph 2; claims	1-13
A	CN 108 998 275 A (XU HONGHUA) 14 December 2018 (2018-12-14) examples 1,2 claims	1-13
A	DE 20 2006 007594 U1 (REMSGOLD CHEMIE GMBH & CO [DE]) 13 July 2006 (2006-07-13) examples 4,7,9 page 4, paragraph 20 - paragraph 21	1-13

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 August 2021	10/09/2021

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/065145

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 2015/275144 A1 (HULSKOTTER FRANK [DE] ET AL) 1 October 2015 (2015-10-01) claims examples page 19, paragraph 176 - paragraph 185 page 16, paragraph 130 - paragraph 135 -----	11,12

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Information on patent family members

International application No
PCT/EP2021/065145

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