

PERACID-GENERATING COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to United States Application No. 61/577,499, filed on 19 December 2011, which is hereby incorporated by reference in its entirety.

BACKGROUND

[0001] There exists a need for whitening strips suitable for home use, having reduced total levels of peroxide, yet providing enhanced whitening activity.

SUMMARY

[0002] The invention provides whitening strips comprising a granular bleaching ingredient in combination with an enzyme having perhydrolytic activity (“perhydrolase”) which comprises the conserved structural motif of the carbohydrate esterase family 7 and an acyl donor, such that upon use, the peroxide released by the granular bleaching ingredient reacts with the acyl donor in the presence of the perhydrolase to form a peracid, in direct proximity to the teeth, without substantial dilution from formulation excipients, thereby permitting enhanced bleaching of the teeth with much lower total amounts of peroxide.

[0003] The strips comprise an adhesive film, either single layer or multiple layers (e.g., two layers), which when hydrated with water or saliva becomes sufficiently adhesive to stick to teeth. Granular bleaching ingredient is attached to the side of the film to be placed in contact with the teeth. Upon application, the bleaching ingredient is placed directly on the teeth (that is, between the teeth and the adhesive layer). The granules then release peroxide by rapidly dissolving in water. The bleaching ingredient can be optionally coated by a quickly dissolving material, such as sodium sulfate, cornstarch or gum Arabic. Optionally, the strips provide a second layer in the film is present to prolong the exposure time. This second layer can be insoluble in water, which would require the user to remove the strip after treatment, or erodible in water, which would cause the strip to dissolve after sufficient treatment. The strip further comprises a perhydrolase (an enzyme capable of catalyzing the reaction of carboxylic acid ester and hydrogen peroxide to form a peracid), which may also be provided in granular form on the

surface of the film, and an acyl donor, e.g., selected from carboxylic acids and acyl compounds, for example, triacetin or sorbitol hexaacetate, wherein the acyl donor reacts with the peroxide source in the strip in the presence of the perhydrolase to form a peracid, which enhances the bleaching action of the strip.

[0004] Some embodiments of the present invention provide a tooth whitening strip comprising a hydratable adhesive film with a first side and a second side, the first side having a granular bleaching ingredient attached thereto, wherein the tooth whitening strip further comprises, in or on the film or in the form of granules attached to the first side of the film;

a) an enzyme having perhydrolytic activity, said enzyme having a carbohydrate esterase family 7 (CE-7) signature motif that aligns with a reference sequence SEQ ID NO: 1, said signature motif comprising:

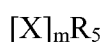
i) an RGQ motif at positions corresponding to positions 118-120 of SEQ ID NO: 1;

ii) a GXSQG motif at positions corresponding to positions 186-190 of SEQ ID NO:1; and

iii) an HE motif at positions corresponding to positions 303-304 of SEQ ID NO:1; and

(b) at least one acyl donor substrate, said substrate selected from the group consisting of:

i) esters having the structure



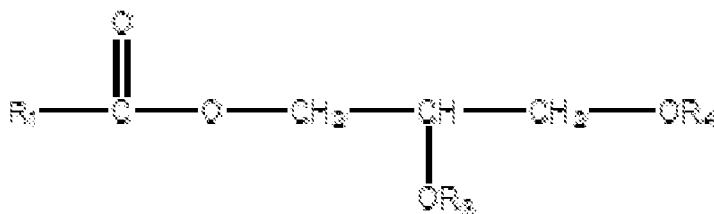
wherein X = an ester group of the formula $R_6C(O)O$

R_6 = C1 to C7 linear, branched or cyclic hydrocarbyl moiety, optionally substituted with hydroxyl groups or C1 to C4 alkoxy groups, wherein R_6 optionally comprises one or more ether linkages for R_6 = C2 to C7;

R_5 = a C1 to C6 linear, branched, or cyclic hydrocarbyl moiety or a five-membered cyclic heteroaromatic moiety or six-membered cyclic aromatic or heteroaromatic moiety optionally substituted with hydroxyl groups; wherein each carbon atom in R_5 individually comprises no more than one hydroxyl group or no more than one ester group or carboxylic acid group; wherein R_5 optionally comprises one or more ether linkages;

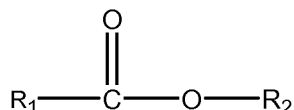
M is an integer ranging from 1 to the number of carbon atoms in R_5 ; and
wherein said esters have solubility in water of at least 5 ppm at 25 °C;

ii) glycerides having the structure



wherein R_1 = C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R_3 and R_4 are individually H or $R_1\text{C}(\text{O})$;

iii) one or more esters of the formula



wherein R_1 is a C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R_2 is a C1 to C10 straight chain or branched chain alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, heteroaryl, $(\text{CH}_2\text{CH}_2\text{O})_n$, or $(\text{CH}_2\text{CH}(\text{CH}_3)\text{-O})_n\text{H}$ and n is 1 to 10; and

iv) acetylated saccharides selected from the group consisting of acetylated monosaccharides, acetylated disaccharides, and acetylated polysaccharide; wherein upon hydration of the hydratable adhesive film hydrogen peroxide is released from the granular bleaching ingredient and said enzyme catalyzes the formation of an effective amount of a peracid.

[0005] Other embodiments of the present invention provide a method of whitening teeth comprising providing a packaging system comprising the tooth whitening strip according to any foregoing claim; removing the tooth whitening strip from the packaging system; and contacting the tooth whitening strip directly to the teeth for a period of time sufficient time

whiten the teeth; wherein the tooth whitening strip is hydrated by moisture present in the oral cavity or on the tooth surface or is hydrated after step (b) but prior to step (c).

[0006] Further areas of applicability of the present invention will become apparent from the detailed description provided hereinafter. It should be understood that the detailed description and specific examples, while indicating the preferred embodiment of the invention, are intended for purposes of illustration only and are not intended to limit the scope of the invention.

BRIEF DESCRIPTION OF THE BIOLOGICAL SEQUENCES

[0007] The following sequences comply with 37 C.F.R. §§ 1.821-1.825 (“Requirements for Patent Applications Containing Nucleotide Sequences and/or Amino Acid Sequence Disclosures - the Sequence Rules”) and are consistent with World Intellectual Property Organization (WIPO) Standard ST.25 (2009) and the sequence listing requirements of the European Patent Convention (EPC) and the Patent Cooperation Treaty (PCT) Rules 5.2 and 49.5(a-bis), and Section 208 and Annex C of the Administrative Instructions. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. § 1.822.

[0008] SEQ ID NO: 1 is the amino acid sequence of *Thermotoga maritima* C277S variant perhydrolase.

[0009] SEQ ID NO: 2 is the amino acid sequence of fusion protein comprising the *Thermotoga maritima* C277S variant perhydrolase coupled to a tooth binding domain (also known as “EZ-7” in International Patent Application Publication No. WO2012/087970A2 to Butterick *et al.*).

[0010] SEQ ID NO: 3 is the nucleic acid sequence encoding a cephalosporin C deacetylase from *Bacillus subtilis* ATCC[®] 31954[™].

[0011] SEQ ID NO: 4 is the amino acid sequence of a cephalosporin C deacetylase from *Bacillus subtilis* ATCC[®] 31954[™].

[0012] SEQ ID NO: 5 is the amino acid sequence of a cephalosporin C deacetylase from *Bacillus subtilis* subsp. *subtilis* strain 168.

[0013] SEQ ID NO: 6 is the amino acid sequence of a cephalosporin C deacetylase from *B. subtilis* ATCC[®] 6633[™].

[0014] SEQ ID NO: 7 is the amino acid sequence of a cephalosporin C deacetylase from *B. licheniformis* ATCC[®] 14580[™].

[0015] SEQ ID NO: 8 is the amino acid sequence of an acetyl xylan esterase from *B. pumilus* PS213.

[0016] SEQ ID NO: 9 is the amino acid sequence of an acetyl xylan esterase from *Clostridium thermocellum* ATCC® 27405™.

[0017] SEQ ID NO: 10 is the amino acid sequence of an acetyl xylan esterase from *Thermotoga neapolitana*.

[0018] SEQ ID NO: 11 is the amino acid sequence of an acetyl xylan esterase from *Thermotoga maritima* MSB8.

[0019] SEQ ID NO: 12 is the amino acid sequence of an acetyl xylan esterase from *Thermoanaerobacterium* sp. JW/SL YS485.

[0020] SEQ ID NO: 13 is the amino acid sequence of a cephalosporin C deacetylase from *Bacillus halodurans* C-125.

[0021] SEQ ID NO: 14 is the amino acid sequence of a cephalosporin C deacetylase from *Bacillus clausii* KSM-K16.

[0022] SEQ ID NO: 15 is the amino acid sequence of a *Thermotoga neapolitana* acetyl xylan esterase variant from U.S. Patent Application Publication No. 2010-0087529 (incorporated herein by reference in its entirety), where the Xaa residue at position 277 is Ala, Val, Ser, or Thr.

[0023] SEQ ID NO: 16 is the amino acid sequence of a *Thermotoga maritima* MSB8 acetyl xylan esterase variant from U.S. Patent Application Publication No. 2010-0087529, where the Xaa residue at position 277 is Ala, Val, Ser, or Thr.

[0024] SEQ ID NO: 17 is the deduced amino acid sequence of a *Thermotoga lettingae* acetyl xylan esterase variant from U.S. Patent Application Publication No. 2010-0087529, where the Xaa residue at position 277 is Ala, Val, Ser, or Thr.

[0025] SEQ ID NO: 18 is the amino acid sequence of a *Thermotoga petrophila* acetyl xylan esterase variant from U.S. Patent Application Publication No. 2010-0087529, where the Xaa residue at position 277 is Ala, Val, Ser, or Thr.

[0026] SEQ ID NO: 19 is the amino acid sequence of a *Thermotoga* sp. RQ2 acetyl xylan esterase variant derived from "RQ2(a)" from U.S. Patent Application Publication No. 2010-0087529, where the Xaa residue at position 277 is Ala, Val, Ser, or Thr.

[0027] SEQ ID NO: 20 is the amino acid sequence of a *Thermotoga* sp. RQ2 acetyl xylan esterase variant derived from “RQ2(b)” from U.S. Patent Application Publication No. 2010-0087529, where the Xaa residue at position 278 is Ala, Val, Ser, or Thr.

[0028] SEQ ID NO: 21 is the amino acid sequence of a *Thermotoga lettingae* acetyl xylan esterase.

[0029] SEQ ID NO: 22 is the amino acid sequence of a *Thermotoga petrophila* acetyl xylan esterase.

[0030] SEQ ID NO: 23 is the amino acid sequence of a first acetyl xylan esterase from *Thermotoga* sp. RQ2 described as “RQ2(a)”.

[0031] SEQ ID NO: 24 is the amino acid sequence of a second acetyl xylan esterase from *Thermotoga* sp. RQ2 described as “RQ2(b)”.

[0032] SEQ ID NO: 25 is the amino acid sequence of a *Thermoaneorobacterium saccharolyticum* cephalosporin C deacetylase.

[0033] SEQ ID NO: 26 is the amino acid sequence of the acetyl xylan esterase from *Lactococcus lactis* (GENBANK[®] accession number ABX75634.1).

[0034] SEQ ID NO: 27 is the amino acid sequence of the acetyl xylan esterase from *Mesorhizobium loti* (GENBANK[®] accession number BAB53179.1).

[0035] SEQ ID NO: 28 is the amino acid sequence of the acetyl xylan esterase from *Geobacillus stearothermophilus* (GENBANK[®] accession number AAF70202.1).

[0036] SEQ ID NOs 29-163 are the amino acid sequences of peptides having affinity to an oral cavity surface.

[0037] SEQ ID NOs: 164-177 are the amino acid sequences of peptide linkers/spacers.

SEQ ID NOs: 178-197 are the amino acid sequences of various targeted perhydrolase fusion constructs comprising a perhydrolytic enzyme couple via a peptide linker to a binding domain having affinity for an oral surface (see International Patent Application Publication No. WO2012/087970A2 to Butterick *et al.*).

DETAILED DESCRIPTION

[0038] The following description of the preferred embodiment(s) is merely exemplary in nature and is in no way intended to limit the invention, its application, or uses.

[0039] As used herein, the articles “a”, “an”, and “the” preceding an element or component of the invention are intended to be nonrestrictive regarding the number of instances (*i.e.*, occurrences) of the element or component. Therefore “a”, “an”, and “the” should be read to include one or at least one, and the singular word form of the element or component also includes the plural unless the number is obviously meant to be singular.

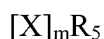
[0040] As used herein, the term “comprising” means the presence of the stated features, integers, steps, or components as referred to in the claims, but that it does not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof. The term “comprising” is intended to include embodiments encompassed by the terms “consisting essentially of” and “consisting of”. Similarly, the term “consisting essentially of” is intended to include embodiments encompassed by the term “consisting of”.

[0041] As used herein, the term “about” modifying the quantity of an ingredient or reactant employed refers to variation in the numerical quantity that can occur, for example, through typical measuring and liquid handling procedures used for making concentrates or use solutions in the real world; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of the ingredients employed to make the compositions or carry out the methods; and the like. The term “about” also encompasses amounts that differ due to different equilibrium conditions for a composition resulting from a particular initial mixture. Whether or not modified by the term “about”, the claims include equivalents to the quantities.

[0042] Where present, all ranges are inclusive and combinable. For example, when a range of “1 to 5” is recited, the recited range should be construed as including ranges “1 to 4”, “1 to 3”, “1-2”, “1-2 & 4-5”, “1-3 & 5”, and the like.

[0043] As used herein, the terms “substrate”, “suitable substrate”, “acyl donor”, and “carboxylic acid ester substrate” interchangeably refer specifically to:

(a) one or more esters having the structure



wherein

X is an ester group of the formula $R_6C(O)O$;

R_6 is a C1 to C7 linear, branched or cyclic hydrocarbyl moiety, optionally substituted with a hydroxyl group or C1 to C4 alkoxy group, wherein R_6 optionally comprises one or more ether linkages where R_6 is C2 to C7;

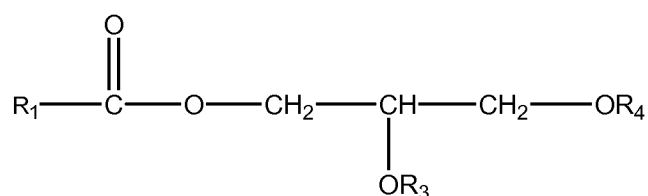
R_5 is a C1 to C6 linear, branched, or cyclic hydrocarbyl moiety or a cyclic five-membered heteroaromatic or six-membered cyclic aromatic or heteroaromatic moiety optionally substituted with a hydroxyl group; wherein each carbon atom in R_5 individually comprises no more than one hydroxyl group or no more than one ester group, and wherein R_5 optionally comprises one or more ether linkages;

m is an integer ranging from 1 to the number of carbon atoms in R_5 ,

said one or more esters having solubility in water of at least 5 ppm at 25

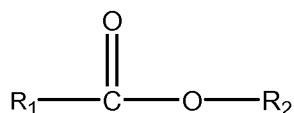
°C; or

(b) one or more glycerides having the structure



wherein R_1 is a C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R_3 and R_4 are individually H or $R_1\text{C}(\text{O})$; or

(c) one or more esters of the formula



wherein R_1 is a C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R_2 is a C1 to C10 straight chain or branched chain alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, heteroaryl, $(\text{CH}_2\text{CH}_2\text{O})_n$, or $(\text{CH}_2\text{CH}(\text{CH}_3)-\text{O})_n\text{H}$ and n is 1 to 10; or

(d) one or more acetylated monosaccharides, acetylated disaccharides, or acetylated polysaccharides; or

(e) any combination of (a) through (d).

[0044] As used herein, the term “peracid” is synonymous with peroxyacid, peroxycarboxylic acid, peroxy acid, percarboxylic acid and peroxy acid.

[0045] As used herein, the term “peracetic acid” is abbreviated as “PAA” and is synonymous with peroxyacetic acid, ethaneperoxy acid and all other synonyms of CAS Registry Number 79-21-0.

[0046] As used herein, the term “monoacetin” is synonymous with glycerol monoacetate, glycerin monoacetate, and glyceryl monoacetate.

[0047] As used herein, the term “diacetin” is synonymous with glycerol diacetate; glycerin diacetate, glyceryl diacetate, and all other synonyms of CAS Registry Number 25395-31-7.

[0048] As used herein, the term “triacetin” is synonymous with glycerin triacetate; glycerol triacetate; glyceryl triacetate, 1,2,3-triacetoxyp propane; 1,2,3-propanetriol triacetate and all other synonyms of CAS Registry Number 102-76-1.

[0049] As used herein, the terms “acetylated sugar” and “acetylated saccharide” refer to mono-, di- and polysaccharides comprising at least one acetyl group. Examples include, but are not limited to glucose pentaacetate; xylose tetraacetate; acetylated xylan; acetylated xylan fragments; β -D-ribofuranose-1,2,3,5-tetraacetate; tri-O-acetyl-D-galactal; and tri-O-acetyl-glucal.

[0050] As used herein, the terms “hydrocarbyl”, “hydrocarbyl group”, and “hydrocarbyl moiety” is meant a straight chain, branched or cyclic arrangement of carbon atoms connected by single, double, or triple carbon to carbon bonds and/or by ether linkages, and substituted accordingly with hydrogen atoms. Such hydrocarbyl groups may be aliphatic and/or aromatic. Examples of hydrocarbyl groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, cyclopropyl, cyclobutyl, pentyl, cyclopentyl, methylcyclopentyl, hexyl, cyclohexyl, benzyl, and phenyl. In a preferred embodiment, the hydrocarbyl moiety is a straight chain, branched or cyclic arrangement of carbon atoms connected by single carbon to carbon bonds and/or by ether linkages, and substituted accordingly with hydrogen atoms.

[0051] As used herein, the terms “monoesters” and “diesters” of 1,2-ethanediol; 1,2-propanediol; 1,3-propanediol; 1,2-butanediol; 1,3-butanediol; 2,3-butanediol; 1,4-butanediol; 1,2-pentanediol; 2,5-pentanediol; 1,5-pentandiol; 1,6-pentanediol; 1,2-hexanediol; 2,5-hexanediol; 1,6-hexanediol; and mixtures thereof, refer to said compounds comprising at least one ester group of the formula $RC(O)O$, wherein R is a C1 to C7 linear hydrocarbyl moiety. In

one embodiment, the carboxylic acid ester substrate is selected from the group consisting of propylene glycol diacetate (PGDA), ethylene glycol diacetate (EDGA), and mixtures thereof.

[0052] As used herein, the term “propylene glycol diacetate” is synonymous with 1,2-diacetoxypropane, propylene diacetate, 1,2-propanediol diacetate, and all other synonyms of CAS Registry Number 623-84-7.

[0053] As used herein, the term “ethylene glycol diacetate” is synonymous with 1,2-diacetoxyethane, ethylene diacetate, glycol diacetate, and all other synonyms of CAS Registry Number 111-55-7.

[0054] As used herein, the terms “suitable enzymatic reaction mixture”, “components suitable for *in situ* generation of a peracid”, “suitable reaction components”, “suitable aqueous reaction mixture”, “reaction mixture”, and “peracid-generating components” refer to the materials and water (from saliva and/or applied by the user to the hydratable adhesive film prior to use) in which the reactants and the perhydrolytic enzyme catalyst come into contact. The peracid-generating components will include at least enzyme having perhydrolytic activity, preferably wherein the perhydrolytic enzyme is at least one CE-7 perhydrolase (optionally in the form of a fusion protein targeted to a body surface), at least one suitable carboxylic acid ester substrate, a source of peroxygen, and water (from saliva and/or applied by the user to the hydratable adhesive film prior to use).

[0055] As used herein, the term “perhydrolysis” is defined as the reaction of a selected substrate with peroxide to form a peracid. Typically, inorganic peroxide is reacted with the selected substrate in the presence of a catalyst to produce the peroxycarboxylic acid. As used herein, the term “chemical perhydrolysis” includes perhydrolysis reactions in which a substrate (a peroxycarboxylic acid precursor) is combined with a source of hydrogen peroxide wherein peroxycarboxylic acid is formed in the absence of an enzyme catalyst. As used herein, the term “enzymatic perhydrolysis” includes perhydrolysis reactions in which a carboxylic acid ester substrate (a peracid precursor; the “acyl donor”) is combined with a source of hydrogen peroxide and water whereby the enzyme catalyst catalyzes the formation of peracid.

[0056] As used herein, the term “perhydrolase activity” refers to the catalyst activity per unit mass (for example, milligram) of protein, dry cell weight, or immobilized catalyst weight.

[0057] As used herein, “one unit of enzyme activity” or “one unit of activity” or “U” is defined as the amount of perhydrolase activity required for the production of 1 μ mol of peroxycarboxylic acid product per minute at a specified temperature.

[0058] As used herein, the terms “enzyme catalyst” and “perhydrolase catalyst” refer to a catalyst comprising an enzyme having perhydrolysis activity and may be in the form of a whole microbial cell, permeabilized microbial cell(s), one or more cell components of a microbial cell extract, partially purified enzyme, or purified enzyme. The enzyme catalyst may also be chemically modified (such as by pegylation or by reaction with cross-linking reagents). The perhydrolase catalyst may also be immobilized on a soluble or insoluble support using methods well-known to those skilled in the art; see for example, Immobilization of Enzymes and Cells; Gordon F. Bickerstaff, Editor; Humana Press, Totowa, NJ, USA; 1997. In one embodiment, the perhydrolase catalyst may be immobilized non-covalently in or on an oral care strip (e.g., a whitening strip) or dental tray. In a further embodiment, the non-covalent immobilization to the strip or dental tray may be through the use of a peptidic binding domain having strong affinity for a material in or on the strip or tray (e.g., a fusion protein comprising a perhydrolytic enzyme coupled through an optional peptide spacer to a peptidic binding domain). In another embodiment, the dental tray is deformable tray. In yet a further embodiment, the perhydrolase catalyst is immobilized in or on the deformable tray after the formation of the dental impression.

[0059] As used herein, “acetyl xylan esterases” refers to an enzyme (E.C. 3.1.1.72; AXEs) that catalyzes the deacetylation of acetylated xylans and other acetylated saccharides.

[0060] As used herein, the terms “cephalosporin C deacetylase” and “cephalosporin C acetyl hydrolase” refer to an enzyme (E.C. 3.1.1.41) that catalyzes the deacetylation of cephalosporins such as cephalosporin C and 7-aminocephalosporanic acid (Mitsushima *et al.*, (1995) *Appl. Env. Microbiol.* 61(6):2224-2229). The amino acid sequences of several cephalosporin C deacetylases having significant perhydrolytic activity are provided herein.

[0061] As used herein, the term “*Bacillus subtilis* ATCC[®] 31954[™]” refers to a bacterial cell deposited to the American Type Culture Collection (ATCC) having international depository accession number ATCC[®] 31954[™]. As described herein, an enzyme having significant perhydrolase activity from *B. subtilis* ATCC[®] 31954[™] is provided as SEQ ID NO: 4 (see United States Patent Application Publication No. 2010-0041752).

[0062] As used herein, the term “*Thermotoga maritima* MSB8” refers to a bacterial cell reported to have acetyl xylan esterase activity (GENBANK[®] NP_227893.1; see U.S. Patent Application Publication No. 2008-0176299). The amino acid sequence of the enzyme having perhydrolase activity from *Thermotoga maritima* MSB8 is provided as SEQ ID NO: 11. Variants of the *Thermotoga maritima* MSB8 perhydrolase are provided as SEQ ID NOs: 1 and 16.

[0063] As used herein, an “isolated nucleic acid molecule”, “isolated polynucleotide”, and “isolated nucleic acid fragment” will be used interchangeably and refer to a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid molecule in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

[0064] The term “amino acid” refers to the basic chemical structural unit of a protein or polypeptide. The following abbreviations are used herein to identify specific amino acids:

<u>Amino Acid</u>	<u>Three-Letter Abbreviation</u>	<u>One-Letter Abbreviation</u>
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P

Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid or as defined herein	Xaa	X

[0065] As used herein, the term “about” modifying the quantity of an ingredient or reactant employed refers to variation in the numerical quantity that can occur, for example, through typical measuring and liquid handling procedures used for making concentrates or As used herein, the terms “signature motif” and “diagnostic motif” refer to conserved structures shared among a family of enzymes having a defined activity. The signature motif can be used to define and/or identify the family of structurally-related enzymes having similar enzymatic activity for a defined family of substrates. The signature motif can be a single contiguous amino acid sequence or a collection of discontinuous, conserved motifs that together form the signature motif. Typically, the conserved motif(s) is represented by an amino acid sequence. In one embodiment, the perhydrolytic enzymes used in the present compositions and methods comprise a CE-7 carbohydrate esterase signature motif.

[0066] As used herein, the term “sequence analysis software” refers to any computer algorithm or software program that is useful for the analysis of nucleotide or amino acid sequences. “Sequence analysis software” may be commercially available or independently developed. Typical sequence analysis software will include, but is not limited to, the GCG suite of programs (Wisconsin Package Version 9.0, Accelrys Software Corp., San Diego, CA), BLASTP, BLASTN, BLASTX (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)), and DNASTAR (DNASTAR, Inc. 1228 S. Park St. Madison, WI 53715 USA), CLUSTALW (for example, version 1.83; Thompson *et al.*, *Nucleic Acids Research*, 22(22):4673-4680 (1994)), and the FASTA program incorporating the Smith-Waterman algorithm (W. R. Pearson, *Comput. Methods Genome Res.*, [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Publisher: Plenum, New York, NY), Vector NTI (Informax, Bethesda, MD) and Sequencher v. 4.05. Within the context of this application it will be understood that where sequence analysis software is used for analysis, that the results of the

analysis will be based on the “default values” of the program referenced, unless otherwise specified. As used herein “default values” will mean any set of values or parameters set by the software manufacturer that originally load with the software when first initialized.

[0067] The term "body surface" refers to any surface of the human body that may serve as the target for a benefit agent, such as a peracid benefit agent. The present methods and compositions are directed to oral care applications and products. As such, the body surface comprises an oral cavity material/surface. In one embodiment, the oral cavity material comprises tooth enamel.

[0068] As used herein, the terms “tooth whitening” and “tooth bleaching” are used interchangeably, to refer to improving the brightness (*e.g.*, whitening) of a tooth or teeth. Whitening strips are described herein comprising ingredients suitable to enzymatically generate an efficacious amount of a peracid to whiten teeth when hydrated.

[0069] As used in herein, “intrinsic stains” in teeth refer to the resulting color from chromogens within the enamel and underlying dentin. The intrinsic color of human teeth tends to become more yellow with aging, due to the thinning of the enamel and darkening of the underlying yellow dentin. Removal of intrinsic stain usually requires the use of peroxides or other oxidizing chemicals, which penetrate the enamel and decolorize the internal chromogens.

[0070] In contrast to intrinsic stains, “extrinsic stains” form on the surface of the teeth when exogenous chromogenic materials bind to the enamel, usually within the pellicle naturally coating the teeth. Most people accumulate some degree of unsightly extrinsic stains on their teeth over time. This staining process is promoted by such factors as: (1) the ingestion of tannin-containing foods and beverages such as coffee, tea, or red wine; (2) the use of tobacco products; and/or (3) exposure to certain cationic substances (*e.g.*, tin, iron, and chlorhexidine). These substances tend to adhere to the enamel's hydroxyapatite structure, which leads to tooth discoloration and a concomitant reduction in tooth whiteness. Over a period of years, extrinsic stains may penetrate the enamel layer and result in intrinsic stains.

[0071] As used herein, the term “destain” or “destaining” refers to the process of removing a stain from an oral cavity surface. The stain(s) may be intrinsic stains, extrinsic stains, or a combination thereof.

[0072] As used herein, “effective amount of perhydrolase enzyme” refers to the quantity of perhydrolase enzyme necessary to achieve the enzymatic activity required in the specific

application. Such effective amounts are readily ascertained by one of ordinary skill in the art and are based on many factors, such as the particular enzyme variant used.

[0073] As used herein, the terms “peroxygen source” and “source of peroxygen” refer to compounds capable of providing hydrogen peroxide at a concentration of about 1 mM or more when in an aqueous solution including, but not limited to, hydrogen peroxide, hydrogen peroxide adducts (*e.g.*, urea-hydrogen peroxide adduct (carbamide peroxide)), perborates, and percarbonates. As described herein, the peroxygen source in the present whitening strips is in the form of granular particles, wherein the user hydrates the granular peroxide particles to release an effective amount of hydrogen peroxide. As described herein, the concentration of the hydrogen peroxide provided by the peroxygen compound in the aqueous reaction formulation is initially at least 0.1 mM or more upon combining the reaction components. In one embodiment, the hydrogen peroxide concentration in the aqueous reaction formulation is at least 0.5 mM. In one embodiment, the hydrogen peroxide concentration in the aqueous reaction formulation is at least 1 mM. In another embodiment, the hydrogen peroxide concentration in the aqueous reaction formulation is at least 10 mM. In another embodiment, the hydrogen peroxide concentration in the aqueous reaction formulation is at least 100 mM. In another embodiment, the hydrogen peroxide concentration in the aqueous reaction formulation is at least 200 mM. In another embodiment, the hydrogen peroxide concentration in the aqueous reaction formulation is 500 mM or more. In yet another embodiment, the hydrogen peroxide concentration in the aqueous reaction formulation is 1000 mM or more. The molar ratio of the hydrogen peroxide to enzyme substrate, *e.g.*, triglyceride, (H_2O_2 :substrate) in the formulation may be from about 0.002 to 20, preferably about 0.1 to 10, and most preferably about 0.5 to 5.

[0074] As used herein, the term “oligosaccharide” refers to compounds containing between 2 and at least 24 monosaccharide units linked by glycosidic linkages. The term “monosaccharide” refers to a compound of empirical formula $(\text{CH}_2\text{O})_n$, where $n \geq 3$, the carbon skeleton is unbranched, each carbon atom except one contains a hydroxyl group, and the remaining carbon atom is an aldehyde or ketone at carbon atom 1. The term “monosaccharide” also refers to intracellular cyclic hemiacetal or hemiketal forms.

[0075] As used herein, the term “hydratable adhesive” will refer to an adhesive material capable of being hydrated. The hydratable adhesive is substantially dry and non-adhesive until hydrated. Upon hydration, the hydratable adhesive becomes sufficiently adhesive to bind the

tooth whitening strip/film to the surface of a tooth. The hydratable adhesive film also comprises a granular bleaching ingredient whereby upon hydration and effective amount of hydrogen peroxide is released to be used in the enzymatic formation of a peracid bleaching agent. The whitening strip/film is typically thin (typically less than 2 mm), shaped and sized to fit within the oral cavity, and sufficiently flexible such that the film can be applied and placed in contact with a plurality of teeth whereby the hydrated adhesive helps to hold the film/strip on the tooth surface and provide a sufficient amount of time for the peracid bleaching agent to whiten the teeth.

[0076] As used herein, the term “effective amount” will refer to the amount of material necessary to achieve the desired effect.

[0077] As used herein, the term “substantially non-adhesive until hydrated” will refer to the lack of adhesive strength sufficient to adhere the tooth whitening film to the surface of a plurality of teeth prior to hydration. As such, the hydratable adhesive film will be easy to handle and manipulate prior to application/hydration by the user.

[0078] By “sequence identity” is meant amino acid identity using a sequence alignment program, e.g., ClustalW or BLAST, e.g., generally as described in Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, "Basic local alignment search tool", J Mol Biol (1990) 215 (3): 403–410, and Goujon M, McWilliam H, Li W, Valentin F, Squizzato S, Paern J, Lopez R, Nucleic Acids Research (2010) 38 Suppl: W695-9.

[0079] Acyl donors for use in the present invention, for example, to form peracids upon reaction with peroxide, are selected from one or more of (i) C₂₋₁₈ carboxylic acids, e.g. C₂₋₆ carboxylic acids (*e.g.*, acetic acid), including lower linear or branched alkyl carboxylic acids, optionally substituted with hydroxy and/or C₁₋₄ alkoxy; (ii) hydrolysable and acceptable esters thereof (*e.g.* mono-, di-, and tri-glycerides and acylated saccharides) and (iii) mixtures thereof. For example, acyl donors include 1,2,3-triacetoxyp propane (sometimes referred to herein as triacetin or glycerin triacetate) and acylated saccharides, *e.g.* acetylated saccharides. In a particular embodiment, esters for this use may, for example, be esters having solubility in water of at least 5 ppm at 25 °C.

[0080] The acyl donors and/or enzymes may optionally be encapsulated. There are a variety of encapsulation options well-known to the art, both natural and synthetic. Modified starches and gum Arabic are particularly well-suited since they are food grade, relatively inexpensive, quick

to dissolve, and can adsorb fairly high levels of liquid oils. Any impact on the final viscosity needs to be considered.

[0081] In some embodiments, the granules comprise an antisensitivity agent capable of desensitizing the nerves or occluding dentine tubules. In some embodiments, the antisensitivity agent is selected from a potassium ion source, a silicate, a stannous ion source, a basic amino acid, a clay, and a combination thereof. In some embodiments, the potassium ion source is an orally- acceptable potassium salt and is present in an amount effective to reduce dentinal sensitivity. In some embodiments, the potassium ion source is selected from potassium chloride, potassium nitrate and a combination thereof. In some embodiments, the basic amino acid is arginine. In some embodiments, the basic amino acid is selected from arginine phosphate, arginine bicarbonate, and arginine hydrochloride. In some embodiments, the silicate is calcium silicate.

CE-7 Perhydrolases

[0082] The present compositions and method comprise enzymes having perhydrolytic activity that are structurally classified as members of the carbohydrate family esterase family 7 (CE-7 family) of enzymes (see Coutinho, P.M., Henrissat, B. “Carbohydrate-active enzymes: an integrated database approach” in Recent Advances in Carbohydrate Bioengineering, H.J. Gilbert, G. Davies, B. Henrissat and B. Svensson eds., (1999) The Royal Society of Chemistry, Cambridge, pp. 3-12.). The CE-7 family of enzymes has been demonstrated to be particularly effective for producing peroxycarboxylic acids from a variety of carboxylic acid ester substrates when combined with a source of peroxygen (U.S. Patents 7,794,378; 7,951,566; 7,723,083; and 7,964,378 and U.S. Patent Application Publication Nos. 2008-0176299, 2010-0087529, 2011-0081693, and 2011-0236335 to DiCosimo *et al.*; each incorporated herein by reference).

[0083] Members of the CE-7 family include cephalosporin C deacetylases (CAHs; E.C. 3.1.1.41) and acetyl xylan esterases (AXEs; E.C. 3.1.1.72). Members of the CE-7 esterase family share a conserved signature motif (Vincent *et al.*, *J. Mol. Biol.*, 330:593-606 (2003)). Perhydrolases comprising the CE-7 signature motif (“CE-7 perhydrolases”) and/or a substantially similar structure are suitable for use in the compositions and methods described herein. Means to identify substantially similar biological molecules are well known in the art (*e.g.*, sequence alignment protocols, nucleic acid hybridizations and/or the presence of a conserved signature motif). In one aspect, the perhydrolase includes an enzyme comprising the

CE-7 signature motif and at least 20%, preferably at least 30%, more preferably at least 33%, more preferably at least 40%, more preferably at least 42%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, and most preferably at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity to one of the sequences provided herein.

[0084] As used herein, the phrase “enzyme is structurally classified as a CE-7 enzyme”, “CE-7 perhydrolase” or “structurally classified as a carbohydrate esterase family 7 enzyme” will be used to refer to enzymes having perhydrolytic activity which are structurally classified as a CE-7 carbohydrate esterase. This family of enzymes can be defined by the presence of a signature motif (Vincent et al., *supra*). The signature motif for CE-7 esterases comprises three conserved motifs (residue position numbering relative to reference sequence SEQ ID NO: 1; a C277S variant of the *Thermotoga maritima* perhydrolase).

Arg118-Gly119-Gln120;

Gly186-Xaa187-**Ser188**-Gln189-Gly190; and

His303-Glu304.

[0085] Typically, the Xaa at amino acid residue position 187 is glycine, alanine, proline, tryptophan, or threonine. Two of the three amino acid residues belonging to the catalytic triad are in bold. In one embodiment, the Xaa at amino acid residue position 187 is selected from the group consisting of glycine, alanine, proline, tryptophan, and threonine.

[0086] Further analysis of the conserved motifs within the CE-7 carbohydrate esterase family indicates the presence of an additional conserved motif (LXD at amino acid positions 272-274 of SEQ ID NO: 1) that may be used to further define a perhydrolase belonging to the CE-7 carbohydrate esterase family. In a further embodiment, the signature motif defined above may include an additional (fourth) conserved motif defined as:

Leu272-Xaa273-**Asp274**.

The Xaa at amino acid residue position 273 is typically isoleucine, valine, or methionine. The fourth motif includes the aspartic acid residue (bold) belonging to the catalytic triad (**Ser188-Asp274-His303**).

[0087] The CE-7 perhydrolases may be in the form of fusion proteins having at least one peptidic component having affinity for at least one body surface. In one embodiment, all alignments used to determine if a targeted perhydrolase (fusion protein) comprises the CE-7

signature motif will be based on the amino acid sequence of the perhydrolytic enzyme without the peptidic component having the affinity for a body surface.

[0088] A number of well-known global alignment algorithms (*i.e.*, sequence analysis software) may be used to align two or more amino acid sequences representing enzymes having perhydrolase activity to determine if the enzyme is comprised of the present signature motif. The aligned sequence(s) are compared to the reference sequence (SEQ ID NO: 1) to determine the existence of the signature motif. In one embodiment, a CLUSTAL alignment (such as CLUSTALW) using a reference amino acid sequence (as used herein the perhydrolase sequence (SEQ ID NO: 1)) is used to identify perhydrolases belonging to the CE-7 esterase family. The relative numbering of the conserved amino acid residues is based on the residue numbering of the reference amino acid sequence to account for small insertions or deletions (for example, typically five amino acids or less) within the aligned sequence.

[0089] Examples of other suitable algorithms that may be used to identify sequences comprising the present signature motif (when compared to the reference sequence) include, but are not limited to, Needleman and Wunsch (*J. Mol. Biol.* 48, 443-453 (1970); a global alignment tool) and Smith-Waterman (*J. Mol. Biol.* 147:195-197 (1981); a local alignment tool). In one embodiment, a Smith-Waterman alignment is implemented using default parameters. An example of suitable default parameters include the use of a BLOSUM62 scoring matrix with GAP open penalty = 10 and a GAP extension penalty = 0.5.

[0090] In one embodiment, suitable perhydrolases include enzymes comprising the CE-7 signature motif and at least 20%, preferably at least 30%, 33%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity to SEQ ID NO: 1.

[0091] Examples of suitable CE-7 carbohydrate esterases having perhydrolytic activity include, but are not limited to, enzymes having an amino acid sequence such as SEQ ID NOs: 1, and 4-28. In one embodiment, the enzyme comprises an amino acid sequence selected from the group consisting of 1, 10, 11, 15, and 16.

[0092] As used herein, the term “CE-7 variant”, “variant perhydrolase” or “variant” will refer to CE-7 perhydrolases having a genetic modification that results in at least one amino acid addition, deletion, and/or substitution when compared to the corresponding enzyme (typically the wild type enzyme) from which the variant was derived; so long as the CE-7 signature motif

and the associated perhydrolytic activity are maintained. CE-7 variant perhydrolases may also be used in the present compositions and methods. Examples of CE-7 variants are provided as SEQ ID NOs: 1, 15, 16, 17, 18, 19, and 20. In one embodiment, the variants may include SEQ ID NOs: 1 and 16.

[0093] The skilled artisan recognizes that substantially similar CE-7 perhydrolase sequences (retaining the signature motifs) may also be used in the present compositions and methods. In one embodiment, substantially similar sequences are defined by their ability to hybridize, under highly stringent conditions with the nucleic acid molecules associated with sequences exemplified herein. In another embodiment, sequence alignment algorithms may be used to define substantially similar enzymes based on the percent identity to the DNA or amino acid sequences provided herein.

[0094] As used herein, a nucleic acid molecule is “hybridizable” to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single strand of the first molecule can anneal to the other molecule under appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known and exemplified in Sambrook, J. and Russell, D., T. Molecular Cloning: A Laboratory Manual, Third Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (2001). The conditions of temperature and ionic strength determine the “stringency” of the hybridization. Stringency conditions can be adjusted to screen for moderately similar molecules, such as homologous sequences from distantly related organisms, to highly similar molecules, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes typically determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent hybridization conditions is 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by a final wash of 0.1X SSC, 0.1% SDS, 65°C.

[0095] Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are

possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (Sambrook and Russell, *supra*). For hybridizations with shorter nucleic acids, *i.e.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (Sambrook and Russell, *supra*). In one aspect, the length for a hybridizable nucleic acid is at least about 10 nucleotides. Preferably, a minimum length for a hybridizable nucleic acid is at least about 15 nucleotides in length, more preferably at least about 20 nucleotides in length, even more preferably at least 30 nucleotides in length, even more preferably at least 300 nucleotides in length, and most preferably at least 800 nucleotides in length. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

[0096] As used herein, the term “percent identity” is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, “identity” also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. “Identity” and “similarity” can be readily calculated by known methods, including but not limited to those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1993); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular Biology (von Heinje, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Stockton Press, NY (1991). Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI), the AlignX program of Vector NTI v. 7.0 (Informax, Inc., Bethesda, MD), or the

EMBOSS Open Software Suite (EMBL-EBI; Rice *et al.*, *Trends in Genetics* 16, (6):276-277 (2000)). Multiple alignment of the sequences can be performed using the CLUSTAL method (such as CLUSTALW; for example version 1.83) of alignment (Higgins and Sharp, *CABIOS*, 5:151-153 (1989); Higgins *et al.*, *Nucleic Acids Res.* 22:4673-4680 (1994); and Chenna *et al.*, *Nucleic Acids Res* 31 (13):3497-500 (2003)), available from the European Molecular Biology Laboratory via the European Bioinformatics Institute) with the default parameters. Suitable parameters for CLUSTALW protein alignments include GAP Existence penalty=15, GAP extension =0.2, matrix = Gonnet (*e.g.*, Gonnet250), protein ENDGAP = -1, protein GAPDIST=4, and KTUPLE=1. In one embodiment, a fast or slow alignment is used with the default settings where a slow alignment is preferred. Alternatively, the parameters using the CLUSTALW method (*e.g.*, version 1.83) may be modified to also use KTUPLE =1, GAP PENALTY=10, GAP extension =1, matrix = BLOSUM (*e.g.*, BLOSUM64), WINDOW=5, and TOP DIAGONALS SAVED=5.

[0097] In one aspect, suitable isolated nucleic acid molecules encode a polypeptide having an amino acid sequence that is at least about 20%, preferably at least 30%, 33%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequences reported herein. In another aspect, suitable isolated nucleic acid molecules encode a polypeptide having an amino acid sequence that is at least about 20%, preferably at least 30%, 33%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequences reported herein. Suitable nucleic acid molecules not only have the above homologies, but also typically encode a polypeptide having about 210 to 340 amino acids in length, about 300 to about 340 amino acids, preferably about 310 to about 330 amino acids, and most preferably about 318 to about 325 amino acids in length wherein each polypeptide is characterized as having perhydrolytic activity.

Targeted Perhydrolases

[0098] As used herein, the term “targeted perhydrolase” and “targeted enzyme having perhydrolytic activity” will refer to a fusion proteins comprising at least one perhydrolytic enzyme (wild type or variant thereof) fused/coupled to at least one peptidic component having affinity for a target surface, preferably a targeted body surface. The perhydrolytic enzyme within the targeted perhydrolase may be any CE-7 carbohydrate esterase having perhydrolytic

activity. The CE-7 perhydrolase may be identified by the presence of the CE-7 signature motif that aligns with a reference sequence SEQ ID NO: 1, said signature motif comprising:

- i) an RGQ motif at positions corresponding to positions 118-120 of SEQ ID NO: 1;
- ii) a GXSQG motif at positions corresponding to positions 186-190 of SEQ ID NO:1; and
- iii) an HE motif at positions corresponding to positions 303-304 of SEQ ID NO:1; and

[0099] In one embodiment, perhydrolytic enzymes may be those having an amino acid sequence that is at least about 20%, preferably at least 30%, 33%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any of the amino acid sequences reported herein (*i.e.*, SEQ ID NOs 1, and 4-28).

[00100] In another embodiment, the fusion protein comprises a perhydrolytic enzyme having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, and 4-28.

[00101] As used herein the terms “peptidic component”, “peptidic component having affinity for an oral cavity surface”, “oral cavity binding domain”, and “OCBD” will refer to component of the fusion protein that is not part of the perhydrolytic enzyme comprising at least one polymer of two or more amino acids joined by a peptide bond; wherein the component has affinity for the target oral cavity surface. In a preferred aspect, the OCBD has affinity for tooth enamel.

[00102] In one embodiment, the peptidic component having affinity for a body surface may be an antibody, an Fab antibody fragment, a single chain variable fragment (scFv) antibody, a *Camelidae* antibody (Muyldermans, S., *Rev. Mol. Biotechnol.* (2001) 74:277-302), a non-antibody scaffold display protein (Hosse *et al.*, *Prot. Sci.* (2006) 15(1): 14-27 and Binz, H. *et al.* (2005) *Nature Biotechnology* 23, 1257-1268 for a review of various scaffold-assisted approaches) or a single chain polypeptide lacking an immunoglobulin fold. In another aspect, the peptidic component having affinity for the oral cavity tissue/surface (such as tooth enamel) is a single chain peptide lacking an immunoglobulin fold.

[00103] The peptidic component having affinity for an oral cavity surface may be separated from the perhydrolytic enzyme by an optional peptide linker. Certain peptide linkers/spacers are from 1 to 100 or 1 to 50 amino acids in length. In some embodiments, the peptide spacers are

about 1 to about 25, 3 to about 40, or 3 to about 30 amino acids in length. In other embodiments are spacers that are about 5 to about 20 amino acids in length. Multiple peptide linkers may be used. In one embodiment, at least one peptide linker is present and may be repeated up to 10 times.

[00104] In one embodiment, the fusion peptide comprises at least one oral cavity surface-binding peptide selected from the group consisting of SEQ ID NOs: 178-197.

[00105] In another embodiment the target surface is a material that is part of the packaging, such as the whitening strip or polymeric backing layer (when using a polymeric backing layer to which the hydratable adhesive is applied) and/or method of delivery to the oral cavity. The peptidic component is selected for its affinity to a material or materials in use such as polymers, plastics and films. The targeted perhydrolase fusion protein design allows for the controlled delivery and removal of the perhydrolase from the user by maintaining it on a removable device such as, but not limited to, a mouth tray or strip.

[00106] The peptidic component having affinity for an oral cavity surface may be separated from the CE-7 perhydrolase by an optional peptide linker. Certain peptide linkers/spacers are from 1 to 100 or 1 to 50 amino acids in length. In some embodiments, the peptide spacers are about 1 to about 25, 3 to about 40, or 3 to about 30 amino acids in length. In other embodiments are spacers that are about 5 to about 20 amino acids in length. Multiple peptide linkers may be used. Examples of peptide linkers are provided as SEQ ID NOs: 164 – 177.

[00107] As such, examples of targeted CE-7 perhydrolases may include, but are not limited to, any of the CE-7 perhydrolases having an amino acid sequence selected from the group consisting of SEQ ID NOs 1, and 4-28 coupled to a peptidic component having affinity for an oral cavity surface. In a preferred embodiment, examples of targeted perhydrolases may include, but are not limited to, any of CE-7 perhydrolases having an amino acid sequence selected from the group consisting of SEQ ID NOs 1, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, and 28 coupled to one or more body surface-binding peptides having affinity for an oral cavity surface (optionally through a peptide spacer). In a preferred embodiment, the targeted perhydrolase includes a CE-7 perhydrolase having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 16.

[00108] In one embodiment, the perhydrolase is a CE-7 perhydrolase in the form of a fusion protein having the following general structure:

PAH-[L]_y-OCBD or OCBD-[L]_y-PAH

wherein PAH is the enzyme having perhydrolytic activity, e.g., having a CE-7 signature motif, e.g., SEQ ID NO:1, and OCBD is a peptidic component having affinity for an oral cavity surface; and L is an optional linker; and y is an integer ranging from 0 to 10. In one embodiment, the linker (L) is present and is a peptide linker ranging from 1 to 100 amino acids in length.

[00109] For example SEQ ID NO: 2 is a fusion protein having a perhydrolase sequence of SEQ ID NO: 1 coupled to a C-terminal targeting domain with an affinity for oral tissues.

[00110] The perhydrolases for use in the products and methods of the invention may be in free, protected (e.g., acetylated), or salt form.

[00111] In another embodiment, the target surface is a material that is part of the packaging and or delivery to the oral cavity. The peptidic component is selected for its affinity to a material or materials in use such as polymers, plastics and films. The targeted CE-7 perhydrolase fusion protein design allows for the controlled delivery and removal of the perhydrolase from the user by maintaining it on a removable device such as a mouth tray or strip.

Binding Affinity

[00112] The peptidic component having affinity for the oral cavity surface comprises a binding affinity for an oral cavity surface of 10^{-5} molar (M) or less. In certain embodiments, the peptidic component is one or more oral cavity surface-binding peptides and/or binding domain(s) having a binding affinity of 10^{-5} molar (M) or less for tooth enamel. In some embodiments, the binding peptides or domains will have a binding affinity value of 10^{-5} M or less in the presence of at least about 50 – 500 mM salt. The term “binding affinity” refers to the strength of the interaction of a binding peptide with its respective substrate. Binding affinity can be defined or measured in terms of the binding peptide’s dissociation constant (“ K_D ”), or “ MB_{50} .”

[00113] “ K_D ” corresponds to the concentration of peptide at which the binding site on the target is half occupied, i.e., when the concentration of target with peptide bound (bound target material) equals the concentration of target with no peptide bound. The smaller the dissociation constant, the more tightly the peptide is bound. For example, a peptide with a nanomolar (nM) dissociation constant binds more tightly than a peptide with a micromolar (μ M) dissociation constant. Certain embodiments of the invention will have a K_D value of 10^{-5} or less.

[00114] “MB₅₀” refers to the concentration of the binding peptide that gives a signal that is 50% of the maximum signal obtained in an ELISA-based binding assay. See, *e.g.*, Example 3 of U.S. Patent Application Publication 2005/022683; hereby incorporated by reference. The MB₅₀ provides an indication of the strength of the binding interaction or affinity of the components of the complex. The lower the value of MB₅₀, the stronger, *i.e.*, “better,” the interaction of the peptide with its corresponding substrate. For example, a peptide with a nanomolar (nM) MB₅₀ binds more tightly than a peptide with a micromolar (μM) MB₅₀. Certain embodiments of the invention will have a MB₅₀ value of 10⁻⁵ M or less.

[00115] In some embodiments, the peptidic component having affinity for an oral cavity surface may have a binding affinity, as measured by K_D or MB₅₀ values, of less than or equal to about 10⁻⁵ M, less than or equal to about 10⁻⁶ M, less than or equal to about 10⁻⁷ M, less than or equal to about 10⁻⁸ M, less than or equal to about 10⁻⁹ M, or less than or equal to about 10⁻¹⁰ M.

[00116] In some embodiments, the oral cavity surface-binding peptides and/or oral cavity surface-binding domains may have a binding affinity, as measured by K_D or MB₅₀ values, of less than or equal to about 10⁻⁵ M, less than or equal to about 10⁻⁶ M, less than or equal to about 10⁻⁷ M, less than or equal to about 10⁻⁸ M, less than or equal to about 10⁻⁹ M, or less than or equal to about 10⁻¹⁰ M.

[00117] As used herein, the term “strong affinity” will refer to a binding affinity having a K_D or MB₅₀ value of less than or equal to about 10⁻⁵ M, preferably less than or equal to about 10⁻⁶ M, more preferably less than or equal to about 10⁻⁷ M, more preferably less than or equal to about 10⁻⁸ M, less than or equal to about 10⁻⁹ M, or most preferably less than or equal to about 10⁻¹⁰ M.

Enzyme Powders

[00118] In some embodiments, the personal care compositions may use an enzyme catalyst in form of a stabilized enzyme powder. Methods to make and stabilize formulations comprising an enzyme powder are described in U.S. Patent Application Publication Nos. 2010-0086534 and 2010-0086535.

[00119] In one embodiment, the enzyme may be in the enzyme powder in an amount in a range of from about 0.5 weight percent (wt%) to about 75 wt%, *e.g.*, 1 wt% to about 60 wt%, based on the dry weight of the enzyme powder. A preferred weight percent range of the enzyme in the enzyme powder/spray-dried mixture is from about 10 wt% to 50 wt%, and a more preferred

weight percent range of the enzyme in the enzyme powder/spray-dried mixture is from about 20 wt% to 33 wt%.

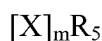
[00120] In one embodiment, the enzyme powder may further comprise an excipient. In one aspect, the excipient is provided in an amount in a range of from about 95 wt% to about 25 wt% based on the dry weight of the enzyme powder. A preferred wt % range of excipient in the enzyme powder is from about 90 wt% to 50 wt%, and a more preferred wt % range of excipient in the enzyme powder is from about 80 wt% to 67 wt%.

[00121] In one embodiment, the excipient used to prepare an enzyme powder may be an oligosaccharide excipient. In one embodiment, the oligosaccharide excipient has a number average molecular weight of at least about 1250 and a weight average molecular weight of at least about 9000. In some embodiments, the oligosaccharide excipient has a number average molecular weight of at least about 1700 and a weight average molecular weight of at least about 15000. Specific oligosaccharides may include, but are not limited to, maltodextrin, xylan, mannan, fucoidan, galactomannan, chitosan, raffinose, stachyose, pectin, insulin, levan, graminan, amylopectin, sucrose, lactulose, lactose, maltose, trehalose, cellobiose, nigerotriose, maltotriose, melezitose, maltotriulose, raffinose, kestose, and mixtures thereof. In a preferred embodiment, the oligosaccharide excipient is maltodextrin. Oligosaccharide-based excipients may also include, but are not limited to, water-soluble non-ionic cellulose ethers, such as hydroxymethyl-cellulose and hydroxypropylmethylcellulose, and mixtures thereof. In yet a further embodiment, the excipient may be selected from, but not limited to, one or more of the following compounds: trehalose, lactose, sucrose, mannitol, sorbitol, glucose, cellobiose, α -cyclodextrin, and carboxymethylcellulose.

Suitable Ester Substrates / Acyl Donors

[00122] Suitable carboxylic acid ester substrates may include esters having the following formula:

(a) one or more esters having the structure



wherein

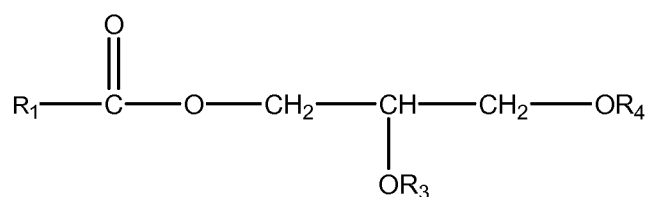
X is an ester group of the formula $R_6C(O)O$;

R₆ is a C1 to C7 linear, branched or cyclic hydrocarbyl moiety, optionally substituted with a hydroxyl group or C1 to C4 alkoxy group, wherein R₆ optionally comprises one or more ether linkages where R₆ is C2 to C7;

R₅ is a C1 to C6 linear, branched, or cyclic hydrocarbyl moiety or a five-membered cyclic heteroaromatic moiety or six-membered cyclic aromatic or heteroaromatic moiety optionally substituted with a hydroxyl group; wherein each carbon atom in R₅ individually comprises no more than one hydroxyl group or no more than one ester group or carboxylic acid group, and wherein R₅ optionally comprises one or more ether linkages;

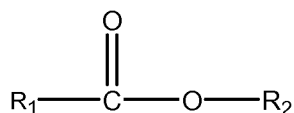
m is an integer ranging from 1 to the number of carbon atoms in R₅, said one or more esters having solubility in water of at least 5 ppm at 25 °C; or

(b) one or more glycerides having the structure



wherein R₁ is a C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R₃ and R₄ are individually H or R₁C(O); or

(c) one or more esters of the formula



wherein R₁ is a C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R₂ is a C1 to C10 straight chain or branched chain alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, heteroaryl, (CH₂CH₂O)_n, or (CH₂CH(CH₃)-O)_nH and n is 1 to 10; or

(d) one or more acetylated monosaccharides, acetylated disaccharides, or acetylated polysaccharides; or

(e) any combination of (a) through (d).

[00123] Suitable substrates may also include one or more acylated saccharides selected from the group consisting of acylated mono-, di-, and polysaccharides. In another embodiment, the acylated saccharides are selected from the group consisting of acetylated xylan; fragments of acetylated xylan; acetylated xylose (such as xylose tetraacetate); acetylated glucose (such as α -D-glucose pentaacetate; β -D-glucose pentaacetate; 1-thio- β -D-glucose-2,3,4,6-tetraacetate); β -D-galactose pentaacetate; sorbitol hexaacetate; sucrose octaacetate; β -D-ribofuranose-1,2,3,5-tetraacetate; β -D-ribofuranose-1,2,3,4-tetraacetate; tri-O-acetyl-D-galactal; tri-O-acetyl-D-glucal; β -D-xylofuranose tetraacetate, β -D-glucopyranose pentaacetate; β -D-glucopyranose-1,2,3,4-tetraacetate; β -D-glucopyranose-2,3,4,6-tetraacetate; 2-acetamido-2-deoxy-1,3,4,6-tetracetyl- β -D-glucopyranose; 2-acetamido-2-deoxy-3,4,6-triacetyl-1-chloride- α -D-glucopyranose; β -D-mannopyranose pentaacetate, and acetylated cellulose. In a preferred embodiment, the acetylated saccharide is selected from the group consisting of β -D-ribofuranose-1,2,3,5-tetraacetate; tri-O-acetyl-D-galactal; tri-O-acetyl-D-glucal; sucrose octaacetate; and acetylated cellulose.

[00124] In another embodiment, additional suitable substrates may also include 5-acetoxymethyl-2-furaldehyde; 3,4-diacetoxy-1-butene; 4-acetoxybenzoic acid; vanillin acetate; propylene glycol methyl ether acetate; methyl lactate; ethyl lactate; methyl glycolate; ethyl glycolate; methyl methoxyacetate; ethyl methoxyacetate; methyl 3-hydroxybutyrate; ethyl 3-hydroxybutyrate; and triethyl 2-acetyl citrate.

[00125] In another embodiment, suitable substrates are selected from the group consisting of: monoacetin; diacetin; triacetin; monopropionin; dipropionin; tripropionin; monobutylin; dibutylin; tributyrin; glucose pentaacetate; xylose tetraacetate; acetylated xylan; acetylated xylan fragments; β -D-ribofuranose-1,2,3,5-tetraacetate; tri-O-acetyl-D-galactal; tri-O-acetyl-D-glucal; monoesters or diesters of 1,2-ethanediol; 1,2-propanediol; 1,3-propanediol; 1,2-butanediol; 1,3-butanediol; 2,3-butanediol; 1,4-butanediol; 1,2-pentanediol; 2,5-pentanediol; 1,5-pentanediol; 1,6-pentanediol; 1,2-hexanediol; 2,5-hexanediol; 1,6-hexanediol; and mixtures thereof. In another embodiment, the substrate is a C1 to C6 polyol comprising one or more ester groups. In a preferred embodiment, one or more of the hydroxyl groups on the C1 to C6 polyol

are substituted with one or more acetoxy groups (such as 1,3-propanediol diacetate; 1,2-propanediol diacetate; 1,4-butanediol diacetate; 1,5-pentanediol diacetate, etc.). In a further embodiment, the substrate is propylene glycol diacetate (PGDA), ethylene glycol diacetate (EGDA), or a mixture thereof.

[00126] In a further embodiment, suitable substrates are selected from the group consisting of monoacetin, diacetin, triacetin, monopropionin, dipropionin, tripropionin, monobutyryn, dibutyryn, and tributyrin. In yet another aspect, the substrate is selected from the group consisting of diacetin and triacetin. In a most preferred embodiment, the suitable substrate comprises triacetin.

[00127] The carboxylic acid ester is present at a concentration sufficient to produce the desired concentration of peroxy-carboxylic acid upon enzyme-catalyzed perhydrolysis. The carboxylic acid ester need not be completely soluble in the reaction formulation, but has sufficient solubility to permit conversion of the ester by the perhydrolase catalyst to the corresponding peroxy-carboxylic acid. The carboxylic acid ester is present in the reaction formulation at a concentration of 0.05 wt % to 40 wt % of the reaction formulation, preferably at a concentration of 0.1 wt % to 20 wt % of the reaction formulation, and more preferably at a concentration of 0.5 wt % to 10 wt % of the reaction formulation.

[00128] The peroxygen source is provided as granules deposited in or on the hydratable adhesive film and may include hydrogen peroxide adducts (*e.g.*, urea-hydrogen peroxide adduct (carbamide peroxide)) perborate salts, percarbonate salts and peroxide salts. The concentration of peroxygen compound in the reaction formulation may range from 0.0033 wt % to about 50 wt %, preferably from 0.033 wt % to about 40 wt %, more preferably from 0.1 wt % to about 30 wt %.

[00129] Many perhydrolase catalysts (whole cells, permeabilized whole cells, and partially purified whole cell extracts) have been reported to have catalase activity (EC 1.11.1.6). Catalases catalyze the conversion of hydrogen peroxide into oxygen and water. In one aspect, the perhydrolysis catalyst lacks catalase activity. In another aspect, a catalase inhibitor may be added to the reaction formulation. One of skill in the art can adjust the concentration of catalase inhibitor as needed. The concentration of the catalase inhibitor typically ranges from 0.1 mM to about 1 M; preferably about 1 mM to about 50 mM; more preferably from about 1 mM to about 20 mM.

[00130] In another embodiment, the enzyme catalyst lacks significant catalase activity or may be engineered to decrease or eliminate catalase activity. The catalase activity in a host cell can be down-regulated or eliminated by disrupting expression of the gene(s) responsible for the catalase activity using well known techniques including, but not limited to, transposon mutagenesis, RNA antisense expression, targeted mutagenesis, and random mutagenesis.

[00131] The concentration of peroxycarboxylic acid generated (*e.g.* peracetic acid) by the perhydrolysis of at least one carboxylic acid ester is at least about 0.1 ppm, preferably at least 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, 20 ppm, 100 ppm, 200 ppm, 300 ppm, 500 ppm, 700 ppm, 1000 ppm, 2000 ppm, 5000 ppm or 10,000 ppm of peracid within 10 minutes, preferably within 5 minutes, of initiating the perhydrolysis reaction. Clearly one of skill in the art can adjust the reaction components to achieve the desired peracid concentration.

[00132] In one aspect, the reaction time required to produce the desired concentration of peracid is not greater than about two hours, preferably not greater than about 30 minutes, more preferably not greater than about 10 minutes, and most preferably in about 5 minutes or less. In other aspects, an oral cavity surface is contacted with the peroxycarboxylic acid formed in accordance with the processes described herein within 5 minutes of hydrating and combining the reaction components. In one embodiment, the tooth enamel is contacted with the peroxycarboxylic acid produced with the processes and compositions described herein within about 5 minutes to about 24 hours or within about 5 minutes to 2 hours of combining (via user hydration) said reaction components present in or on the whitening strip/film.

HPLC Assay Method for Determining the Concentration of Peroxycarboxylic acid and Hydrogen Peroxide

[00133] A variety of analytical methods can be used to analyze the reactants and products including, but not limited to, titration, high performance liquid chromatography (HPLC), gas chromatography (GC), mass spectroscopy (MS), capillary electrophoresis (CE), the analytical procedure described by U. Pinkernell *et al.*, (*Anal. Chem.*, 69(17):3623-3627 (1997)), and the 2,2'-azino-bis (3-ethylbenzothiazoline)-6-sulfonate (ABTS) assay (U. Pinkernell *et. al. Analyst*, 122: 567-571 (1997) and Dinu *et. al. Adv. Funct. Mater.*, 20: 392-398 (2010)) as described in the present examples.

Exemplary Embodiments:

[00134] Embodiment 1. A tooth whitening strip (Strip 1) comprising a hydratable adhesive film with a first side and a second side, the first side having a granular bleaching ingredient attached thereto,

wherein the strip comprises, in or on the film or in granules attached to the first side of the film,

(i) a protein having perhydrolytic activity which contains the signature motif of a member of the carbohydrate esterase family 7;

(ii) an acyl donor, e.g., selected from carboxylic acid esters and acyl compounds,

[00135] Embodiment 2. Strip 1 wherein the protein having perhydrolase activity comprises an amino acid sequence selected from:

a) MAFFDLPLEELKKYRPERYEEKDFDEFWEETLAESEKFPLDPVFERMESHK
TVEAYDVTFSGYRGQRIKGWLLVPKLEEEKLPCVVQYIGYNGGRGFPHDWL
FWPSMGYICFVMDTRGQGSGWLKGDTPDYPEGVPDPQYPGFMTRGILDPRT
YYYRRVFTDAVRAVEAAASFPQVDQERIVIAGGSQGGGIALAVSALSKKAK
ALLCDVPFLCHFRRVQLVDTHPYAEITNFLKTHRDKEEIVFRTLSTYFDGVN
FAARAKIPALFSVGLMDNISPPSTVFAAYNNYAGPKEIRIYPYNNHEGGGSFQ
AVEQVKFLKKLFEKG (SEQ ID NO: 1) ,

b) an amino acid sequence having i) an RGQ motif at positions corresponding to positions 118-120 of SEQ ID NO: 1; ii) a GXSQG motif at positions corresponding to positions 186-190 of SEQ ID NO: 1; and iii) an HE motif at positions corresponding to positions 303-304 of SEQ ID NO: 1; and

c) an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 1.

[00136] Embodiment 3. Embodiment 1 or 2 wherein the protein having perhydrolytic activity comprises an amino acid sequence which has affinity to, e.g., binds to or complexes with oral surfaces or alternatively has affinity, e.g., binds to or complexes with, one or more components of the whitening strip.

[00137] Embodiment 4. Any of the foregoing strips comprising a protein having perhydrolase activity which binds to or complexes with oral surfaces (e.g., tooth pellicle or enamel) comprising an amino acid sequence selected from

a) MAFFDLPLEELKKYRPERYEEKDFDEFWEETLAESEKFPLDPVFERMESHK
TVEAYDVTFSGYRGQRIKGWLLVPKLEEEKLPCVVQYIGYNGGRGFPHDWL

FWPSMGYICFVMDTRGQGSGWLKGDTPDYPEGVPDPQYPGFMTRGILDPR
 YYYRRVFTDAVRAVEAAASFPQVDQERIVIAGGSQGGGIALAVSALSKKAK
 ALLCDVPFLCHFERRAVQLVDTHPYAEITNFLKTHRDKEEIVFRTLSTYFDGVN
 FAARAKIPALFSVGLMDNISPPSTVFAAYNNYAGPKEIRIYPYNNHEGGGSFQ
 AVEQVKFLKKLFEKGGPGSGGAGSPGSAGGPGSTKPPRTPTANTSRPHHNFG
 SGGGGSPHHHHH (SEQ ID NO: 2), and

b) an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 2.

[00138] Embodiment 5. Any of the foregoing strips wherein the protein having perhydrolytic activity is provided in granular form on the first surface of the film.

[00139] Embodiment 6. Any of the foregoing strips wherein the second side of the hydratable adhesive film is attached to a layer which inhibits dissolution of the hydratable adhesive film.

[00140] Embodiment 7. Any of the foregoing strips wherein the granular bleaching ingredient is coated with a quickly dissolving material, such as cornstarch, sodium sulfate gum arabic, and combinations thereof.

[00141] Embodiment 8. Any of the foregoing strips wherein the granular bleaching ingredient is selected from granules comprising organic and/or inorganic oxidizers, e.g., selected from hydrogen peroxide, urea peroxide, percarbonates, perborates, peroxyphosphates, peroxydisulfates, peroxyacids, and peracetic acid.

[00142] Embodiment 9. Any of the foregoing strips wherein the granular bleaching ingredient is selected from solid peroxides and solid peroxide donors, e.g., selected from peroxide salts or complexes (e.g., such as peroxyphosphate, peroxyphosphate, perborate, peroxyphosphate, or persulfate salts; for example calcium peroxyphosphate, sodium perborate, sodium carbonate peroxide, sodium peroxyphosphate, and potassium persulfate); hypochlorites; urea peroxide; hydrogen peroxide polymer complexes such as hydrogen peroxide-polyvinyl pyrrolidone polymer complexes; metal peroxides e.g. zinc peroxide and calcium peroxide; peracids, and combinations thereof.

[00143] Embodiment 10. Any of the foregoing strips wherein the granular bleaching ingredient comprises urea peroxide, a hydrogen peroxide-polyvinyl pyrrolidone polymer complex, sodium percarbonate, or a combination of two or more thereof.

[00144] Embodiment 11. Any of the foregoing strips where the particle size (D_{50}) of the granules on the first surface of the film, e.g., the granular bleaching ingredient, or perhydrolase or acyl donor in granular form, is 0.1-300 microns, e.g. 10-275 microns, e.g., 100-250 microns.

[00145] Embodiment 12. Any of the foregoing strips wherein the granular bleaching ingredient comprises greater than 0.01%, e.g. 0.01-0.1%, e.g. 0.02-0.08%, of the total weight of the hydratable adhesive film and a granular bleaching ingredient attached thereto.

[00146] Embodiment 13. Any of the foregoing strips wherein the amount of granular bleaching agent on the first side of the hydratable adhesive film is $0.001 - 10 \text{ mg/cm}^2$, e.g., $0.001 - 1 \text{ mg/cm}^2$, for example $0.005 - 0.015 \text{ mg/cm}^2$.

[00147] Embodiment 14. Any of the foregoing strips wherein the acyl donor is selected from (i) one or more C_{2-18} carboxylic acid esters, e.g C_{2-6} carboxylic acid esters (e.g., acetyl esters), including lower linear or branched alkyl carboxylic acid esters, optionally substituted with hydroxy and/or C_{1-4} alkoxy; (ii) one or more acylated glycerides (e.g. mono-, di-, and tri-glycerides), (iii) acylated saccharides, and (iv) mixtures thereof.

[00148] Embodiment 15. Any of the foregoing strips the acyldonor is selected from 1,2,3-triacetoxyp propane (sometimes referred to herein as triacetin or glycerin triacetate) and acylated saccharides, e.g. acylated saccharides.

[00149] Embodiment 16. Any of the foregoing strips comprising an acyl donor which comprises an ester compound having solubility in water of at least 5 ppm at 25 °C.

[00150] Embodiment 17. Any of the foregoing strips which comprises a peracid or which generates a peracid upon use.

[00151] Embodiment 18. Any of the foregoing strips wherein the ingredients are present in amounts sufficient to provide, upon mixing, a bleaching agent in an amount and concentration effective to whiten teeth.

[00152] Embodiment 19. Any of the foregoing strips wherein the hydratable adhesive film comprises one or more water-soluble polymers selected from hydrophilic cellulose ethers (e.g. carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose,), polyvinyl acetates, carbomers (e.g., CARBOPOL[®] 971P), polysaccharide gums (e.g. xanthan gum), modified food starches, gelatin (e.g. animal or fish-based gelatin), cross-linked carboxyvinyl copolymers, cross-linked polyvinylpyrrolidones, polyethylene oxide (e.g. POLYOX[™]),

polyacrylic acids and polyacrylates, polyvinyl alcohols, alginate, casein, pullulan, and combinations thereof.

[00153] Embodiment 20. Any of the foregoing strips wherein the hydratable adhesive film comprises one or more water-soluble polymers selected from hydrophilic cellulose ethers (*e.g.* hydroxypropylmethyl cellulose or hydroxypropyl cellulose), polyethylene oxide, polyvinyl acetates, and carbomers (*e.g.*, CARBOPOL[®] 971P); and combinations thereof.

[00154] Embodiment 21. Any of the foregoing strips wherein the hydratable adhesive film comprises hydroxypropylmethyl cellulose, polyvinyl acetates, and a carbomer, for example, in a dry weight ratio of 10- 20 HPMC : 2- 10 PVAc : 1 carbomer.

[00155] Embodiment 22. Any of the foregoing strips wherein the hydratable adhesive film further comprises a plasticizer, *e.g.* propylene glycol, polyethylene glycol or triacetin.

[00156] Embodiment 23. Any of the foregoing strips wherein the first side of the hydratable adhesive film is covered by a protective cover prior to use.

[00157] Embodiment 24. Any of the foregoing strips wherein the hydratable adhesive film has a viscosity of at least 100,000 cps upon activation, *e.g.*, viscosity of 100,000 to 200,000 cps.

[00158] Embodiment 25. Any of the foregoing strips wherein the hydratable adhesive film is substantially dry prior to application.

[00159] Embodiment 26. Any of the foregoing strips wherein the thickness of the hydratable adhesive film is 0.1-5 mm, *e.g.*, 0.5-5 mm.

[00160] Embodiment 27. Any of the foregoing strips wherein the approximate overall dimensions are 2-10 cm long x 0.5-2 cm wide x 0.1-10 mm thick, *e.g.*, 1-10 mm thick, for example a strip wherein the surface area of one side is 5 – 20 cm², *e.g.*, about 5 – 15 cm², *e.g.*, about 10 cm².

[00161] Embodiment 28. Any of the foregoing strips comprising coated granules of a hydrogen peroxide-polyvinyl pyrrolidone polymer complex, urea peroxide and/or sodium percarbonate and granules of perhydrolase on the first surface of the film, with triacetin dispersed in the film.

[00162] Embodiment 29. Any of the foregoing strips, further comprising granules of an antisensitivity agent, *e.g.* potassium nitrate or arginine.

[00163] Embodiment 30. A method (Method 2) of whitening teeth comprising applying the first side of a strip as hereinbefore described, *e.g.* Strip 1 et seq. directly to the teeth, and leaving

it on for a sufficient time, e.g., at least 5 minutes, for example 10-60 minutes, e.g., 10-30 minutes, to whiten the teeth.

[00164] Embodiment 31. A method (Method 3) of making a strip for tooth whitening, e.g., a strip as hereinbefore described, according to Strip 1 et seq., comprising providing a semi-dry hydratable adhesive film, e.g., as hereinbefore described, e.g., which film has been cast from water and not fully dried, or which film has been moistened, adding to one surface of the film granules of a granular bleaching ingredient, e.g., as hereinbefore described, and drying the film with the granules added to one surface.

[00165] For example, the strips may be made by first making the hydratable adhesive film, using conventional means and then adding the granulated whitening ingredient to one surface. The hydratable adhesive film strips can be cast from water in a variety of ways known in the art, such as by extrusion, or by casting from a water suspension (for example at a solids level of 10-30%) onto a heated belt, from which the water is evaporated. Alternatively, the film is dried, but then remoistened. The granules can be added to the surface of this film while the film is semi-dry, i.e. just moist enough to be tacky, so that the granules stick to the surface of the film. Once the film is fully dry and cooled to room temperature, the granules continue to adhere to the surface of the film. Prior to use, therefore, the hydratable adhesive film and the strip as a whole are substantially dry. Because the peroxide is on the surface of the film only, a relatively small quantity of granules are required to provide an effective concentration at the surface.

Preferred Embodiments:

[00166] When exposed to saliva or other sources of water (such as tap water), the granules dissolve and release the reaction components to enzymatically produce the desired peracid. Hydration of the hydratable adhesive layer increases the tackiness of the film, enabling the whitening film/strip to bind to the target surface (*i.e.*, tooth enamel).

[00167] The hydratable adhesive film comprises one or more water soluble, orally acceptable polymers selected from hydrophilic cellulose ethers (*e.g.*, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose,), polyvinyl acetates, carbomers (*e.g.*, CARBOPOL[®] 971P), polysaccharide gums (*e.g.*, xanthan gum), modified food starches, gelatin (*e.g.*, animal or fish-based gelatin), cross-linked carboxyvinyl copolymers, cross-linked polyvinylpyrrolidones, polyethylene oxide (*e.g.*, POLYOX[™]), polyacrylic acids and polyacrylates, polyvinyl alcohols, alginate, casein, pullulan, and combinations thereof.

Adhesive gel formulations for use with tooth whitening agents are known in the art, for example, as described in US Patents 7,862,801; 5,746,598; 6,730,316; and 7,128,899; each incorporated by reference in its entirety. The adhesive film allows the peracid bleaching agent to stay in contact with the teeth for extended periods of time and protects soft tissues, and thus should provide a high viscosity, for example, a viscosity upon application of at least 100,000 centipoise (cps) (about 100 Pascal-second (Pa·s)), preferable 100,000 to 200,000 cps (100 to 200 Pa·s).

[00168] Where a second film layer is used to protect the hydratable adhesive film from rapid degradation or dissolution, the carrier or backing material may be made from textiles, cloth, wood composite, resin, elastomer, paper, insoluble or less soluble cellulose derivatives such as ethyl cellulose and cellulose acetate, polyvinyl chloride, wax, PARAFILMTM, polyethylene, polyvinyl alcohol, TEFLONTM, polyvinyl chloride, polyvinyl acetate and their derivatives.

[00169] The granular bleaching ingredient may be a solid peroxide or solid peroxide donor selected from peroxide salts or complexes (such as peroxyphosphate, peroxycarbonate, perborate, peroxysilicate, or persulphate salts; for example calcium peroxyphosphate, sodium perborate, sodium carbonate peroxide, sodium peroxyphosphate, and potassium persulfate), hypochlorites; urea peroxide; hydrogen peroxide polymer complexes such as hydrogen peroxide-polyvinyl pyrrolidone polymer complexes, and metal peroxides, for example, zinc peroxide and calcium peroxide; a solid peracid; and combinations thereof. In particular embodiments, the granular bleaching ingredient is urea peroxide or a hydrogen peroxide polyvinylpyrrolidone polymer complex. The granular bleaching ingredient may be optionally coated to provide improved storage stability (for example, coated with sodium sulfate, corn starch or gum arabic).

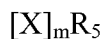
Listing of the Preferred Embodiments:

[00170] Preferred Embodiment 1. A tooth whitening strip comprising a hydratable adhesive film with a first side and a second side, the first side having a granular bleaching ingredient attached thereto, wherein the tooth whitening strip further comprises, in or on the film or in the form of granules attached to the first side of the film;

a) an enzyme having perhydrolytic activity, said enzyme having a carbohydrate esterase family 7 (CE-7) signature motif that aligns with a reference sequence SEQ ID NO: 1, said signature motif comprising:

- i) an RGQ motif at positions corresponding to positions 118-120 of SEQ ID NO: 1;
 - ii) a GXSQG motif at positions corresponding to positions 186-190 of SEQ ID NO:1; and
 - iii) an HE motif at positions corresponding to positions 303-304 of SEQ ID NO:1; and
- (b) at least one acyl donor substrate, said substrate selected from the group consisting of:

- i) esters having the structure



wherein X = an ester group of the formula $R_6C(O)O$

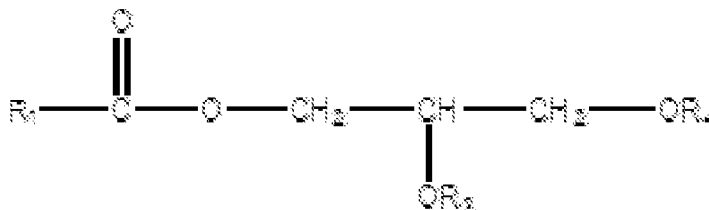
R_6 = C1 to C7 linear, branched or cyclic hydrocarbyl moiety, optionally substituted with hydroxyl groups or C1 to C4 alkoxy groups, wherein R_6 optionally comprises one or more ether linkages for R_6 = C2 to C7;

R_5 = a C1 to C6 linear, branched, or cyclic hydrocarbyl moiety or a five-membered cyclic heteroaromatic moiety or six-membered cyclic aromatic or heteroaromatic moiety optionally substituted with hydroxyl groups; wherein each carbon atom in R_5 individually comprises no more than one hydroxyl group or no more than one ester group or carboxylic acid group; wherein R_5 optionally comprises one or more ether linkages;

M is an integer ranging from 1 to the number of carbon atoms in R_5 ; and

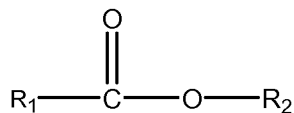
wherein said esters have solubility in water of at least 5 ppm at 25 °C;

- ii) glycerides having the structure



wherein R_1 = C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R_3 and R_4 are individually H or $R_1C(O)$;

- iii) one or more esters of the formula



wherein R₁ is a C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R₂ is a C1 to C10 straight chain or branched chain alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, heteroaryl, (CH₂CH₂O)_n, or (CH₂CH(CH₃)-O)_nH and n is 1 to 10; and

iv) acetylated saccharides selected from the group consisting of acetylated monosaccharides, acetylated disaccharides, and acetylated polysaccharide; wherein upon hydration of the hydratable adhesive film hydrogen peroxide is released from the granular bleaching ingredient and said enzyme catalyzes the formation of an effective amount of a peracid.

[00171] Preferred Embodiment 2. The tooth whitening strip according to preferred embodiment 1 wherein the enzyme having perhydrolytic activity comprises an amino acid sequence selected from:

- a) SEQ ID NO: 1; and
- b) an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO: 1.

[00172] Preferred Embodiment 3. The tooth whitening strip according to Preferred Embodiment 1 wherein the enzyme having perhydrolytic activity further comprises a binding domain fused to the N- or C-terminus of the enzyme, said binding domain having affinity for an oral tissue or for the tooth whitening strip.

[00173] Preferred Embodiment 4. The tooth whitening strip according to Preferred Embodiment 3 wherein the binding domain having affinity for an oral tissue comprises an amino acid sequence selected from the group consisting of SEQ D NOs: 178-197.

[00174] Preferred Embodiment 5. The tooth whitening strip according to any of the above preferred embodiments wherein the enzyme having perhydrolytic activity has affinity for an oral tissue and comprises an amino acid sequence selected from

- a) SEQ ID NO: 2, and
- b) an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO: 2.

[00175] Preferred Embodiment 6. The tooth whitening strip according to any of the above preferred embodiments further comprising a backing layer attached to said second side of the hydratable adhesive film, said backing layer capable of inhibiting dissolution of the hydratable adhesive film.

[00176] Preferred Embodiment 7. The tooth whitening strip according to any of the above preferred embodiments wherein the granular bleaching ingredient is coated with a water soluble coating capable of dissolving upon hydration.

[00177] Preferred Embodiment 8. The tooth whitening strip according to any of the above preferred embodiments wherein the granular bleaching ingredient is selected from solid peroxides and solid peroxide donors.

[00178] Preferred Embodiment 9. The tooth whitening strip according to any of the above preferred embodiments wherein the granular bleaching ingredient is selected from peroxide salts, peroxide complexes, peroxyphosphate, peroxycarbonate, perborate, peroxysilicate, persulphate salts, calcium peroxyphosphate, sodium perborate, sodium carbonate peroxide, sodium peroxyphosphate, potassium persulfate, hypochlorites, urea peroxide, hydrogen peroxide polymer complexes, hydrogen peroxide-polyvinyl pyrrolidone polymer complexes, metal peroxides, zinc peroxide, calcium peroxide, and combinations thereof.

[00179] Preferred Embodiment 10. The tooth whitening strip according to any of the above preferred embodiments wherein the granular bleaching ingredient comprises urea peroxide. In some embodiments, the granular bleaching ingredient comprises a hydrogen peroxide-polyvinyl pyrrolidone polymer complex. In some embodiments, the hydrogen peroxide-polyvinyl pyrrolidone polymer complex is a hydrogen peroxide-crosslinked polyvinyl pyrrolidone polymer complex.

[00180] Preferred Embodiment 11. The tooth whitening strip according to any of the above preferred embodiments wherein the particle size median diameter (D50) of the granular

bleaching ingredient ranged from 10 microns to 300 microns, e.g., 10 microns to 200 microns.

[00181] Preferred Embodiment 12. The tooth whitening strip according to any of the above preferred embodiments wherein the tooth whitening strip comprises from about 0.01 wt% to about 0.1 wt% of a peracid. In some embodiments, the granular bleaching ingredient comprises from about 0.1 wt% to about 30 wt% of a peroxygen source.

[00182] Preferred Embodiment 13. The tooth whitening strip according to any of the above preferred embodiments wherein the amount of granular bleaching agent on the first side of the hydratable adhesive film ranges from 0.001 mg/cm² to 10 mg/cm², e.g., 0.001 mg/cm² to 1 mg/cm².

[00183] Preferred Embodiment 14. The tooth whitening strip according to any of the above preferred embodiments wherein the acyl donor substrate is 1,2,3-triacetoxyp propane.

[00184] Preferred Embodiment 15. The tooth whitening strip according to any of the above preferred embodiments wherein the hydratable adhesive film comprises one or more water-soluble polymers selected from a hydrophilic cellulose ether, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, a polyvinyl acetate, a carbomer, a polysaccharide gum, xanthan gum, a modified food starch, gelatin, animal or fish-based gelatin, a cross-linked carboxyvinyl copolymer, a cross-linked polyvinylpyrrolidone, polyethylene oxide, a polyacrylic acid, a polyacrylate, a polyvinyl alcohol, alginate, casein, pullulan, and a combination of two or more thereof.

[00185] Preferred Embodiment 16. The tooth whitening strip according to any of the above preferred embodiments wherein the hydratable adhesive film comprises one or more water-soluble polymers selected from a hydrophilic cellulose ether, a polyvinyl acetate, a carbomer, and a combination of two or more thereof.

[00186] Preferred Embodiment 17. The tooth whitening strip according to any of the above preferred embodiments wherein the hydratable adhesive film comprises hydroxypropylmethyl cellulose (HPMC), polyvinyl acetate (PVAc), and a carbomer in a dry weight ratio for HPMC : PVAc : carbomer of 10-20 : 2-10 : 1.

[00187] Preferred Embodiment 18. The tooth whitening strip according to any of the above preferred embodiments wherein the hydratable adhesive film further comprises a plasticizer.

[00188] Preferred Embodiment 19. The tooth whitening strip according to any of the above preferred embodiments further comprising propylene glycol.

[00189] Preferred Embodiment 20. A method of whitening teeth comprising

- a) providing a packaging system comprising the tooth whitening strip according to any of the above preferred embodiments;
- b) removing the tooth whitening strip from the packaging system; and
- c) contacting the tooth whitening strip directly to the teeth for a period of time sufficient time whiten the teeth; wherein the tooth whitening strip is hydrated by moisture present in the oral cavity or on the tooth surface or is hydrated after step (b) but prior to step (c).

[00190] Preferred Embodiment 21. The method of Preferred Embodiment 20 wherein the whitening strip further comprises a backing layer attached to said second side of the hydratable adhesive film, said backing layer capable of inhibiting dissolution of the hydratable adhesive film.

[00191] Preferred Embodiment 22. The method according to any of the above preferred embodiments wherein the particle size median diameter (D50) of the granular bleaching ingredient ranged from 10 microns to 200 microns.

[00192] Preferred Embodiment 23. The method according to any of the above preferred embodiments wherein the granular bleaching ingredient comprises greater than 0.01 wt% of the total weight of the hydratable adhesive film and a granular bleaching ingredient attached thereto. In some embodiments, the granular bleaching ingredient comprises greater than 0.05 wt% of the total weight of the hydratable adhesive film and a granular bleaching ingredient attached thereto. In some embodiments, the granular bleaching ingredient comprises from about 0.01 wt% to about 0.1 wt% of the total weight of the hydratable adhesive film and a granular bleaching ingredient attached thereto.

[00193] Preferred Embodiment 24. The method according to any of the above preferred embodiments wherein the amount of granular bleaching agent on the first side of the hydratable adhesive film ranges from 0.001 mg/cm² to 1 mg/cm².

[00194] Preferred Embodiment 25. The method according to any of the above preferred embodiments wherein the acyl donor substrate is 1,2,3-triacetoxyp propane.

[00195] Preferred Embodiment 26. The method according to any of the above preferred embodiments wherein the hydratable adhesive film comprises one or more water-soluble

polymers selected from a hydrophilic cellulose ether, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, a polyvinyl acetate, a carbomer, a polysaccharide gum, xanthan gum, a modified food starch, gelatin, animal or fish-based gelatin, a cross-linked carboxyvinyl copolymer, a cross-linked polyvinylpyrrolidone, polyethylene oxide, a polyacrylic acid, a polyacrylate, a polyvinyl alcohol, alginate, casein, pullulan, and a combination of two or more thereof.

[00196] Preferred Embodiment 27. The method according to any of the above preferred embodiments wherein the hydratable adhesive film comprises one or more water-soluble polymers selected from a hydrophilic cellulose ether, a polyvinyl acetate, a carbomer, and a combination of two or more thereof.

[00197] Preferred Embodiment 28. The method according to any of the above preferred embodiments wherein the hydratable adhesive film comprises hydroxypropylmethyl cellulose (HPMC), polyvinyl acetate (PVAc), and a carbomer in a dry weight ratio for HPMC : PVAc : carbomer of 10-20 : 2-10 : 1.

[00198] Preferred Embodiment 29. The method according to any of the above preferred embodiments wherein the hydratable adhesive film further comprises a plasticizer.

[00199] Preferred Embodiment 30. The method according to any of the above preferred embodiments wherein the hydratable adhesive film further comprises propylene glycol.

[00200] Preferred Embodiment 31. The method according to any of the above preferred embodiments wherein the granular bleaching ingredient is coated with a water soluble coating capable of dissolving upon hydration.

[00201] Preferred Embodiment 32. A method of making a tooth whitening strip comprising:

- a) providing a semi-dry hydratable adhesive film,
- b) applying to one surface of the film granules of a granular bleaching ingredient whereby the granules adhere to the surface, and
- c) drying the film.

[00202] In some embodiments, the particle size median diameter (D50) of the granular bleaching ingredient is from 10 microns to 300 microns. In some embodiments, the particle size median diameter (D50) of the granular bleaching ingredient is from 25 microns to 200 microns. In some embodiments, the particle size median diameter (D50) of the granular bleaching ingredient is from 35 microns to 150 microns. In some embodiments, the particle size median

diameter (D50) of the granular bleaching ingredient is from 50 microns to 125 microns. In some embodiments, the particle size median diameter (D50) of the granular bleaching ingredient is from 60 microns to 100 microns. In some embodiments, the particle size median diameter (D50) of the granular bleaching ingredient is about 64 microns. In some embodiments, the particle size median diameter (D50) of the granular bleaching ingredient is about 94 microns.

[00203] In some embodiments, the particle size median diameter (D50) of the enzyme having perhydrolytic activity is from 100 microns to 300 microns. In some embodiments, the particle size median diameter (D50) of the enzyme having perhydrolytic activity is from 150 microns to 275 microns. In some embodiments, the particle size median diameter (D50) of the enzyme having perhydrolytic activity is from 175 microns to 250 microns.

[00204] All ingredients for use in the strips described herein should be orally acceptable. By “orally acceptable” as the term is used herein is meant an ingredient which is present in a strip as described in an amount and form which does not render the strip unsafe for use in the oral cavity.

[00205] As used throughout, ranges are used as shorthand for describing each and every value that is within the range. Any value within the range can be selected as the terminus of the range. In addition, all references cited herein are hereby incorporated by referenced in their entireties. In the event of a conflict in a definition in the present disclosure and that of a cited reference, the present disclosure controls.

[00206] Unless otherwise specified, all percentages and amounts expressed herein and elsewhere in the specification should be understood to refer to percentages by weight. The amounts given are based on the active weight of the material.

EXAMPLES

[00207] The following examples are provided to demonstrate preferred aspects of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples follow techniques to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the

specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the presently disclosed methods and examples.

[00208] All reagents and materials were obtained from DIFCO Laboratories (Detroit, MI), GIBCO/BRL (Gaithersburg, MD), TCI America (Portland, OR), Roche Diagnostics Corporation (Indianapolis, IN), Thermo Scientific (Pierce Protein Research Products) (Rockford, IL) or Sigma/Aldrich Chemical Company (St. Louis, MO), unless otherwise specified.

[00209] The following abbreviations in the specification correspond to units of measure, techniques, properties, or compounds as follows: “sec” or “s” means second(s), “min” means minute(s), “h” or “hr” means hour(s), “μL” means microliter(s), “mL” means milliliter(s), “L” means liter(s), “mM” means millimolar, “M” means molar, “mmol” means millimole(s), “ppm” means part(s) per million, “wt” means weight, “wt%” means weight percent, “g” means gram(s), “mg” means milligram(s), “μg” means microgram(s), and “ng” means nanogram(s).

Example 1

[00210] A strip is prepared as described above, forming the hydratable adhesive film and then while the film is still tacky, adding the granulated whitening agent and granulated enzyme to the surface of one side, using the ingredients in Table 1. The strip will erode slowly in the mouth upon application, and so does not need to be removed.

Table 1

Ingredients in Strip	Dry Strip Concentration (wt%)
Hydroxypropylmethylcellulose (HPMC)	59
Polyvinylacetate (PVAc)	30
Carbopol, 971	5
Triacetin	5
Titanium dioxide	1
Total	100
Ingredients in Bleach Granule	Concentration (wt%)
Urea peroxide	100
Ingredients in Enzyme Granule	Concentration

	(wt%)
Cornstarch	99
Perhydrolase enzyme	1

[00211] The strip in example 1 is cast from water by extrusion, or by casting from a water suspension (for example at a solids level of 10-30%) onto a heated belt, from which the water is evaporated. The granules are added to the surface of this film when the film is semi-dry or dry, but still tacky. Once cooled to room temperature, the granules adhere to the surface of the film. For a strip with an area of 10 cm² and a weight of 10 mg/cm², this formula delivers 5 mg triacetin. Assuming that 1.4 mg of bleach granules are distributed on the strip, along with 0.3 mg of enzyme granules, this dose is sufficient to produce enough peracetic acid directly at the tooth surface to significantly outperform a peroxide-only whitening strip. (Peroxide only strips typically have a total dose of approximately 3-10 mg peroxide.)

[00212] When exposed to saliva or other sources of water (such as tap water), the granules immediately dissolve and become active. The adhesive layer also is activated and sticks to the teeth effectively. Example 1 is designed to slowly erode in the mouth over time, so the user does not need to remove it.

Example 2

[00213] A strip is prepared as described above, forming the hydratable adhesive film and then while the strip is still tacky, adding the granulated agents to one side and the protective backing layer to the other side, using the ingredients in Table 2. Because the backing layer will not dissolve, the user should remove it after a sufficient period has passed to permit whitening to take place, typically about 10-30 minutes. The two layers can also be produced simultaneously by extrusion or solvent-based casting, then the granulated whitening agent can be added to the surface of the hydratable adhesive film.

Table 2

Ingredients in Layer #1	Dry Strip Concentration (wt%)
Ethyl cellulose	94
Propylene glycol	5
Titanium dioxide	1
Total	100

Ingredients in Layer #2	Dry Strip Concentration (wt%)
Hydroxypropylmethylcellulose (HPMC)	69
Polyvinylacetate (PVAc)	15
Carbopol, 971	5
Triacetin	5
Titanium dioxide	1
Flavor	5
Total	100
Ingredients in Bleach Granule	Concentration (wt%)
Gum arabic	10
Urea peroxide	90
Ingredients in Enzyme Granule	Concentration (wt%)
Cornstarch	99
Perhydrolase enzyme	1

Example 3

[00214] Various particle sizes of hydrogen peroxide-polyvinylpyrrolidone complex and an enzyme having perhydrolytic activity (“enzyme”) were evaluated for their ability to generate peracetic acid uniformly across the surface of a hydratable adhesive strip containing triacetin.

[00215] To evaluate the generation of peracetic acid from this product, 3/8” discs were cut from the film. Each disc was hydrated with 20 μ L of 50 mM sodium phosphate buffer, pH 7.2 and incubated at 37 °C for 15 min. 380 μ L of 0.1 M phosphoric acid was added to the film to quench the enzyme reaction and dilute the sample for detection. The solution was analyzed for peracetic acid with HPLC analyses for peracetic acid using the method described previously in U.S. Patent 7,829,315 to DiCosimo et al. For each evaluation, a minimum of three samples were cut from the strip product across a 24 inch length of film.

[00216] Strip samples with the enzyme and hydrogen peroxide-polyvinylpyrrolidone coating were made by first incorporating the enzyme in a pullulan polymer film. The dried film containing the enzyme was milled and sieved to a particle size of 60 to 80 mesh (177 μ m to 250 μ m). The particulate form of enzyme was then blended with hydrogen peroxide-

polyvinylpyrrolidone (PEROXYDONE™ XL-10, Ashland Inc., Wilmington, DE). This blend was deposited onto a two-layer film structure with a hydratable adhesive layer containing primarily polyethylene oxide and triacetin and a backing layer of polyvinyl alcohol to provide a non-dissolvable support layer. The results of evaluation of peracetic acid generation for two consecutive runs are listed in Table 3. Samples were evaluated at the beginning and end of the production run and demonstrate poor consistency for peracetic acid.

Table 3: Peracetic acid production from hydratable adhesive strips produced with a coating of perhydrolase enzyme in pullulan and PEROXYDONE™ XL-10.

Sample	Start of Run		End of Run	
	Avg PAA (ppm)	Std Dev (3 replicates)	Avg PAA (ppm)	Std Dev (3 replicates)
1	304	27	110	5
2	132	16	55	3

[00217] The PEROXYDONE™ XL-10 was further processed to form larger particles with ethanol high shear granulation. After granulation the sample was sieved to a particle size of 60 to 200 mesh (75 µm to 250 µm). The particle size distribution of PEROXYDONE™ XL-10, as used to produce samples described in Table 3, and the PEROXYDONE™ XL-10 following high shear granulation and sieving are described in Table 4. The samples were measured on a Beckman Coulter LS13320 equipped with a Tornado dry feeder. The powders were analyzed directly without being dispersed in a liquid.

Table 4: Particle size distribution for PEROXYDONE™ XL-10 used in deposition before and after granulation.

PEROXYDONE™ XL-10 Sample	Particle Size Distribution Statistics (µm)			
	Mean	Median	D10	D90
As received	75.9	64.0	28.5	143.5
After granulation	100.5	93.8	49.9	165.1

[00218] Another strip production run was completed by first preparing an enzyme sample loaded into the pullulan matrix at twice the concentration of samples from Table 3. The pullulan film was milled and sieved to 60 to 80 mesh (177 μm to 250 μm) and then combined with the granulated PEROXYDONE™ XL-10. The blend was then coated onto a similar two-layer film structure as described above. The evaluation of this sample for peracetic acid generation is provided in Table 4, and demonstrated higher and more consistent production of peracetic acid throughout the production run.

Table 5: Peracetic acid production from hydratable adhesive strips produced with a coating of perhydrolase enzyme in pullulan and high shear granulated PEROXYDONE™ XL-10.

Sample	Start of Run		End of Run	
	Avg PAA (ppm)	Std Dev (3 replicates)	Avg PAA (ppm)	Std Dev (3 replicates)
3	828	51	1039	162

CLAIMS

1. A tooth whitening strip comprising a hydratable adhesive film with a first side and a second side, the first side having a granular bleaching ingredient attached thereto, wherein the tooth whitening strip further comprises, in or on the film or in the form of granules attached to the first side of the film;

a) an enzyme having perhydrolytic activity, said enzyme having a carbohydrate esterase family 7 (CE-7) signature motif that aligns with a reference sequence SEQ ID NO: 1, said signature motif comprising:

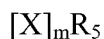
i) an RGQ motif at positions corresponding to positions 118-120 of SEQ ID NO: 1;

ii) a GXSQG motif at positions corresponding to positions 186-190 of SEQ ID NO:1; and

iii) an HE motif at positions corresponding to positions 303-304 of SEQ ID NO:1; and

(b) at least one acyl donor substrate, said substrate selected from the group consisting of:

i) esters having the structure



wherein X = an ester group of the formula $R_6C(O)O$

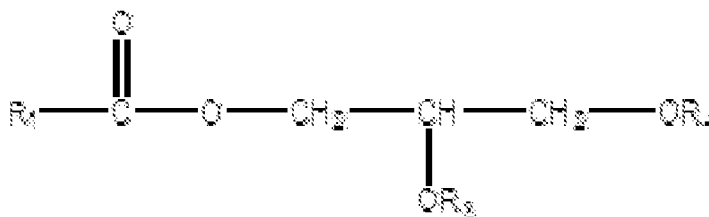
R_6 = C1 to C7 linear, branched or cyclic hydrocarbyl moiety, optionally substituted with hydroxyl groups or C1 to C4 alkoxy groups, wherein R_6 optionally comprises one or more ether linkages for R_6 = C2 to C7;

R_5 = a C1 to C6 linear, branched, or cyclic hydrocarbyl moiety or a five-membered cyclic heteroaromatic moiety or six-membered cyclic aromatic or heteroaromatic moiety optionally substituted with hydroxyl groups; wherein each carbon atom in R_5 individually comprises no more than one hydroxyl group or no more than one ester group or carboxylic acid group; wherein R_5 optionally comprises one or more ether linkages;

M is an integer ranging from 1 to the number of carbon atoms in R_5 ; and

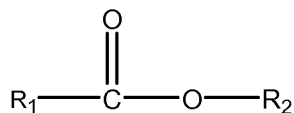
wherein said esters have solubility in water of at least 5 ppm at 25 °C;

ii) glycerides having the structure



wherein R_1 = C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R_3 and R_4 are individually H or $\text{R}_1\text{C}(\text{O})$;

iii) one or more esters of the formula



wherein R_1 is a C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R_2 is a C1 to C10 straight chain or branched chain alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, heteroaryl, $(\text{CH}_2\text{CH}_2\text{O})_n$, or $(\text{CH}_2\text{CH}(\text{CH}_3)-\text{O})_n\text{H}$ and n is 1 to 10; and

iv) acetylated saccharides selected from the group consisting of acetylated monosaccharides, acetylated disaccharides, and acetylated polysaccharide; wherein upon hydration of the hydratable adhesive film hydrogen peroxide is released from the granular bleaching ingredient and said enzyme catalyzes the formation of an effective amount of a peracid.

2. The tooth whitening strip according to claim 1 wherein the enzyme having perhydrolytic activity comprises an amino acid sequence selected from:

- a) SEQ ID NO: 1; and
- b) an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO: 1.

3. The tooth whitening strip according to claim 1 wherein the enzyme having perhydrolytic activity further comprises a binding domain fused to the N- or C-terminus of the enzyme, said binding domain having affinity for an oral tissue or for the tooth whitening strip.
4. The tooth whitening strip according to claim 3 wherein the binding domain having affinity for an oral tissue comprises an amino acid sequence selected from the group consisting of SEQ D NOs: 29-163.
5. The tooth whitening strip according to claim 4 wherein the enzyme having perhydrolytic activity has affinity for an oral tissue and comprises an amino acid sequence selected from
 - a) SEQ ID NO: 2, and
 - b) an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO: 2.
6. The tooth whitening strip according to any foregoing claim, further comprising a backing layer attached to said second side of the hydratable adhesive film, said backing layer capable of inhibiting dissolution of the hydratable adhesive film.
7. The tooth whitening strip according to any foregoing claim, wherein the granular bleaching ingredient is coated with a water soluble coating capable of dissolving upon hydration.
8. The tooth whitening strip according to any foregoing claim, wherein the granular bleaching ingredient is selected from solid peroxides and solid peroxide donors.
9. The tooth whitening strip according to any foregoing claim, wherein the granular bleaching ingredient is selected from peroxide salts, peroxide complexes, peroxyphosphate, peroxycarbonate, perborate, peroxysilicate, persulphate salts, calcium peroxyphosphate, sodium perborate, sodium carbonate peroxide, sodium peroxyphosphate, potassium persulfate, hypochlorites, urea peroxide, hydrogen peroxide polymer complexes, hydrogen peroxide-

polyvinyl pyrrolidone polymer complexes, metal peroxides, zinc peroxide, calcium peroxide, and combinations thereof.

10. The tooth whitening strip according to any foregoing claim, wherein the granular bleaching ingredient is selected from urea peroxide, sodium percarbonate, a hydrogen peroxide-polyvinylpyrrolidone polymer complex, and a combination of two or more thereof.

11. The tooth whitening strip according to any foregoing claim, wherein the particle size median diameter (D50) of the granular bleaching ingredient is from 10 microns to 300 microns.

12. The tooth whitening strip according to any foregoing claim, wherein the particle size median diameter (D50) of the granular bleaching ingredient is from 100 microns to 250 microns.

13. The tooth whitening strip according to any foregoing claim, wherein the particle size median diameter (D50) of the enzyme having perhydrolytic activity is from 100 microns to 250 microns.

14. The tooth whitening strip according to any foregoing claim, wherein the strip generates at least about 0.1 wt% of a peracid.

15. The tooth whitening strip according to any foregoing claim, wherein the amount of granular bleaching agent on the first side of the hydratable adhesive film ranges from 0.001 mg/cm² to 1 mg/cm².

16. The tooth whitening strip according to any foregoing claim, wherein the acyl donor substrate is 1,2,3-triacetoxypropane.

17. The tooth whitening strip according to any foregoing claim, wherein the hydratable adhesive film comprises one or more water-soluble polymers selected from a hydrophilic cellulose ether, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl

methycellulose, a polyvinyl acetate, a carbomer, a polysaccharide gum, xanthan gum, a modified food starch, gelatin, animal or fish-based gelatin, a cross-linked carboxyvinyl copolymer, a cross-linked polyvinylpyrrolidone, polyethylene oxide, a polyacrylic acid, a polyacrylate, a polyvinyl alcohol, alginate, casein, pullulan, and a combination of two or more thereof.

18. The tooth whitening strip according to any foregoing claim, wherein the hydratable adhesive film comprises one or more water-soluble polymers selected from a hydrophilic cellulose ether, a polyvinyl alcohol, a carbomer, and a combination of two or more thereof.

19. The tooth whitening strip according to any foregoing claim, wherein the hydratable adhesive film further comprises a plasticizer.

20. The tooth whitening strip according to any foregoing claim, wherein the hydratable adhesive film further comprises propylene glycol.

21. The tooth whitening strip according to any foregoing claim, wherein the granular bleaching ingredient is coated with a water soluble coating capable of dissolving upon hydration.

22. The tooth whitening strip according to any foregoing claim, wherein an effective amount of a peracid is generated at a substantially uniform concentration across the surface of the tooth whitening strip.

22. A method of whitening teeth comprising

- a) providing a packaging system comprising the tooth whitening strip according to any foregoing claim;
- b) removing the tooth whitening strip from the packaging system; and
- c) contacting the tooth whitening strip directly to the teeth for a period of time sufficient time whiten the teeth; wherein the tooth whitening strip is hydrated by moisture present in the oral cavity or on the tooth surface or is hydrated after step (b) but prior to step (c).