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(54) **Title**: DETERGENT COMPOSITION AND USE OF DETERGENT COMPOSITION

(57) **Abstract**: The present invention concerns the use of an enzyme exhibiting lactase phlorizin hydrolase activity for removing or releasing a flavonoid stain from a textile, a dish or a hard surface having a flavonoid stain, a composition comprising the enzyme and a method for removing or releasing a flavonoid stain from a textile, a dish or a hard surface having a flavonoid stain.

Detergent Composition and Use of Detergent Composition

Reference to a Sequence Listing

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

Field of the Invention

The present invention concerns a composition comprising an enzyme exhibiting hydrolase activity, use of the composition, a method for removing stains by use of the composition and a textile treated according to the method.

Background of the Invention

Anthocyanins are a group of reddish water-soluble pigments that are very widespread in the plant kingdom. Many fruits, vegetables, and flowers owe their attractive coloration to this group of water-soluble compounds, which exist in the cell sap. The structure of the anthocyanin group is fairly well known.

Flavonoids are a major class of plant phenolics found widely in fruits and vegetables, tea and red wine. Although the range of plant phenolics is diverse, the majority of the flavonoids are glycosylated.

Glycoside hydrolases (EC 3.2.1.-) are a widespread group of enzymes which hydrolyse the glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety. Glycoside hydrolases (GH) are divided is several subgroups where family GH1 has been reported to exhibit various activities: b-glucosidase (EC 3.2.1.21); b-galactosidase (EC 3.2.1.23); b-mannosidase (EC 3.2.1.25); b-glucuronidase (EC 3.2.1.31); b-D-fucosidase (EC 3.2.1.38); phlorizin hydrolase (EC 3.2.1.62); exo-b-1,4-glucanase (EC 3.2.1.74); 6-phospho-b-galactosidase (EC 3.2.1.85); 6-phospho-b-glucosidase (EC 3.2.1.86); strictosidine b-glucosidase (EC 3.2.1.105); lactase (EC 3.2.1.108); amygdalin b-glucosidase (EC 3.2.1.117); prunasin b-glucosidase (EC 3.2.1.118); raucaffricine b-glucosidase (EC 3.2.1.125); thioglucosidase (EC 3.2.1.147); b-primeverosidase (EC 3.2.1.149); isoflavonoid 7-O-b-apiosyl-b-glucosidase (EC 3.2.1.161); hydroxyisourate hydrolase (EC 3.-.-.-); b-glycosidase (EC 3.2.1.-).

The enzyme lactase phlorizin hydrolase (LPH) (EC 3.2.1.23) belonging to the GH1 family has been shown to hydrolyse the following flavonoids: quercetin-4P-glucoside, quercetin-3-glucoside, quercetin-3,4P-diglucoside,3P-methylquercetin-3-glucoside, genistein-7-glucoside and daidzein-7-glucoside.

International patent application WO 2006/12904 discloses functional polypeptides encoded by polynucleotides comprised in the mRNA of *Diplodia gossypina, syn. Botryospaeria rhodina*

deposited under deposit accession number CBS 247.96. The sequence SEQ ID NO: 34 is an enzyme exhibiting lactase phlorizin hydrolase activity, although describes a beta-glucosidase in the publication.

A.J. Day et al./FEBS Letters 468 (2000) 166-170) reports that Lactase Phlorizin Hydrolase purified from sheep small intestine was capable of hydrolyzing a range of flavonol and isoflavone glycosides.

Summary of the Invention

The present invention concerns the use of an enzyme exhibiting hydrolase activity for removing or releasing a flavonoid stain from a textile having a flavonoid stain.

In addition, the invention concerns a detergent composition comprising a surfactant and an enzyme exhibiting hydrolase activity.

The invention further concerns a method for removing or releasing a flavonoid stain from a textile having a flavonoid stain which method comprises exposing the textile to an aqueous solution of an enzyme exhibiting lactase phlorizin hydrolase activity and a textile treated by the method.

Brief Description of the Figures

Figure 1 shows the delta remission data at 460 nm after a TOM wash (example 1).

Figure 2 shows removal of color from flavonoid containing fruit extract (example 2).

Definitions

Enzyme Detergency benefit: The term “enzyme detergency benefit” is defined herein as the advantageous effect an enzyme may add to a detergent compared to the same detergent without the enzyme. Important detergency benefits which can be provided by enzymes are stain removal with no or very little visible soils after washing and/or cleaning, prevention or reduction of redeposition of soils released in the washing process (an effect that also is termed anti-redeposition), restoring fully or partly the whiteness of textiles which originally were white but after repeated use and wash have obtained a greyish or yellowish appearance (an effect that also is termed whitening). Textile care benefits, which are not directly related to catalytic stain removal or prevention of redeposition of soils, are also important for enzyme detergency benefits. Examples of such textile care benefits are prevention or reduction of dye transfer from one fabric to another fabric or another part of the same fabric (an effect that is also termed dye transfer inhibition or anti-backstaining), removal of protruding or broken fibers from a fabric surface to decrease pilling tendencies or remove already existing pills or fuzz (an effect that also is termed anti-pilling), improvement of the fabric-softness, colour clarification of the fabric and removal of particulate soils

which are trapped in the fibers of the fabric or garment. Enzymatic bleaching is a further enzyme detergency benefit where the catalytic activity generally is used to catalyze the formation of bleaching components such as hydrogen peroxide or other peroxides.

Delta enzyme performance value: The term “Delta enzyme remission value” or “delta Enz” or “ΔEnz” is defined herein as the result of a reflectance or remission measurement at 460 nm. The swatch is measured with one swatch of similar color as background, preferably a swatch from a repetition wash. A swatch representing each swatch type is measured before wash. The delta enzyme performance value is the remission value of the swatch washed in detergent with an enzyme present minus the remission value of a similar swatch washed in a detergent without enzyme present.

Dish washing composition: The term “dish washing composition" refers to compositions intended for cleaning dishes, table ware, pots, pans, cutlery and all forms of compositions for cleaning hard surfaces areas in kitchens. The present invention is not restricted to any particular type of dish wash composition or any particular detergent.

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). The textile or fabric may be in the form of knits, wovens, denims, non-wovens, felts, yarns, and toweling. 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.

Hard surface cleaning: The term “Hard surface cleaning” is defined herein as cleaning of hard surfaces wherein hard surfaces may include floors, tables, walls, roofs etc. as well as surfaces of hard objects such as cars (car wash) and dishes (dish wash). Dish washing includes but are not limited to cleaning of plates, cups, glasses, bowls, cutlery such as spoons, knives, forks, serving utensils, ceramics, plastics, metals, china, glass and acrylics.

Improved wash performance: The term “improved wash performance” is defined herein as the detergent or ADW composition comprising LPH enzyme displaying an increased wash performance relative to the wash performance of a detergent or ADW composition without LPH enzyme, e.g. by increased stain removal. The term “wash performance” includes wash performance in laundry but also e.g. in hard surface cleaning such as automated dish wash (ADW).

Detergent Composition: the term “detergent composition” refers to compositions that find use in the removal of undesired compounds from items to be cleaned, such as textiles, dishes, and hard surfaces. The detergent composition may be used to e.g. clean textiles, dishes and hard surfaces for both household cleaning and industrial cleaning. The terms encompass any materials/compounds selected for the particular type of cleaning 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 (e.g., liquid and/or solid laundry detergents and fine fabric detergents; hard surface cleaning formulations, such as for glass, wood, ceramic and metal counter tops and windows; carpet cleaners; oven cleaners; fabric fresheners; fabric softeners; and textile and laundry pre-spotters, as well as dish wash detergents). In addition to containing a bifunctional compound 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, peroxidaes, haloperoxygenases, catalases and mannanases, or any mixture thereof), and/or components 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, transferase(s), hydrolytic enzymes, oxido reductases, bluing agents and fluorescent dyes, antioxidants, and solubilizers.

Dish wash: The term “dish wash" refers to all forms of washing dishes, e.g. by hand or automatic dish wash (ADW). Washing dishes includes, but is not limited to, the cleaning of all forms of crockery such as plates, cups, glasses, bowls, all forms of cutlery such as spoons, knives, forks and serving utensils as well as ceramics, plastics, metals, china, glass and acrylics.

Hard surface cleaning: The term “Hard surface cleaning” is defined herein as cleaning of hard surfaces wherein hard surfaces may include floors, tables, walls, roofs etc. as well as surfaces of hard objects such as cars (car wash) and dishes (dish wash). Dish washing includes but are not limited to cleaning of plates, cups, glasses, bowls, cutlery such as spoons, knives, forks, serving utensils, ceramics, plastics, metals, china, glass and acrylics.

Hard surface cleaning: The term "Hard surface cleaning" is defined herein as cleaning of hard surfaces, wherein hard surfaces may include floors, tables, walls, roofs etc. as well as surfaces of hard objects such as cars (car wash).

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.

Detailed Description of the Invention

The present invention concerns the use of an enzyme exhibiting hydrolase activity for removing or releasing a flavonoid stain from a textile having a flavonoid stain, from a dish having a flavonoid stain or from a hard surface having a flavonoid stain. Flavonoid stains are stains selected from the group consisting of fruit stains, berry stains and/or vegetable stains. The stains can be selected from the group consisting of stains of strawberry, blueberry, cherry, juice, jam, and forest fruit jam.

The enzyme may be selected from the GH1 familiy comprised by the enzyme classification EC 3.2.1.-. The enzyme of the invention may further exhibits lactase activity (EC 3.2.1.108). In one embodiment of the invention the enzyme exhibits lactase phlorizin hydrolase activity (EC 3.2.1.62) and may belong to the enzyme classisification (EC 3.2.1.23).

The enzyme lactase phlorizin hydrolase has been shown to hydrolyze some flavonoid compounds by cleaving the glucoside unit. The inventors have found that lactase phlorizin hydrolase can be used for removing or releasing flavonoid stain from textiles, dishes or hard surfaces.

The enzyme of the invention exhibits exhibiting lactase phlorizin hydrolase activity and comprises an amino acid sequence which has at least 90% identity with amino acids 1 to 603 of SEQ ID NO: 1. In one embodiment, the enzyme has at least 95% identity with amino acids 1 to 603 of SEQ ID NO: 1. In one embodiment, the enzyme has at least 96% identity with amino acids 1 to 603 of SEQ ID NO: 1. In one embodiment, the enzyme has at least 97% identity with amino acids 1 to 603 of SEQ ID NO: 1. In one embodiment, the enzyme has at least 98% identity with amino acids 1 to 603 of SEQ ID NO: 1. In one embodiment, the enzyme has at least 99% identity with amino acids 1 to 603 of SEQ ID NO: 1. In one embodiment, the enzyme has at least 100% identity with amino acids 1 to 603 of SEQ ID NO: 1.

In one embodiment, the enzyme exhibiting lactase phlorizin hydrolase activity comprises amino acids 1 to 603 of SEQ ID NO: 1.

The enzyme of the invention may be used as an aqueous solution of the enzyme. The pH of the aqueous solution may be in the range of 7 to 10, preferably 7 to 9 such as 7.5.

In one embodiment of the invention, the enzyme may be used in combination with at least one additional enzyme. The additional enzyme can be selected from the group consisting of protease, lipase, cutinase, amylase, carbohydrase, cellulase, chlorophyllase, pectate lyase, pectinase, mannanase, arabinase, galactanase, and/or xylanase. In one embodiment of the invention the additional enzyme is pectate lyase.

The capability of the enzyme to remove or release stains from textile, dish or hard surfaces can be evaluated by the use of the TOM assay. In one embodiment ΔEnz is at least 1 when measured with the TOM assay. In one embodiment ΔEnz is at least 1.5, ΔEnz is at least 2.0, ΔEnz is at least 2.5, ΔEnz is at least 3.0, ΔEnz is at least 3.5, ΔEnz is at least 4.0, ΔEnz is at least 4.5, ΔEnz is at least 5.0, ΔEnz is at least 5.5, ΔEnz is at least 6.0, ΔEnz is at least 6.5, ΔEnz is at least 7.0, ΔEnz is at least 7.5, ΔEnz is at least 8.0 or ΔEnz is at least 8.5, ΔEnz is at least 9.0, ΔEnz is at least 9.5 or ΔEnz is at least 10.0 when measured with the TOM assay.

In one embodiment of the invention, the textile is made of cotton fabric. And the ΔEnz is at least 1 when measured with the TOM assay. In one embodiment ΔEnz is at least 1.5, ΔEnz is at least 2.0, ΔEnz is at least 2.5, ΔEnz is at least 3.0, ΔEnz is at least 3.5, ΔEnz is at least 4.0, ΔEnz is at least 4.5, ΔEnz is at least 5.0, ΔEnz is at least 5.5, ΔEnz is at least 6.0, ΔEnz is at least 6.5, ΔEnz is at least 7.0, ΔEnz is at least 7.5, ΔEnz is at least 8.0 or ΔEnz is at least 8.5, ΔEnz is at least 9.0, ΔEnz is at least 9.5 or ΔEnz is at least 10.0 when measured with the TOM assay.

In one embodiment of the invention, the enzyme is used at a temperature in the range of 10-60°C, such as 15-50°C, or 30-45°C.

The invention further concerns a composition comprising a surfactant and an enzyme exhibiting hydrolase activity. The composition can be a detergent composition, a dish washing composition, such as an ADW composition or a composition for cleaning of hard surfaces.

The enzyme may be selected from the GH1 family comprised by the enzyme classification EC 3.2.1.-. The enzyme of the invention may further exhibits lactase activity (EC 3.2.1.108). In one embodiment of the invention the enzyme exhibits lactase phlorizin hydrolase activity (EC 3.2.1.62) and may belong to the enzyme classification (EC 3.2.1.23).

The enzyme of the invention exhibits exhibiting lactase phlorizin hydrolase activity and comprises an amino acid sequence which has at least 90% identity with SEQ ID NO: 1. In one embodiment the enzyme has at least 95% identity with SEQ ID NO: 1. In one embodiment the enzyme has at least 96% identity with SEQ ID NO: 1. In one embodiment the enzyme has at least 97% identity with SEQ ID NO: 1. In one embodiment the enzyme has at least 98% identity with

SEQ ID NO: 1. In one embodiment, the enzyme has at least 99% identity with SEQ ID NO: 1. In one embodiment, the enzyme has at least 100% identity with SEQ ID NO: 1.

In one embodiment, the enzyme exhibiting lactase phlorizin hydrolase activity comprises amino acids 1 to 603 of SEQ ID NO: 1.

The enzyme lactase phlorizin hydrolase has been shown to hydrolyze some flavonoid compounds by cleaving the glucoside unit. The inventors have found that a compound comprising a surfactant and a lactase phlorizin hydrolase can be used for removing or releasing flavonoid stain from textiles, dishes or hard surfaces. The composition of the invention may further comprise hydrotropes, builders, co-builders, a bleaching systems, polymers, fabric hueing agents, mediators and/or enzymes.

In one embodiment of the invention, the composition may comprise at least one additional enzyme. The additional enzyme can be selected from the group consisting of protease, lipase, cutinase, amylase, carbohydrase, cellulase, chlorophyllase, pectate lyase, pectinase, mannanase, arabinase, galactanase, and/or xylanase. In one embodiment of the invention the at least one additional enzyme is pectate lyase.

In one embodiment of the invention, the composition comprises an enzyme exhibiting hydrolase activity in a concentration wherein the concentration of the enzyme exhibiting hydrolase activity is in the range of 0.05-1.0 % w/w enzyme per detergent composition. In one embodiment of the invention the concentration of the enzyme exhibiting hydrolase activity is in the range of 0.1-0.9 % w/w enzyme per detergent composition, in the range of 0.2-0.8 % w/w enzyme per detergent composition, in the range of 0.3-0.7 % w/w enzyme per detergent composition or in the range of 0.4-0.6 % w/w enzyme per detergent composition.

The composition according to invention may further comprise an enzyme exhibiting pectin lyase activity, which is present in a concentration in the range of 0.05-1.0 % w/w enzyme per detergent composition. In one embodiment the concentration of the invention the enzyme exhibiting pectin lyase activity is in the range of 0.1-0.9 % w/w enzyme per detergent composition, in the range of 0.2-0.8 % w/w enzyme per detergent composition, in the range of 0.3-0.7 % w/w enzyme per detergent composition or in the range of 0.4-0.6 % w/w enzyme per detergent composition.

In one embodiment of the invention, the composition is in the form of a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a unit dose, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid.

The invention further concerns a method for removing or releasing a flavonoid stain from a textile having a flavonoid stain which method comprises exposing the textile to an aqueous solution of an enzyme exhibiting hydrolase activity. The invention further concerns a method for removing or

releasing a flavonoid stain from a dish having a flavonoid stain which method comprises exposing the dish to an aqueous solution of an enzyme exhibiting hydrolase activity.

The method also concerns a method for removing or releasing a flavonoid stain from a hard surface having a flavonoid stain which method comprises exposing the hard surface to an aqueous solution of an enzyme exhibiting hydrolase activity.

Flavonoid stains are stains selected from the group consisting of fruit stains, berry stains and/or vegetable stains. The stains can be selected from the group comprising of stains of strawberry, blueberry, cherry, juice, jam, and forest fruit jam.

The enzyme may be selected from the GH1 family comprised by the enzyme classification EC 3.2.1.-. The enzyme of the invention may further exhibits lactase activity (EC 3.2.1.108). In one embodiment of the invention, the enzyme exhibits lactase phlorizin hydrolase activity (EC 3.2.1.62) and may belong to the enzyme classification (EC 3.2.1.23).

The enzyme of the invention exhibits lactase phlorizin hydrolase activity and comprises an amino acid sequence which has at least 90% identity with SEQ ID NO: 1. In one embodiment the enzyme has at least 95% identity with SEQ ID NO: 1. In one embodiment the enzyme has at least 96% identity with SEQ ID NO: 1. In one embodiment the enzyme has at least 97% identity with SEQ ID NO: 1. In one embodiment the enzyme has at least 98% identity with SEQ ID NO: 1. In one embodiment the enzyme has at least 99% identity with SEQ ID NO: 1. In one embodiment, the enzyme has at least 100% identity with SEQ ID NO: 1.

In one embodiment, the enzyme exhibiting lactase phlorizin hydrolase activity comprises amino acids 1 to 603 of SEQ ID NO: 1.

The enzyme lactase phlorizin hydrolase has been shown to hydrolyze some flavonoid compounds by cleaving the glucoside unit. The inventors have found that in the methods of the invention a lactase phlorizin hydrolase can be used for removing or releasing flavonoid stain from textiles, dishes or hard surfaces.

The method of the invention comprises using the enzyme exhibiting lactase phlorizin hydrolase activity as an aqueous solution of the enzyme. The pH of the aqueous solution may be in the range of 7 to 10, preferably 7 to 9 such as 7.5.

In one embodiment, the method comprises using the enzyme in combination with at least one additional enzyme. The at least one additional enzyme can be selected from the group consisting of protease, lipase, cutinase, amylase, carbohydrase, cellulase, chlorophyllase, pectate lyase, pectinase, mannanase, arabinase, galactanase, and/or xylanase. In one embodiment of the invention the at least one additional enzyme is pectate lyase.

The capability of the enzyme to remove or release stains from textile, dish or hard surfaces can be evaluated by the use of the TOM assay. In one embodiment, ΔEnz is at least 1 when

measured with the TOM assay. In one embodiment ΔEnz is at least 1.5, ΔEnz is at least 2.0, ΔEnz is at least 2.5, ΔEnz is at least 3.0, ΔEnz is at least 3.5, ΔEnz is at least 4.0, ΔEnz is at least 4.5, ΔEnz is at least 5.0, ΔEnz is at least 5.5, ΔEnz is at least 6.0, ΔEnz is at least 6.5, ΔEnz is at least 7.0, ΔEnz is at least 7.5, ΔEnz is at least 8.0 or ΔEnz is at least 8.5, ΔEnz is at least 9.0, ΔEnz is at least 9.5 or ΔEnz is at least 10.0 when measured with the TOM assay.

In one embodiment of the invention, the textile is made of cotton fabric. And the ΔEnz is at least 1 when measured with the TOM assay. In one embodiment ΔEnz is at least 1.5, ΔEnz is at least 2.0, ΔEnz is at least 2.5, ΔEnz is at least 3.0, ΔEnz is at least 3.5, ΔEnz is at least 4.0, ΔEnz is at least 4.5, ΔEnz is at least 5.0, ΔEnz is at least 5.5, ΔEnz is at least 6.0, ΔEnz is at least 6.5, ΔEnz is at least 7.0, ΔEnz is at least 7.5, ΔEnz is at least 8.0 or ΔEnz is at least 8.5, ΔEnz is at least 9.0, ΔEnz is at least 9.5 or ΔEnz is at least 10.0 when measured with the TOM assay.

In one embodiment of the invention, the method may be carried out at a temperature in the range of 10-60°C, such as 15-50°C, or 30-45°C.

In one embodiment, the method comprises applying mechanical stress to the textile.

The invention further concerns a textile treated according to the inventive method.

Enzyme of the present invention

SEQ ID NO: 1: lactase phlorizin hydrolase (LPH) (EC 3.2.1.23)

SEQ ID NO: 2: *B. rhodina* beta-glucosidase DNA

LPH is a glucohydrolase that belongs to GH1 family

In a particular embodiment, the polypeptide of the invention is a glucohydrolase belonging to the GH1 family and comprising or consisting of an amino acid sequence which has at least 90%, particularly at least 95%, more particularly at least 96%, more particularly at least 97%, more particularly at least 98%, more particularly at least 99% or most particularly 100% identity with a glucohydrolase obtainable from *Botryosphaeria rhodina*, in particular that strain of *Botryosphaeria rhodina* deposited under deposit accession number CBS 247.96, more particularly the mature beta-glucosidase comprised in SEQ ID NO: 1. More specifically the mature glucohydrolase comprise or consists of the sequences from position 1 to 603 of SEQ ID NO: 1. In the present context the beta-glucosidase is defined as a lactase phlorizin hydrolase (LPH) (EC 3.2.1.23) which hydrolyse the glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety and which hydrolyse the following flavonoids: quercetin-4P-glucoside, quercetin-3-glucoside, quercetin-3,4P-diglucoside,3P-methylquercetin-3-glucoside, genistein-7-glucoside and daidzein-7-glucoside.

For purposes of the present invention, the lactase phlorizin hydrolase activity can be tested as described in Assay I.

In one embodiment of the present invention, the a polypeptide of the present invention may be added to a detergent composition in an amount corresponding to 0.001-200 mg of protein, such as 0.005-100 mg of protein, preferably 0.01-50 mg of protein, more preferably 0.05-20 mg of protein, even more preferably 0.1-10 mg of protein per liter of wash liquor.

The enzyme(s) of the detergent composition of the invention 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, for example, WO 92/19709 and WO 92/19708.

The enzyme of SEQ ID NO: 1 can be produced as described in WO 2006/012904, see especially example 1.

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).

For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) 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 Deoxyribonucleotides x 100)/(Length of Alignment – Total Number of Gaps in Alignment).

Surfactants

The detergent 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(s) is typically present at a level of from about 0.1% to 60% by weight, such as about 1% to about 40%, or about 3% to about 20%, or about 3% to about 10%. The surfactant(s) is chosen based on the desired cleaning application, and may include any conventional surfactant(s) known in the art.

When included therein the detergent will usually contain from about 1% to about 40% by weight of an anionic surfactant, such as from about 5% to about 30%, including from about 5% to about 15%, or from about 15% to about 20%, or from about 20% to about 25% of an anionic surfactant. Non-limiting examples of anionic surfactants include sulfates and sulfonates, in particular, 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% 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 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.

Hydrotropes

A hydrotrope is a compound that solubilises hydrophobic compounds in aqueous solutions (or oppositely, polar substances in a non-polar environment). Typically, hydrotropes have both hydrophilic and a hydrophobic character (so-called amphiphilic properties as known from surfactants); however the molecular structure of hydrotropes generally do not favor spontaneous self-aggregation, see e.g. review by Hodgdon and Kaler (2007), Current Opinion in Colloid & Interface Science 12: 121-128. Hydrotropes do not display a critical concentration above which self-aggregation occurs as found for surfactants and lipids forming miceller, lamellar or other well defined meso-phases. Instead, many hydrotropes show a continuous-type aggregation process where the sizes of aggregates grow as concentration increases. However, many hydrotropes alter the phase behavior, stability, and colloidal properties of systems containing substances of polar and non-polar character, including mixtures of water, oil, surfactants, and polymers. Hydrotropes are classically used across industries from pharma, personal care, food, to technical applications. Use of hydrotropes in detergent compositions allow for example more concentrated formulations of surfactants (as in the process of compacting liquid detergents by removing water) without inducing undesired phenomena such as phase separation or high viscosity.

The detergent 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.

Builders and Co-Builders

The detergent composition may contain about 0-65% by weight, such as about 5% to about 50% 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

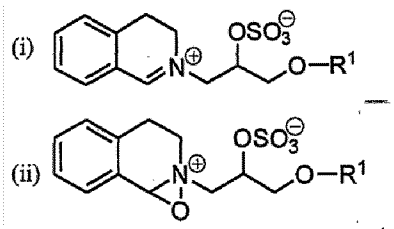
chelating agent that forms water-soluble complexes with Ca and Mg. Any builder and/or co-builder known in the art for use in laundry detergents may be utilized. Non-limiting examples of builders include zeolites, diphosphates (pyrophosphates), triphosphates such as sodium triphosphate (STP or STPP), 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 iminodiethanol), triethanolamine (TEA, also known as 2,2',2"-nitrilotriethanol), 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, or a mixture thereof. The detergent composition may include 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)-ethylidenediamine-*N*, *N'*, *N'*-triacetate (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 detergent may contain 0-50% of a bleaching system. Any bleaching system known in the art for use in laundry detergents may be utilized. Suitable bleaching system components include bleaching catalysts, photobleaches, bleach activators, sources of hydrogen peroxide such as sodium

percarbonate and sodium perborates, preformed peracids and mixtures thereof. Suitable preformed peracids include, but are not limited to, peroxycarboxylic acids and salts, percarbonic acids and salts, perimidic acids and salts, peroxymonosulfuric acids and salts, for example, Oxone (R), and mixtures thereof. Non-limiting examples of bleaching systems include peroxide-based bleaching systems, which may comprise, for example, an inorganic salt, including alkali metal salts such as sodium salts of perborate (usually mono- or tetra-hydrate), percarbonate, persulfate, perphosphate, persilicate salts, in combination with a peracid-forming bleach activator. The term bleach activator is meant herein as a compound which reacts with peroxygen bleach like hydrogen peroxide to form a peracid. The peracid thus formed constitutes the activated bleach. Suitable bleach activators to be used herein include those belonging to the class of esters amides, imides or anhydrides. Suitable examples are tetracetylethylene diamine (TAED), sodium 4-[(3,5,5-trimethylhexanoyl)oxy]benzene sulfonate (ISONOBS), diperoxy dodecanoic acid, 4-(dodecanoyloxy)benzenesulfonate (LOBS), 4-(decanoyloxy)benzenesulfonate, 4-(decanoyloxy)benzoate (DOBS), 4-(nonanoyloxy)-benzenesulfonate (NOBS), and/or those disclosed in WO 98/17767. A particular family of bleach activators of interest was disclosed in EP624154 and particulary preferred in that family is acetyl triethyl citrate (ATC). ATC or a short chain triglyceride like triacetin has the advantage that it is environmental friendly as it eventually degrades into citric acid and alcohol. Furthermore acetyl triethyl citrate and triacetin has a good hydrolytical stability in the product upon storage and it is an efficient bleach activator. Finally ATC provides a good building capacity to the laundry additive. Alternatively, the bleaching system may comprise peroxyacids of, for example, the amide, imide, or sulfone type. The bleaching system may also comprise peracids such as 6-(phthalimido)peroxyhexanoic acid (PAP). The bleaching system may also include a bleach catalyst. In some embodiments the bleach component may be an organic catalyst selected from the group consisting of organic catalysts having the following formulae:



and mixtures thereof; wherein each R^1 is independently a branched alkyl group containing from 9 to 24 carbons or linear alkyl group containing from 11 to 24 carbons, preferably each R^1 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 R^1 is independently selected from the group consisting of 2-propylheptyl, 2-butyloctyl, 2-pentylnonyl, 2-hexyldecyl, n-dodecyl, n-tetradecyl, n-hexadecyl, n-octadecyl, iso-nonyl, iso-decyl, iso-tridecyl and iso-pentadecyl. Other exemplary bleaching systems

are described, e.g. in WO 2007/087258, WO 2007/087244, WO 2007/087259 and WO 2007/087242. Suitable photobleaches may for example be sulfonated zinc phthalocyanine.

Polymers

The detergent 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). 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.

Fabric hueing agents

The detergent 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 WO 2005/03274, WO 2005/03275, WO 2005/03276 and EP 1876226 (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 WO 2007/087243.

Additional enzymes

The detergent additive as well as the detergent composition may comprise one or more additional enzymes such as a protease, lipase, cutinase, an amylase, carbohydrase, cellulase, pectinase, mannanase, arabinase, galactanase, pectate lyase, xylanase, oxidase, *e.g.*, a laccase, and/or peroxidase.

In general, the properties of the selected enzyme(s) should be compatible with the selected detergent, (*i.e.*, pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.), and the enzyme(s) should be present in effective amounts.

Cellulases: 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*, *Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757 and WO 89/09259.

Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. 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 and PCT/DK98/00299.

Commercially available cellulases include Celluzyme™, and Carezyme™ (Novozymes A/S), Clazinase™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

Lyases: The lyase may be a pectate lyase derived from *Bacillus*, particularly *B. licherniformis* or *B. agaradhaerens*, or a variant derived of any of these, e.g. as described in US 6124127, WO 99/027083, WO 99/027084, WO 02/006442, WO 02/092741, WO 03/095638, A commercially available pectate lyase is XPect, Pectawash and Pectaway (Novozymes A/S).

Mannanase: 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 99/064619. A commercially available mannanase is Mannaway (Novozymes A/S).

Proteases: Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants are included. The protease may be a serine protease or a metalloprotease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus*, *e.g.*, subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (*e.g.*, of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270 and WO 94/25583.

Examples of useful proteases are the variants described in WO 92/19729, WO 98/20115, WO 98/20116, and WO 98/34946, especially the variants with substitutions in one or more of the following positions: 27, 36, 57, 76, 87, 97, 101, 104, 120, 123, 167, 170, 194, 206, 218, 222, 224, 235, and 274.

Preferred commercially available protease enzymes include Alcalase™, Savinase™, Primase™, Duralase™, Esperase™, and Kannase™ (Novozymes A/S), Maxatase™, Maxacal™, Maxapem™, Properase™, Purafect™, Purafect OxP™, FN2™, and FN3™ (Genencor International Inc.).

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 EP 258068 and EP 305216, cutinase from *Humicola*, e.g. *H. insolens* (WO 96/13580), lipase from strains of *Pseudomonas* (some of these now renamed to *Burkholderia*), e.g. *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218272), *P. cepacia* (EP 331376), *P. sp.* strain SD705 (WO 95/06720 & WO 96/27002), *P. wisconsinensis* (WO 96/12012), GDSL-type *Streptomyces* lipases (WO 10/065455), cutinase from *Magnaporthe grisea* (WO 10/107560), cutinase from *Pseudomonas mendocina* (US 5,389,536), lipase from *Thermobifida fusca* (WO 11/084412), *Geobacillus stearothermophilus* lipase (WO 11/084417), lipase from *Bacillus subtilis* (WO 11/084599), and lipase from *Streptomyces griseus* (WO 11/150157) and *S. pristinaespiralis* (WO 12/137147).

Other examples are lipase variants such as those described in EP 407225, WO 92/05249, WO 94/01541, WO 94/25578, WO 95/14783, WO 95/30744, WO 95/35381, WO 95/22615, WO 96/00292, WO 97/04079, WO 97/07202, WO 00/34450, WO 00/60063, WO 01/92502, WO 07/87508 and WO 09/109500.

Preferred commercial lipase products include 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 (WO 10/111143), acyltransferase from *Mycobacterium smegmatis* (WO 05/56782), perhydrolases from the CE 7 family (WO 09/67279), 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 (WO 10/100028).

Amylases:

Suitable amylases which can be used together with XX of the invention 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: 3 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. More preferred variants are those having a deletion in positions 181 and 182 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.

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 WO 01/66712: 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 WO 2011/098531, WO 2013/001078 and WO 2013/001087.

Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™, Stainzyme ™, Stainzyme Plus™, Natalase™ , Liquozyme X and BAN™ (from Novozymes A/S), and Rapidase™ , Purastar™ , and Powerase (from Genencor International Inc.).

Peroxidases/Oxidases: 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™ (Novozymes A/S).

The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, *i.e.*, a separate additive or a combined additive, can be formulated, for example, as a granulate, liquid, slurry, etc. Preferred detergent additive formulations are granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids, or slurries.

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- and 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.

Adjunct materials

Any detergent components known in the art for use in laundry detergents may also be utilized. Other optional detergent components include anti-corrosion agents, anti-shrink agents, anti-soil redeposition agents, anti-wrinkling agents, bactericides, binders, corrosion inhibitors, disintegrants/disintegration agents, dyes, enzyme stabilizers (including boric acid, borates, CMC, and/or polyols such as propylene glycol), fabric conditioners including clays, fillers/processing aids, fluorescent whitening agents/optical brighteners, foam boosters, foam (suds) regulators, perfumes, soil-suspending agents, softeners, suds suppressors, tarnish inhibitors, and wicking agents, either alone or in combination. Any ingredient known in the art for use in laundry detergents may be utilized. The choice of such ingredients is well within the skill of the artisan.

Dispersants - The detergent 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 detergent 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 detergent compositions of the present invention will preferably also contain additional components that may tint articles being cleaned, such as fluorescent whitening agent 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-(2*H*-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 detergent 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 are 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). 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 detergent 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.

Other suitable adjunct materials 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.

Formulation of detergent products

The detergent 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 multi compartments. 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. Ref: (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.

Definition/characteristics of the forms:

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 be non-aqueous.

Laundry soap bars

The enzymes of the invention may be added to laundry soap bars and used for hand washing laundry, fabrics and/or textiles. The term laundry soap bar includes laundry bars, soap bars, combo bars, syndet bars and detergent bars. The types of bar usually differ in the type of surfactant they contain, and the term laundry soap bar includes those containing soaps from fatty acids and/or synthetic soaps. The laundry soap bar has a physical form which is solid and not a liquid, gel or a powder at room temperature. The term solid is defined as a physical form which does not significantly change over time, i.e. if a solid object (e.g. laundry soap bar) is placed inside a container, the solid object does not change to fill the container it is placed in. The bar is a solid typically in bar form but can be in other solid shapes such as round or oval.

The laundry soap bar may contain one or more additional enzymes, protease inhibitors such as peptide aldehydes (or hydrosulfite adduct or hemiacetal adduct), boric acid, borate, borax and/or phenylboronic acid derivatives such as 4-formylphenylboronic acid, one or more soaps or synthetic surfactants, polyols such as glycerine, pH controlling compounds such as fatty acids, citric acid, acetic acid and/or formic acid, and/or a salt of a monovalent cation and an organic anion wherein the monovalent cation may be for example Na^+, K^+ or NH_4^+ and the organic anion may be for example formate, acetate, citrate or lactate such that the salt of a monovalent cation and an organic anion may be, for example, sodium formate.

The laundry soap bar may also contain complexing agents like EDTA and HEDP, perfumes and/or different type of fillers, surfactants e.g. anionic synthetic surfactants, builders, polymeric soil release agents, detergent chelators, stabilizing agents, fillers, dyes, colorants, dye transfer inhibitors, alkoxylated polycarbonates, suds suppressers, structurants, binders, leaching agents, bleaching

activators, clay soil removal agents, anti-redeposition agents, polymeric dispersing agents, brighteners, fabric softeners, perfumes and/or other compounds known in the art.

The laundry soap bar may be processed in conventional laundry soap bar making equipment such as but not limited to: mixers, plodders, e.g. a two stage vacuum plodder, extruders, cutters, logo-stampers, cooling tunnels and wrappers. The invention is not limited to preparing the laundry soap bars by any single method. The premix of the invention may be added to the soap at different stages of the process. For example, the premix containing a soap, an enzyme, optionally one or more additional enzymes, a protease inhibitor, and a salt of a monovalent cation and an organic anion may be prepared and the mixture is then plodded. The enzyme and optional additional enzymes may be added at the same time as the protease inhibitor for example in liquid form. Besides the mixing step and the plodding step, the process may further comprise the steps of milling, extruding, cutting, stamping, cooling and/or wrapping.

Granular detergent formulations

A granular detergent may be formulated as described in WO 09/092699, EP 1705241, EP 1382668, WO 07/001262, US 6472364, WO 04/074419 or WO 09/102854. Other useful detergent formulations are described in WO 09/124162, WO 09/124163, WO 09/117340, WO 09/117341, WO 09/117342, WO 09/072069, WO 09/063355, WO 09/132870, WO 09/121757, WO 09/112296, WO 09/112298, WO 09/103822, WO 09/087033, WO 09/050026, WO 09/047125, WO 09/047126, WO 09/047127, WO 09/047128, WO 09/021784, WO 09/010375, WO 09/000605, WO 09/122125, WO 09/095645, WO 09/040544, WO 09/040545, WO 09/024780, WO 09/004295, WO 09/004294, WO 09/121725, WO 09/115391, WO 09/115392, WO 09/074398, WO 09/074403, WO 09/068501, WO 09/065770, WO 09/021813, WO 09/030632, WO 09/015951, WO 2011025615, WO 2011016958, WO 2011005803, WO 2011005623, WO 2011005730, WO 2011005844, WO 2011005904, WO 2011005630, WO 2011005830, WO 2011005912, WO 2011005905, WO 2011005910, WO 2011005813, WO 2010135238, WO 2010120863, WO 2010108002, WO 2010111365, WO 2010108000, WO 2010107635, WO 2010090915, WO 2010033976, WO 2010033746, WO 2010033747, WO 2010033897, WO 2010033979, WO 2010030540, WO 2010030541, WO 2010030539, WO 2010024467, WO 2010024469, WO 2010024470, WO 2010025161, WO 2010014395, WO 2010044905, WO 2010145887, WO 2010142503, WO 2010122051, WO 2010102861, WO 2010099997, WO 2010084039, WO 2010076292, WO 2010069742, WO 2010069718, WO 2010069957, WO 2010057784, WO 2010054986, WO 2010018043, WO 2010003783, WO 2010003792, WO 2011023716, WO 2010142539, WO 2010118959, WO 2010115813, WO 2010105942, WO 2010105961, WO 2010105962, WO 2010094356, WO 2010084203, WO 2010078979, WO 2010072456, WO 2010069905, WO 2010076165, WO

2010072603, WO 2010066486, WO 2010066631, WO 2010066632, WO 2010063689, WO 2010060821, WO 2010049187, WO 2010031607, WO 2010000636.

Unit Dose

In one aspect, the automatic dishwashing detergent composition of the invention is in unit dose form. Automatic dishwashing detergent products in unit dose form include tablets, capsules, sachets, pouches, etc. In one aspect, for use herein are tablets wrapped with a water- soluble film and water-soluble pouches. The weight of the composition of the invention is from about 10 to about 25 grams, from about 12 to about 24 grams or even from 14 to 22 grams. These weights are extremely convenient for automatic dishwashing detergent product dispenser fit. In the cases of unit dose products having a water-soluble material enveloping the automatic dishwashing detergent composition, the water-soluble material is not considered as part of the composition. In one aspect, the unit dose form is a water-soluble pouch (i.e., water-soluble film enveloping an automatic dishwashing detergent composition), in one aspect, a multi-compartment pouch having a plurality of films forming a plurality of compartments. This configuration contributes to the flexibility and optimization of the composition. It allows for the separation and controlled release of different ingredients. In one aspect, one compartment contains an automatic dishwashing detergent composition in solid form and another compartment contains an automatic dishwashing detergent composition in liquid form.

In one aspect, multi-compartment pouch embodiments two different compartments could contain two different cleaning agents. In one aspect, the films of these two compartments have different dissolution profiles, allowing the release of the same or different agents at different times. For example, the agent from one compartment (first compartment) can be delivered early in the washing process to help with soil removal and a second agent from another compartment (second compartment) can be delivered at least two minutes, or even at least five minutes later than the agent from the first compartment.

In one aspect, a multi-compartment pouch comprising two side-by-side compartments superposed onto another compartment, wherein at least two different compartments contain two different automatic dishwashing detergent compositions is disclosed.

According to another aspect of the invention, there is provided an automatic dishwashing detergent dosing element for use in an auto-dosing device the dosing element comprising an automatic dishwashing detergent composition according to any of the preceding claims. By "auto-dosing device" herein is meant a device that is placed into the dishwasher holding a plurality of doses to be delivered in different washes. The user does not need to charge the detergent for each

wash, the auto-dosing device delivers them automatically. Each wash can use a single or more doses.

A multi-compartment pack is formed by a plurality of water-soluble enveloping materials which form a plurality of compartments, one of the compartments would contain the automatic dishwashing detergent composition of the invention, another compartment can contain a liquid composition, the liquid composition can be aqueous (i.e. comprises more than 10 percent of water by weight of the liquid composition) and the compartment can be made of warm water soluble material. In some embodiments the compartment comprising the automatic dishwashing detergent composition of the invention is made of cold water soluble material. It allows for the separation and controlled release of different ingredients. In other embodiments all the compartments are made of warm water soluble material.

Suitable packs comprise at least two side-by-side compartments superposed (i.e. placed above) onto another compartment, especially suitable are pouches. This disposition contributes to the compactness, robustness and strength of the pack, additionally, it minimises the amount of water-soluble material required. It only requires three pieces of material to form three compartments. The robustness of the pack allows also for the use of very thin films without compromising the physical integrity of the pack. The pack is also very easy to use because the compartments do not need to be folded to be used in machine dispensers of fix geometry. At least two of the compartments of the pack contain two different automatic dishwashing detergent compositions. By "different compositions" herein is meant automatic dishwashing detergent compositions that differ in at least one ingredient.

In one aspect, at least one of the compartments contains a solid automatic dishwashing detergent composition and another compartment an aqueous liquid automatic dishwashing detergent composition, the compositions are typically in a solid to liquid weight ratio of from about 20:1 to about 1:20, from about 18: 1 to about 2:1 or from about 15:1 to about 5: 1. This kind of pack is very versatile because it can accommodate compositions having a broad spectrum of values of solid:liquid ratio. Pouches having a high solid:liquid ratio because many of the detergent ingredients are particularly suitable for use in solid form, in one aspect in powder form. The ratio solid:liquid defined herein refers to the relationship between the weight of all the solid compositions and the weight of all the liquid compositions in the pack.

Suitable solid:liquid weight ratios are from about 2:1 to about 18:1, or from about 5:1 to about 15:1. These weight ratios are suitable in cases in which most of the ingredients of the detergent are in liquid form.

In one aspect, the two side-by-side compartments contain liquid automatic dishwashing detergent compositions, which can be the same or different and another compartment contains a

solid automatic dishwashing detergent composition, for example in powder form, in one aspect, a densified powder. The solid composition contributes to the strength and robustness of the pack.

For dispenser fit reasons, especially in an automatic dishwasher, the unit dose form products herein have a square or rectangular base and a height of from about 1 to about 5 cm, or from about 1 to about 4 cm. In one aspect, the weight of the solid composition is from about 5 to about 20 grams, or from about 10 to about 15 grams and the weight of the liquid compositions is from about 0.5 to about 4 grams, or from about 0.8 to about 3 grams. In one aspect, at least two of the films which form different compartments have different solubilities, under the same conditions. This enables the release of the compositions which they partially or totally envelope at different times.

Controlled release of the ingredients of a multi-compartment pouch can be achieved by modifying the thickness of the film and/or the solubility of the film material. The solubility of the film material can be delayed by for example cross-linking the film as described in WO 2002/102955. Other water-soluble films designed for rinse release are described in US 4,765,916 and US 4,972,017. Waxy coating (see US 5,453,216) of films can help with rinse release. pH controlled release means are described in US 5,453,216, in particular amino- acetylated polysaccharide having selective degree of acetylation.

Other means of obtaining delayed release by multi-compartment pouches with different compartments, where the compartments are made of films having different solubility are taught in US 6,727,215.

Uses

The present invention is also directed to methods for using the enzyme of the present invention or compositions thereof in house hold laundry washing or industry laundry washing. The present invention is also directed to the use of enzyme of the present invention or compositions thereof in hard surface cleaning such as cleaning of floor, tables, walls and the like. The present invention is also directed to the use of enzyme of the present invention or compositions thereof cleaning of plates, cups, glasses, bowls and cutlery.

Use in detergents. The polypeptides of the present invention may be added to and thus become a component of a detergent composition.

The detergent 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 a polypeptide of the present invention as described herein.

Assays

Activity assay

The activity of the LPH was tested at pH 5.5 and 7.5 respectively by reducing ends, with each substrate quercetin-3-glucoside (Q3) and quercetin-4´-glucoside (Q4) at 0.05%.

Assay I: Activity towards quercetin compounds by LC-MS

The analysis is performed using an LTQ Deca max IonTrap equipped with an ESI source and an Accela HPLC system with a PDA detector. A BEH Acquity CSH C18 column (2.1 x 100 mm) is used for the separation at a flow rate of 250 µL/min and 65˚C. The gradient is as follows: A: 0.15% HCOOH in water, B: HCOOH in MeCN; 0 min 17%B, 4 min 17%B, 14 min 24.3%B, 15 min 95%B, 16 min 17%B, 20 min 17%B. 5 µL of samples is injected and UV detection is at 245 nm. The spray settings are a follows: Capillary temp 275˚C, sheath gas flow 40 l/min and source voltage of 5 kV.

Preparation of samples for LC-MS:

A flavonoid solution of the respective quercetin Q3 (1%) and Q4 (2%) referred as quercetin 3 glucoside and quercetin 4 glucoside (in MilliQ (MQ)) were prepared. The reaction mixture was made in Buffer Phosphate 20 mM pH 7.0 up to 5 mL

The enzyme *LPH was diluted to 0.2 mg/mL in the reaction mixture.*

As Blanks: Q3, Q4, LPH and MQ were run alone.The Standards used were: Glucose, Lactose

The respective enzyme and substrate mix was incubated at room T. At determined times aliquots were precipitated with ethanol as follows:

1. Precipitation with EtOH (96%) At different intervals of time, 500 ul of sample were precipitated with 1 mL EtOH, followed by centrifugation at 15000xg 20 min at 4°C. The supernantant is saved and the EtOH is evaporated and each sample redissolved in 500 ul of 20 mM Phosphate buffer pH 7.0.
2. PMP-Derivatization: Place 200 µL sample to a suitable vial, then add 20 µL of 4 M NaOH followed by 200 µL 0.5 M PMP solution in methanol. Close vial and mix.

Incubate at 70°C for 30 min. Cool to room temperature. Add 20 µL 4 M HCl and mix.

Finally Add 1,5 mL MQ and run on LC-MS.

Assay II: Activity assay Reducing ends

Substrates: - 1) 0.05 % Quercetin 3 glucoside in MilliQ Water (MQ)

Stock: 10mg/ml (1%)

100 µl stock + 1900 ml MQ

- 2) 0,05% Quercetin 4´- glucoside in MQ

Stock: 20mg/ml (2%)

50 µl stock + 1950 ml MQ

Activity buffer pH 5,5: *100mM acetate, 100mM MES, in 0.01% Triton X100, 1mM CaCl2, pH 5.5*

Stock	Add for 100mL
1M Acetate	10 ml
0,5M MES	20 ml
1M CaCl2	100µl
10% Triton X100	100µl
MilliQ	adjust pH and fill up to 100mL,

Activity buffer pH 7,5: *100mM acetate, 100mM MES, 100mM Glycine in 0.01% Triton X100, 1mM CaCl2, pH 7.5*

Stock	Add for 100mL
1M Acetate	10 ml
0,5M MES	20 ml
1 M Glycine	10ml
1M CaCl2	100µl
10% Triton X100	100µl
MilliQ	adjust pH and fill up to 100mL,

Enzyme dilution buffer: *10mM acetate, 1mM CaCl2, 0.01% Triton X100, pH 7.4*

Stock	Add for 500mL
1M Acetate	5 ml
1M CaCl2	120µl

10% Triton X100	1 ml
MilliQ	adjust pH to 7.4 and fill up to 500ml

Ka-Na-tartrate/NaOH *For 1l Ka-Na-tartrate/NaOH solution. Store at 4°C*

Stock	Add for 1 liter
K-Na-tartrate (Merck 8087)	50 g
NaOH (Merck 1.06498) 20g	20 g
MilliQ	Up to 1 liter

Stop solution: Dissolve PAHBAH (Sigma H-9882) in Ka-Na-tartrate/NaOH solution to 15mg/ml, make fresh. Note that this stop solution should be collected for 2% NaOH disposal.

PCR-MTP ThermoFast 96 non-skirted, Thermo Scientific, cat. no. AB-0600

Procedure

Two samples of the LPH enzyme named as pool 1 and pool 2 were diluted to 0,232mg/ml in 2 ml enzyme solutions.

Pool 1)3,13mg/ml - 154µl + 1846µl MQ

Pool 2)2,32mg/ml - 200µl + 1800µl MQ

The stock solutions were transferred to a new MTPlate and a 2-fold-dilutions with dilution buffer were made.

Setup in 96-well PCR-MTP:

1. Add 50µl Activity buffer pH 5.5 and 7.5 to PCR-MTP and 50µl substrate, mix.
2. The reaction is started by adding 50µl enzyme solutions from MTPlate, mix.
3. Incubate in PCR machine at 37°C for 15 min. Use lit heat and sealing tape. Immediately lower temperature to 10 °C.
4. Dissolved 511 mg PAHBAH in 33 ml Ka-Na-tartrate/NaOH solution (15mg/ml).
5. Add 75 µl of the Stopsolution, mix, and discarded 75 µl of the mix.

6. The mix was incubated in 10 min. at 95°C, then 1 min. 10°C.

7. Transfer 150µl to new 96 MTP, measure absorbance at 405nm.

Results:

	Pool 1				Pool 2			
	Q3		Q4		Q3		Q4	
	pH 5,5	pH 7,5	pH 5,5	pH 7,5	pH 5,5	pH 7,5	pH 5,5	pH 7,5
Dil 160	2,6047	2,5642	1,4305	1,4666	3,0306	2,4062	1,5	1,3737
Dil 320	2,5713	2,2487	1,5071	1,3443	2,8531	2,3368	1,4728	1,4279
Dil 640	2,5718	2,3286	1,4736	1,181	2,6438	2,1905	1,4686	1,2819
Blank	2,0364	1,8801	0,7523	0,7477	2,0981	1,6761	0,748	0,7581

The activity of the LPH was tested at pH 5.5 and 7.5 respectively by reducing ends, with each substrate quercetin-3-glucoside (Q3) and quercetin-4´-glucoside (Q4) at 0.05%. The structure of the two substrates is as indicated in Figure 1.

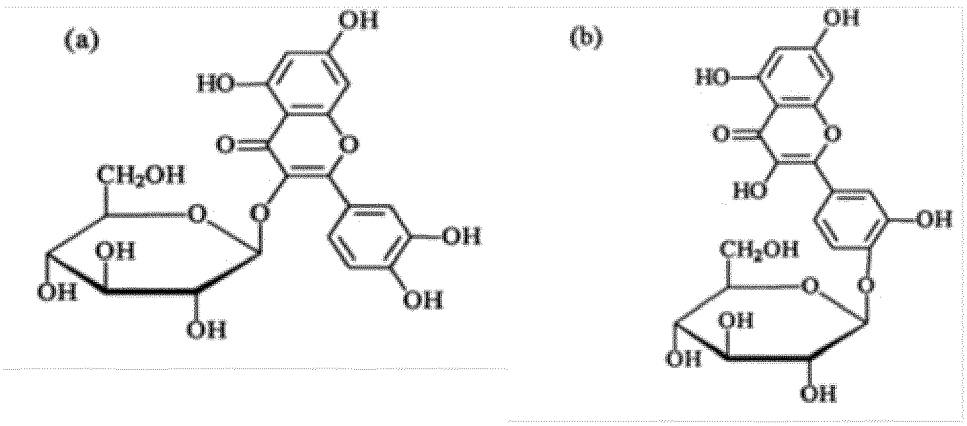


Figure 1: Structure of two flavonoid compounds (a) quercetin-3-glucoside and (b) quercetin-4´-glucoside respectively.

Because the blank (absorbance of substrate without enzyme) was higher than expected, the activity assay was extended by LC-MS (Liquid Chromatography coupled to Mass Spectrophotometer).

Wash assays

Wash conditions in various regions for normal heavy duty wash***

Region	Latin America	North America	Europe	Asia excl. Japan	Japan
Temperature	20-25°C	16-32°C	30-60°C	15-30°C	15-20°C
Main Wash time	14-16 min	12 min	20-40 min	14-20 min	9 min
Total wash time	55 min	50 min	90-120 min	60 min	45-50 min
Water hardness*	6-12°dH	6°-8,4 dH	15°dH	14°dH	3°dH
Detergent dosage	1.5-4 g/l	1.0-1.5 g/l	4-10 g/l	1.5-2.5 g/l	0.5-0.7 g/l
Washing pH	As it is	As it is	As it is	As it is	As it is

* °dH: adjusted by adding $CaCl_2*2H_2O$, $MgCl_2*6H_2O$ and $NaHCO_3$ to Milli-Q water.

Note (this is estimations and not absolutes, there is speciel conditions for different regions and also with different machines)

Automatic Mechanical Stress Assay (AMSA) for laundry

In order to assess the wash performance in laundry, washing experiments are performed using the Automatic Mechanical Stress Assay (AMSA). With the AMSA, the wash performance of many small volume enzyme-detergent solutions can be examined. The AMSA plate has a number of slots for test solutions and a lid that firmly squeezes the textile to be washed against the slot openings. During the wash, the plate, test solutions, textile and lid are vigorously shaken to bring the test solution in contact with the textile and apply mechanical stress in a regular, periodic, oscillating manner. For further description see WO02/42740 especially the paragraph "Special method embodiments" at page 23-24.

The laundry experiments are conducted under the experimental conditions specified below:

Detergent dosage	5 g/L (liquid detergent) 2.5 g/L (powder detergent)
Test solution volume	160 micro L
pH	Adjusted to pH 7 or pH 6 (liquid detergent) As is (powder detergent)
Wash time	20 minutes
Temperature	60°C, 40°C and 20°C or 15°C
Water hardness	15°dH

Model detergents and test materials were as follows:

Laundry liquid model detergent	Sodium alkylethoxy sulfate (C-9-15, 2EO) 6.0%

	Sodium dodecyl benzene sulfonate 3.0% Sodium toluene sulfonate 3.0% Oleic acid 2.0% Primary alcohol ethoxylate (C12-15, 7EO) 3.0% Primary alcohol ethoxylate (C12-15, 3EO) 2.5% Ethanol 0.5% Monopropylene glycol 2.0% Tri-sodium citrate dihydrate 4.0% Triethanolamine 0.4% De-ionized water ad 100% pH adjusted to 8.5 with NaOH
Laundry powder model detergent	Sodium citrate dihydrate 32.3% Sodium-LAS 24.2% Sodium lauryl sulfate 32.2% Neodol 25-7 (alcohol ethoxylate) 6.4% Sodium sulfate 4.9%
Test material	EMPA112 (Cocoa on cotton) PC-05 (Blood/milk/ink on cotton/polyester) WFK10PPM (Vegetable oil/milk/pigment on cotton) C-10 (Oil/milk/pigment on cotton)

Test materials are obtained from EMPA Testmaterials AG, Mövenstrasse 12, CH-9015 St. Gallen, Switzerland, from Center For Testmaterials BV, P.O. Box 120, 3133 KT Vlaardingen, the Netherlands, and WFK Testgewebe GmbH, Christenfeld 10, D-41379 Brüggen, Germany.

Water hardness was adjusted to 15°dH by addition of $CaCl_2$, $MgCl_2$, and $NaHCO_3$ (Ca^{2+}:Mg^{2+} = 4:1:7.5) to the test system. After washing the textiles were flushed in tap water and dried.

The wash performance is measured as the brightness of the colour of the textile washed. Brightness can also be expressed as the intensity of the light reflected from the sample when illuminated with white light. When the sample is stained the intensity of the reflected light is lower, than that of a clean sample. Therefore the intensity of the reflected light can be used to measure wash performance.

Colour measurements are made with a professional flatbed scanner (Kodak iQsmart, Kodak, Midtager 29, DK-2605 Brøndby, Denmark), which is used to capture an image of the washed textile.

To extract a value for the light intensity from the scanned images, 24-bit pixel values from the image are converted into values for red, green and blue (RGB). The intensity value (Int) is calculated by adding the RGB values together as vectors and then taking the length of the resulting vector:

$$Int = \sqrt{r^2 + g^2 + b^2}$$

Automatic Mechanical Stress Assay (AMSA) for Automatic Dish Wash (ADW)

Washing experiments are performed in order to asses the wash performance of the enzyme in dishwash detergent compositions. The enzyme of the present application was tested using the Automatic Mechanical Stress Assay (AMSA). With the AMSA, the wash performance of many small volume enzyme-detergent solutions can be examined. The AMSA plate has a number of slots for test solutions and a lid that firmly squeezes the melamine tile to be washed against the slot openings. During the wash, the plate, test solutions, melamine tile and lid are vigorously shaken to bring the test solution in contact with the soiled melamine tile and apply mechanical stress in a regular, periodic oscillating manner. For further description see WO 02/42740 especially the paragraph "Special method embodiments" at page 23-24.

The experiment was conducted under the experimental conditions as specified in the table(s) below:

ADW model detergent with MGDA	MGDA (40 %) 30 % Sodium carbonate 20 % Sodium percarbonate 10 % Sodium disilicate 5 % TAED 5% Sokalan CP5 (39.5 %) 10 % Surfac 23-6.5 (100 %) 5 % Sodium Sulfate 15 %
Detergent dosage	3.33 g/L
Test solution volume	160 micro L
pH	As is
Wash time	20 minutes

Temperature	50 °C
Water hardness	17 °dH
Enzyme concentration in test solution	0.925, 1.85, 5.55, 11 mg enzyme protein/liter
Test material	Egg yolk melamine tile (DM-21)

ADW model detergent with STPP	STPP 50 % Sodium carbonate 20% Sodium percarbonate 10% Sodium disilicate 5% TAED 2% Sokalan CP5 (39.5%) 5% Surfac 23-6.5 (100%) 2% Phosphonate 6%
Detergent dosage	3.33 g/L
Test solution volume	160 micro L
pH	As is
Wash time	20 minutes
Temperature	50°C
Water hardness	17°dH
Enzyme concentration in test solution	0.925, 1.85, 5.55, 11mg enzyme protein/liter
Test material	Egg yolk melamine tile (DM-21)

Water hardness was adjusted to 17°dH by addition of $CaCl_2$, $MgCl_2$, and $NaHCO_3$ (Ca^{2+}:Mg^{2+} = 4:1:10) to the test system. After washing the egg yolk melamine tiles were flushed in tap water and dried.

The performance of the enzyme variant is measured as the brightness of the color of the melamine tile washed with that specific enzyme. Brightness can also be expressed as the intensity of the light reflected from the sample when illuminated with white light. When the sample is stained the intensity of the reflected light is lower, than that of a clean sample. Therefore the intensity of the reflected light can be used to measure wash performance of an enzyme.

Color measurements are made with a professional flatbed scanner (Kodak iQsmart, Kodak, Midtager 29, DK-2605 Brøndby, Denmark), which is used to capture an image of the washed melamine tiles.

To extract a value for the light intensity from the scanned images, a special designed software application is used (*Novozymes Color Vector Analyzer*). The program retrieves the 24 bit pixel values from the image and converts them into values for red, green and blue (RGB). The intensity value (Int) is calculated by adding the RGB values together as vectors and then taking the length of the resulting vector:

$$Int = \sqrt{r^2 + g^2 + b^2}$$

Terg-O-tometer (TOM) wash assay

The Tergo-To-Meter (TOM) is a medium scale model wash system that can be applied to test 12 different wash conditions simultaneously. A TOM is basically a large temperature controlled water bath with up to 12 open metal beakers submerged into it. Each beaker constitutes one small top loader style washing machine and during an experiment, each of them will contain a solution of a specific detergent/enzyme system and the soiled and unsoiled fabrics its performance is tested on. Mechanical stress is achieved by a rotating stirring arm, which stirs the liquid within each beaker. Because the TOM beakers have no lid, it is possible to withdraw samples during a TOM experiment and assay for information on-line during wash.

The TOM model wash system is mainly used in medium scale testing of detergents and enzymes at US or LA/AP wash conditions. In a TOM experiment, factors such as the ballast to soil ratio and the fabric to wash liquor ratio can be varied. Therefore, the TOM provides the link between small scale experiments, such as AMSA and mini-wash, and the more time consuming full scale experiments in top loader washing machines.

Equipment: The water bath with 12 steel beakers and 1 rotating arm per beaker with capacity of 500 or 1200mL of detergent solution. Temperature ranges from 5 to 80ºC. The water bath has to be filled up with deionised water. Rotational speed can be set up to 70 to 120rpm/min.

1. Set temperature in the Terg-O-Tometer and start the rotation in the water bath. Wait for the temperature to adjust (tolerance is +/- 0,5°C)
2. All beakers shall be clean and without traces of prior test material.
3. Prepare wash solution with desired amount of detergent, temperature and water hardness in a bucket. Let detergent dissolve during magnet stirring for 10 min. Wash solution shall be used within 30 to 60 min after preparation.
4. Add 800ml wash solution into a TOM beaker
5. Start agitation at 120rpm and optionally add enzymes to the beaker.
6. Sprinkle the swatches into the beaker and then the ballast load.

7. Time measurement start when the swatches and ballast are added to the beaker.
8. Wash for 20 minutes
9. Stop agitation
10. Transfer the wash load from TOM beaker to a sieve and rinse with cold tap water
11. Separate the soil swatches from the ballast load. The soil swatches are transferred to a 5L beaker with cold tap water under running water. Keep the ballast load separately for the coming inactivation.
12. Set the timer to 5 minutes.
13. Press gently the water out by hand and place the test swatches on a tray covered with a paper. Add another paper on top of the swatches.
14. Let the swatches dry over night and then measure at the Color Eye as described below.

*There can be variations from this description always ask chemist.

Wash compositions

Model B detergent Composition

Ingredient (abbreviation)	Explanation	Purity	Wt%
LAS	linear alkylbenzenesulfonate	91	7.2
AEOS	alkyl ethoxysulfate	70,5	4.2
Soy soap		100	2.75
Coco soap		100	2.75
AEO	alcohol ethoxylate	100	6.6
NaOH	Sodium hydroxide	100	1.2
Ethanol		100	3
MPG	monopropylene glycol	100	6
glycerol		85	2
TEA	triethanolamine	100	3
Sodium formiate		100	1
Sodium citrate		100	2

DTMPA	Diethylenetriaminepenta (methylenephosphonic acid)	100	0.2
PCA	polycarboxylic acid type polymer	100	0.2
Ion exchanged water		100	57.9

Example 1

The experimental conditions for the experiments are specified in Table 1 below.

Table 1: Experimental conditions for TOM

Test solution	1g/L Model liquid detergent B or Phosphate buffer, 20mM pH7.50
Test solution volume	40 mL
pH	7.50 in buffer, unadjusted in Model B
Wash time	30 minutes
Temperature	40°C
Water hardness	16°dH
Ca^{2+}:Mg^{2+}:CO_3^{2-} ratio	4:1:7.5
Enzyme dosage	8.5ppm
Swatches	3 halves of each C-H045 Strawberry Jam, C-H047 Cherry Jam, C-H147 Forest fruit jam, (all three are on cotton)
Ballast	30g of wfk80A (100% knitted cotton)
Mechanics	Rotation by 100 rpm

Water hardness was adjusted by addition of $CaCl_2$, $MgCl_2$, and $NaHCO_3$ to the test system. After washing, the textiles were rinsed in a Miele washing machine using a rinse program (STIVN). The stained swatches are commercially available from CFT, Center for Testmaterials BV,

AC Vlaardingen, the Netherlands. The ballast is commercially available from WFK, wfk Testgewebe GmbH, Christenfeld 10, D-41379 Brüggen, Germany.

The performance of the enzyme is evaluated by measuring the remission of the textile swatches using the ColorEye at 460 nm, and expressed as the delta enzyme performance value (Delta Enz) as shown in the below table 2.

Table 2:

DELTA Enz	Strawberry jam	Cherry jam	Forest fruit jam
Model B Control	0.00	0.00	0.00
Phosphate Control	0.00	0.00	0.00
Model B + LPH	-0.29	3.11	2.18
Phosphate + LPH	1.57	2.85	1.47

Example 2

Demonstration of the removal of color from flavonoid containing fruit extract

Preparation of blueberry and strawberry extracts:

Fresh fruit was cut into small pieces and placed into an Erlenmeyer with 50mL methanol. The samples were incubated at room T with shaking for 45 min. The supernatant was transferred to a new bottle for further analysis and the rest of fruit flesh was discarded. The methanol extracts were aliquoted into 1mL samples and evaporated on the Speed Vac, until dried. The solid was redissolved in phosphate buffer pH 7.0 and kept in fridge for further use.

Assay for testing LPH on MTP (micro titer plate) with commercial products (red wine, tea) and blueberry and strawberry extracts prepared as described in Assay I above.

A sample of 100ul was placed into a MTP and 100ul of phosphate buffer pH 7 was added. The LPH enzyme (50ul) was added to a final concentration of 4mg/mL and let it at room T overnight. The color was completely removed on the wells containing blueberry and strawberry extracts + LPH as seen from figure 2, where well number 2 from the left is blueberry and well number 4 from the left is strawberry. The other samples showed no change (grass) or an increase in color (tea, wine and apple juice).

Example 3

The wash performance is measured as the brightness of the color of the textile washed. Brightness can also be expressed as the intensity of the light reflected from the sample when illuminated with white light. When the sample is stained the intensity of the reflected light is lower, than that of a clean sample. Therefore the intensity of the reflected light can be used to measure wash performance.

Color measurements are made with a Digieye, which is used to capture an image of the washed textile.

To extract a value for the light intensity from the scanned images, 24-bit pixel values from the image are converted into values for red, green and blue (RGB). The intensity value (Int) is calculated by adding the RGB values together as vectors and then taking the length of the resulting vector:

$$Int=\sqrt{r^2+g^2+b^2}$$

Mini-Launder-O-Meter (mini-LOM) Model Wash System

The Mini-Launder-O-Meter (mini-LOM) is a small scale model wash system that can be applied to test up to 14 different wash conditions simultaneously. A mini-LOM consists of 14 closed plastic tubes in a rotating rotissiere-like setup which is placed in a temperature controlled cabinet. Each beaker constitutes one small washing machine and during an experiment, each will contain a solution of a specific detergent/enzyme system to be tested along with the soiled and unsoiled fabrics it is tested on. Mechanical stress is achieved by the beakers being rotated quickly in air.

The experimental conditions for the experiments are specified in Table 1 below.

Table 3: Experimental conditions for mini-LOM

Test solution	1g/L Model liquid detergent B or Phosphate buffer, 20 mM pH7.50
Test solution volume	40 mL
pH	7.50 in buffer, unadjusted in Model B
Wash time	30 minutes
Temperature	40°C
Water hardness	15 °dH

Ca^{2+}:Mg^{2+}:CO_3^{2-} ratio	4:1:7.5
Enzyme dosage	8.5ppm
Swatches	4 x 2 cm circles of each of the following Blackberry juice CS21 Red Currant Juice CS11
Mechanics	Rotation by 100 rpm, addition of 5 ball bearings to simulate EU washing machine

Water hardness was adjusted by addition of $CaCl_2$, $MgCl_2$, and $NaHCO_3$ to the test system. After washing, the textiles were rinsed in running tap water. The stained swatches are commercially available from CFT, Center for Testmaterials BV, AC Vlaardingen, the Netherlands. The ballast is commercially available from WFK, wfk Testgewebe GmbH, Christenfeld 10, D-41379 Brüggen, Germany.

The performance of the enzyme is evaluated by measuring the intensity of the textile swatches using the Digieye, and expressed as the delta enzyme performance value (Delta Enz) as shown in the below table 2.

Table 4:

DELTA Enz	**Blackberry juice CS21**	**Red Currant Juice CS11**
Model B Control	0	0
Phosphate Control	0	0
Model B + LPH	3.16	-1.49
Phosphate + LPH	2.04	2.33

Claims

1. Use of an enzyme exhibiting hydrolase activity for removing or releasing a flavonoid stain from a textile having a flavonoid stain.
2. Use of an enzyme exhibiting hydrolase activity for removing or releasing a flavonoid stain from a dish having a flavonoid stain.
3. Use of an enzyme exhibiting hydrolase activity for removing or releasing a flavonoid stain from a hard surface having a flavonoid stain.
4. Use according to any of the preceding claims, wherein the enzyme belongs to the GH1 family comprised by the enzyme classification EC 3.2.1.-.
5. Use according to any of the preceding claims, wherein the enzyme further exhibits lactase activity (EC 3.2.1.108).
6. Use according to any of the preceding claims, wherein the enzyme exhibits lactase phlorizin hydrolase activity (EC 3.2.1.62).
7. Use according to any of the preceding claims, wherein the enzyme belongs to the enzyme classification (EC 3.2.1.23).
8. Use according to any of the preceding claims, wherein the enzyme exhibiting lactase phlorizin hydrolase activity comprises an amino acid sequence which has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 1.
9. Use according to any of the preceding claims, wherein the enzyme exhibiting lactase phlorizin hydrolase activity comprises amino acids 1 to 603 of SEQ ID NO: 1.
10. Use according to any of the preceding claims, wherein the flavonoid stain is selected from the group consisting of fruit stains, berry stains and/or vegetable stains.
11. Use according to any of the preceding claims, wherein the flavonoid stain is selected from the group consisting of stains of strawberry, blueberry, cherry, juice, jam, and forest fruit jam.
12. Use according to any of the preceding claims, wherein the enzyme exhibiting hydrolase activity used as an aqueous solution of the enzyme.
13. Use according to any of the preceding claims, wherein the enzyme exhibiting hydrolase is used in combination with at least one additional enzyme.
14. Use according to any of the preceding claims, wherein the additional enzyme is selected from the group consisting of protease, lipase, cutinase, amylase, carbohydrase, cellulase, pectate lyase, pectinase, mannanase, arabinase, galactanase, and/or xylanase.

15. Use according to any of the preceding claims, wherein the additional enzyme is a pectate lyase.
16. Use according to any of the preceding claims, wherein ΔEnz is at least 1 when measured with the TOM assay.
17. Use according to any of the preceding claims, wherein ΔEnz is at least 1.5, ΔEnz is at least 2.0, ΔEnz is at least 2.5, ΔEnz is at least 3.0, ΔEnz is at least 3.5, ΔEnz is at least 4.0, ΔEnz is at least 4.5, ΔEnz is at least 5.0, ΔEnz is at least 5.5, ΔEnz is at least 6.0, ΔEnz is at least 6.5, ΔEnz is at least 7.0, ΔEnz is at least 7.5, ΔEnz is at least 8.0 or ΔEnz is at least 8.5, ΔEnz is at least 9.0, ΔEnz is at least 9.5 or ΔEnz is at least 10.0 when measured with the TOM assay.
18. Use according to claims 1 and 4-17, wherein the textile is made of cotton, cotton/polyester or nylon.
19. Use according to any of the preceding claims, wherein the pH of the aquous solution is in the range of 7 to 10, preferably 7 to 9 such as 7.5.
20. Use according to any of the preceding claims, wherein the temperature is in the range of 10-60°C, such as 15-50°C, or 30-45°C.
21. A composition comprising a surfactant and an enzyme exhibiting hydrolase activity.
22. Composition according to claim 21, wherein the enzyme belongs to the GH1 familiy comprised by the enzyme classification EC 3.2.1.-.
23. Composition according to any of the preceding composition claims, wherein the enzyme further exhibits lactase activity
24. Composition according to any of the preceding composition claims, wherein the enzyme exhibits lactase phlorizin hydrolase activity.
25. Composition according to any of the preceding composition claims, wherein the enzyme belongs to the enzyme classisification (EC 3.2.1.23).
26. Composition according to any of the preceding composition claims, wherein the enzyme exhibiting lactase phlorizin hydrolase activity comprises an amino acid sequence which has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 1.
27. Composition according to any of the preceding composition claims, wherein the enzyme exhibiting lactase phlorizin hydrolase activity comprises amino acids 1 to 603 of SEQ ID NO: 1.
28. Composition according to any of the preceding composition claims, wherein the composition further comprises: hydrotropes, builders, co-builders, a bleaching systems, polymers, fabric hueing agents, mediators and/or enzymes.

29. Composition according to any of the preceding composition claims, wherein composition comprises at least one additional enzyme.
30. Composition according to any of the preceding composition claims, wherein the additional enzyme is selected from the group consisting of protease, lipase, cutinase, amylase, carbohydrase, cellulase, pectate lyase, pectinase, mannanase, arabinase, galactanase, and/or xylanase.
31. Composition according to any of the preceding composition claims, wherein the additional enzyme is pectate lyase.
32. Composition according to any of the preceding composition claims, wherein the concentration of the enzyme exhibiting hydrolase activity is in the range of 0.05-1.0% w/w enzyme per detergent composition.
33. Composition according to any of the preceding composition claims, wherein the concentration of the an enzyme exhibiting lactase phlorizin hydrolase activity is in the range of 0.1-0.9% w/w enzyme per detergent composition, in the range of 0.2-0.8% w/w enzyme per detergent composition, in the range of 0.3-0.7% w/w enzyme per detergent composition or in the range of 0.4-0.6% w/w enzyme per detergent composition.
34. Composition according to any of the preceding composition claims, wherein the concentration of the enzyme exhibiting pectin lyase activity is in the range of 0.05-1.0% w/w enzyme per detergent composition.
35. Composition according to any of the preceding composition claims, wherein the concentration of the an enzyme exhibiting pectin lyase activity is in the range of 0.1-0.9% w/w enzyme per detergent composition, in the range of 0.2-0.8% w/w enzyme per detergent composition, in the range of 0.3-0.7% w/w enzyme per detergent composition or in the range of 0.4-0.6% w/w enzyme per detergent composition.
36. Composition to any of the preceding composition claims, wherein the detergent is 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.
37. Composition according to any of the preceding composition claims, wherein the composition is a detergent composition.
38. Composition according to any of the preceding composition claims, wherein the composition is an ADW composition.
39. Composition according to any of the preceding composition claims, wherein the composition is a composition for hard surface cleaning.

40. A method for removing or releasing a flavonoid stain from a textile having a flavonoid stain which method comprises exposing the textile to an aqueous solution of an enzyme exhibiting hydrolase activity.
41. A method for removing or releasing a flavonoid stain from a dish having a flavonoid stain which method comprises exposing the dish to an aqueous solution of an enzyme exhibiting hydrolase activity.
42. A method for removing or releasing a flavonoid stain from a hard surface having a flavonoid stain which method comprises exposing the hard surface to an aqueous solution of an enzyme exhibiting hydrolase activity.
43. Method according to any of the preceding method claims, wherein the enzyme belongs to the GH1 familiy comprised by the enzyme classification EC 3.2.1.-.
44. Method according to any of the preceding method claims, wherein the enzyme further exhibits lactase activity.
45. Method according to any of the preceding method claims, wherein the enzyme exhibits lactase phlorizin hydrolase activity.
46. Method according to any of the preceding method claims, wherein the enzyme belongs to the enzyme classisification (EC 3.2.1.23).
47. Method according to any of the preceding method claims, wherein the enzyme exhibiting lactase phlorizin hydrolase activity comprises an amino acid sequence which has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 1.
48. Method according to any of the preceding method claims, wherein the enzyme exhibiting lactase phlorizin hydrolase activity comprises amino acids 1 to 603 of SEQ ID NO: 1.
49. Method according to any of the preceding method claims, wherein the flavonoid stain is selected from the group consisting of fruit stains, berry stains and/or vegetable stains.
50. Method according to any of the preceding method claims, wherein the flavonoid stain is selected from the group consisting of stains of wherein the flavonoid stain is selected from the group consisting of stains of strawberry, blueberry, cherry, juice, jam, and forest fruit jam.
51. Method according to any of the preceding method claims, wherein the enzyme exhibiting lactase phlorizin hydrolase activity used as an aqueous solution of the enzyme.
52. Method according to any of the preceding method claims, wherein the enzyme exhibiting hydrolase activity is used in combination with at least one additional enzyme.
53. Method according to any of the preceding method claims, wherein the at least one additional enzyme is selected from the group consisting of protease, lipase, cutinase,

amylase, carbohydrase, cellulase, chlorophyllase, pectate lyase, pectinase, mannanase, arabinase, galactanase, and/or xylanase.

54. Method according to any of the preceding method claims, wherein the at least one additional enzyme is a pectate lyase.

55. Method according to any of the preceding method claims, wherein ΔEnz is at least 1 when measured with the TOM assay.

56. Method according to any of the preceding method claims, wherein ΔEnz is at least 1.5, ΔEnz is at least 2.0, ΔEnz is at least 2.5, ΔEnz is at least 3.0, ΔEnz is at least 3.5, ΔEnz is at least 4.0, ΔEnz is at least 4.5, ΔEnz is at least 5.0, ΔEnz is at least 5.5, ΔEnz is at least 6.0, ΔEnz is at least 6.5, ΔEnz is at least 7.0, ΔEnz is at least 7.5, ΔEnz is at least 8.0 or ΔEnz is at least 8.5, ΔEnz is at least 9.0, ΔEnz is at least 9.5 or ΔEnz is at least 10.0 when measured with the TOM assay.

57. Method according to any of the preceding method claims, wherein the textile is made of cotton, cotton/polyester or nylon.

58. Method according to any of the preceding method claims, wherein the pH is in the interval of 7 to 10, preferably 7 to 9 such as 7.5.

59. Method according to any of the preceding method claims, wherein the temperature is in the interval of 10-60°C, preferably 15-50°C, preferably 30-45°C.

60. Method according to any of the preceding method claims, wherein the method comprises applying mechanical stress to the textile.

61. Textile treated according to the method of claims 40-60.

Figure 1

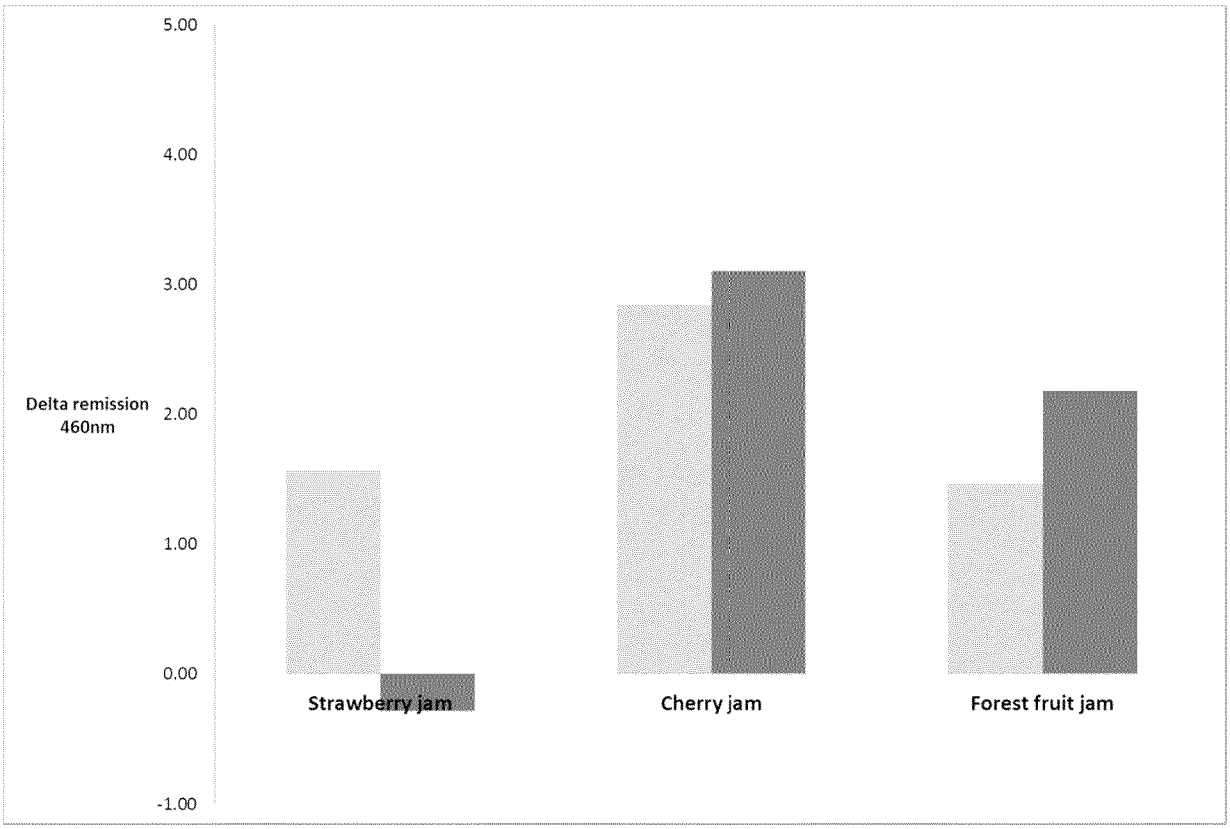
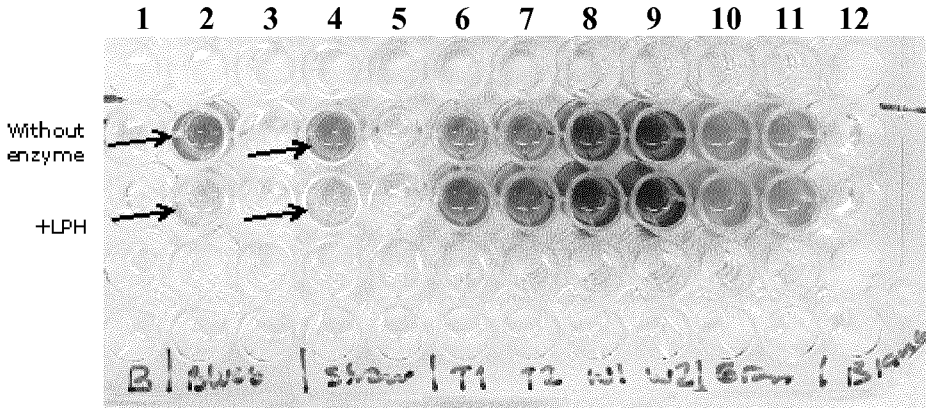


Figure 2



Blanc| blue | straw | T1 T2 W1 W2| Grass |Blanc

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/060703

A. CLASSIFICATION OF SUBJECT MATTER
INV. C11D3/386
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, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/012904 A1 (NOVOZYMES AS [DK]; SCHNORR KIRK MATTHEW [DK]; LANGE LENE [DK]; STEPHEN) 9 February 2006 (2006-02-09) cited in the application see whole doc. esp. p. 42, l.7 ff.	1-61
X	WO 97/28243 A1 (NOVO NORDISK AS [DK]; VON DER OSTEN CLAUS [DK]; CHERRY JOEL R [US]; BJ) 7 August 1997 (1997-08-07) the whole document	1-3,21, 40-42,61
X	US 2009/176682 A1 (BOUTIQUE JEAN-POL [BE] ET AL) 9 July 2009 (2009-07-09) the whole document	1,21, 40-42,61

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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Date of the actual completion of the international search	Date of mailing of the international search report
8 July 2014	16/07/2014

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