

SYSTEM PROVIDING PERHYDROLASE-CATALYZED REACTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to United States Application No. 61/577,529, filed on 19 December 2011, which is hereby incorporated by reference in its entirety.

BACKGROUND

[0001] It is frequently desirable to keep formulation components separate prior to use, for example because the components may be too unstable for long-term storage if combined. It is desirable in such cases to be able to mix the formulation components at the point of use in an efficient and simple way.

[0002] One example of a formulation where it may be desirable to keep formulation components separate is tooth-whitening formulations comprising reactive ingredients such as peroxides or peroxyacids or their precursors. For example, one may want to combine A+B or A+B+C to obtain an unstable bleach X, but keep A and B separate up to that point. The difficulty arises in that during use the mixing must be rapid, and diffusion of the bleaching agent, X, to the tooth surface must be efficient. Unfortunately, combining multiple gels or other moderately viscous materials is not generally an efficient way to quickly mix chemicals; if a typical consumer were to mix by hand, it would lead to regions of well-mixed and poorly-mixed sample. One has only to hand-mix two viscous house paints together to easily see the problem: rather than efficient blending of the two colors, laminar flow causes the colors to exist in adjacent streaks. To overcome this problem directly would require more time and mixing effort than the typical user would be willing to devote to the task, and where the reactive species X begins to break down within minutes, such a method would be unworkable.

[0003] There is thus a need for products that permit ingredients to be efficiently and effectively combined at the point of use.

SUMMARY

[0004] The invention provides a multi-chamber system, wherein one chamber contains a a low viscosity liquid solution and another contains a liquid, powder or mixture of powders, the

chambers being separated by a frangible or tearable barrier, such that upon squeezing one chamber, the barrier breaks and the components of the chambers can mix, to form a solution, emulsion, suspension or extrudable gel, which can be dispensed through an outlet in the second chamber, wherein the low viscosity liquid solution comprises a protein having perhydrolase activity which contains the carbohydrate esterase family 7 signature motif, and the other chamber or chambers contains an acyl donor, e.g., a carboxylic acid ester, and a peroxide source, such that upon mixing of the contents of the chambers, the protein having perhydrolase activity catalyzes a reaction between the peroxide released by the peroxide source and the acyl donor to form a peracid. Applied to the teeth, such a peracid is highly effective for bleaching the teeth, so that effective bleaching action can be achieved in a shorter period and with lower peroxide levels.

[0005] In a particular embodiment, one chamber contains a low viscosity aqueous solution comprising a protein having perhydrolytic activity (*i.e.*, a family 7 carbohydrate esterase), and another chamber contains a gellant, a peroxide, and carboxylic acid ester compound, all in powder form, such that when the barrier is broken and the contents of the chambers allowed to mix, the peroxide and the carboxylic acid ester can react, the reaction being catalyzed by the perhydrolase, to form a peracid, in an extrudable gel formed by the liquid and the gellant, which extrudable gel can then be extruded and applied to the teeth, *e.g.*, using a tray or strip, for sufficient time, *e.g.*, 10-30 minutes, to allow the teeth to bleach.

[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.

[0038] 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.*).

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] The present invention will become more fully understood from the detailed description and the accompanying drawings, wherein:

[0040] Figure 1 depicts one embodiment of the invention which is a two-chambered package in accordance with the invention, the package being heat-sealed about the perimeter (1), and having a first chamber (2) which contains a liquid component and a second chamber (4) comprising a powder component, separated by a frangible seal (3), such that when the first chamber (2) is squeezed, the frangible seal (3) ruptures and the liquid flows into the second chamber (4) and mixes with the powder, which resulting mixture can then be dispensed by breaking the scored edge (5) to allow the mixture to flow or be squeezed out of the nozzle (6).

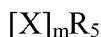
[0041] Figure 2 depicts another embodiment of the invention, permitting mixing of components just prior to use, as described for Figure 1, but utilizing a three-chambered package having a nozzle which can be opened by the consumer for dispensing product. In this embodiment, the package comprises a first chamber (7), a second chamber (8), a third chamber (9), the chambers being separated by frangible seals (3), and a nozzle with a break-away tip (6) to dispense the materials after mixing.

DETAILED DESCRIPTION

[0042] 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.

[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;

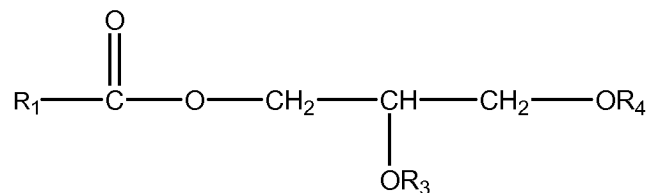
R_5 is a C1 to C6 linear, branched, or cyclic hydrocarbyl moiety or a cyclic five-membered heteroaromatic or six-membered cyclic aromatic or heteroaromatic moiety optionally substituted with a hydroxyl group; wherein each carbon atom in R_5 individually comprises no more than one hydroxyl group or no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

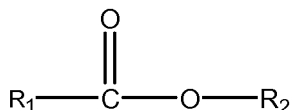
°C; or

(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 $R_1C(O)$; 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).

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

[0044] 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.

[0045] As used herein, the term “monoacetin” is synonymous with glycerol monoacetate, glycerin monoacetate, and glyceryl monoacetate.

[0046] 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.

[0047] 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.

[0048] 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.

[0049] 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.

[0050] 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.

[0051] 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.

[0052] 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.

[0053] As used herein, the terms “suitable enzymatic reaction mixture”, “suitable reaction components”, “suitable aqueous reaction mixture”, “reaction mixture”, and “peracid-generating components” refer to the materials and water in which the reactants and the perhydrolytic enzyme catalyst come into contact. The peracid-generating components will include at least enzyme having perhydrolytic activity, 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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.

[0058] 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.

[0059] 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.

[0060] 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).

[0061] 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.

[0062] 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.

[0063] 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

[0064] 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. 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.

[0065] 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.

[0066] 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.

[0067] 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.

[0068] 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.

[0069] 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.

[0070] 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.

[0071] 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.

[0072] 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 0.1 to 1.

[0073] 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.

[0074] 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 to the surface of a tooth.

[0075] As used herein, the term “effective amount” will refer to the amount of material necessary to achieve the desired effect.

[0076] As used herein, the term “substantially non-adhesive until hydrated” will refer to the lack of adhesive strength sufficient to adhere to the surface of teeth prior to hydration.

[0077] 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.

[0078] 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.

[0079] 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.

[0080] 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

[0081] 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).

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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**.

[0086] 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.

[0099] 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. 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] 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.

[00106] 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.

[00107] 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.

[00108] 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.

[00109] The perhydrolases for use in the products and methods of the invention may be in free, protected (e.g., acetylated), or salt form.

[00110] 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

[00111] 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} .”

[00112] “ 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.

[00113] “ MB_{50} ” 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 MB_{50} provides an indication of the strength of the binding interaction or affinity of the components of the complex. The lower the value of MB_{50} , the stronger, *i.e.*, “better,” the interaction of the peptide with its corresponding substrate. For example, a peptide with a nanomolar (nM) MB_{50} binds more tightly than a peptide with a micromolar (μ M) MB_{50} . Certain embodiments of the invention will have a MB_{50} value of 10^{-5} M or less.

[00114] In some embodiments, the peptidic component having affinity for an oral cavity surface may have a binding affinity, as measured by K_D or MB_{50} 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.

[00115] 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 MB_{50} 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] As used herein, the term “strong affinity” will refer to a binding affinity having a K_D or MB_{50} 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.

[00117] 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 5 weight percent (wt%) to about 75 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%.

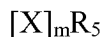
[00121] In one embodiment, the excipient used to prepare an enzyme powder may be an oligosaccharide excipient. In one embodiment, the oligosaccharide excipient has a number average molecular weight of at least about 1250 and a weight average molecular weight of at least about 9000. In some embodiments, 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, pullulan, 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, pullulan, and carboxymethylcellulose.

[00122] Suitable Ester Substrates / Acyl Donors

[00123] 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 $R_6C(O)O$;

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;

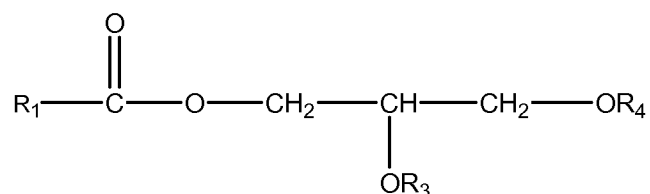
R_5 is a C1 to C6 linear, branched, or cyclic hydrocarbyl moiety or a five-membered cyclic heteroaromatic moiety or six-membered cyclic aromatic or heteroaromatic moiety optionally substituted with a hydroxyl group; wherein each carbon atom in R_5 individually comprises no more than one hydroxyl group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

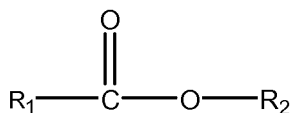
°C; or

(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 $R_1C(O)$; 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).

[00124] 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.

[00125] In another embodiment, additional suitable substrates may also include 5-acetoxymethyl-2-furaldehyde; 3,4-diacetoxy-1-butene; 4-acetoxymethylbenzoic 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.

[00126] 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.

[00127] In a further embodiment, suitable substrates are selected from the group consisting of monoacetin, diacetin, triacetin, monopropionin, dipropionin, tripropionin, monobutylin, dibutylin, 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.

[00128] The carboxylic acid ester is present at a concentration sufficient to produce the desired concentration of peroxydicarboxylic 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 perhydrolyase catalyst to the corresponding peroxydicarboxylic 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.

[00129] The peroxygen source is provided as granules 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 %, more preferably from 0.033 wt % to about 40 wt %, and more preferably from 0.1 wt % to about 30 wt %.

[00130] 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.

[00131] 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.

[00132] 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.

[00133] 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.

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

[00134] 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.

[00135] In one embodiment, the invention provides a package for an oral care product which comprises multiple chambers and is designed to keep the ingredients in each chamber separate and non-reactive until the point of use. For example, this invention provides a chemically-stable structural package design which permits an enzyme catalyzed tooth whitening product to reach pre-steady state kinetics in milliseconds after the ingredients are exposed to each other and mixed. The contents of the container are dispensed via an opening means, e.g., through a nozzle with a removable cap or plug or which becomes functional when a preferentially scored section of the container is broken off by the consumer, permitting clean and convenient dispensing of product through a shaped nozzle.

[00136] Each chamber has the capacity to store, e.g., 0.1 – 30 grams of an ingredient. The oral care product is a tooth whitening product providing a total quantity of product delivered from all chambers, e.g., between 1.0 to 5.0 grams, for example 1-2 grams to provide the intended benefit. The volumetric capacity of the chambers is designed to accommodate ingredients with a specific gravity of e.g., 1.0 to 1.1 and preferably with a specific gravity range of 1.02 to 1.05.

[00137] In one embodiment, the package is manufactured using a thermoforming process of at least two flexible films with a thickness of 50 micron to 500 micron and preferably 300 micron thick. The two films may be opaque, translucent or transparent and can be any combination when assembled in the thermoforming process. Both materials provide water vapor barrier characteristics, e.g., with less than 3% moisture loss over a three year time frame, e.g., less than 1% moisture loss over the same period. The films also provide flavor barrier. The flavor loss can be determined both by gas chromatography and by organoleptic evaluation.

[00138] The films are chemically resistant to the materials comprised therein. For example, in one embodiment they are resistant to 0.1 % to 10% hydrogen peroxide solution by weight, e.g. up to 0.3% hydrogen peroxide solution by weight.

[00139] In one embodiment, one of the two flexible materials is a polymeric laminate and the inner layer of the laminate has been selected to bond with the first flexible material but will delaminate when pressure is manually applied to the chamber with a frangible seal. The force required to break the seal is manually applied and can vary between 2 inch-lbf and 5 inch-lbf.

[00140] After the frangible seal between the compartments are broken, the ingredients in each chamber will come into intimate contact with each other. The consumer is permitted to mix the individual ingredients for a period of time to exceed the pre-steady state kinetic rate or the burst

phase. The time for pre-steady state kinetics or burst phase can be in milliseconds. This provides sufficient time for the formation and consumption of enzyme-substrate intermediates until their steady state concentrations are reached. After steady state has been achieved, the consumer can break a preferentially scored section of the multi chamber container and dispense the mixture onto a dental tray. The tray is applied to the teeth for a period of time of 15 minutes to 45 minutes and provides an effective whitening benefit.

[00141] Exemplary embodiments of the invention thus include for example packages, oral care compositions, and methods of whitening teeth, e.g.:

1. Package 1, a package comprising a deformable material configured to form at least two sealed chambers, having a first chamber, a second chamber, and optionally additional chambers, the chambers being separated by one or more barriers which are frangible or tearable,

wherein

the first chamber contains a low viscosity liquid solution comprising 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 GX SQG 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

the second chamber comprises at least one acyl donor substrate, said substrate selected from the group consisting of:

 - i) esters having the structure

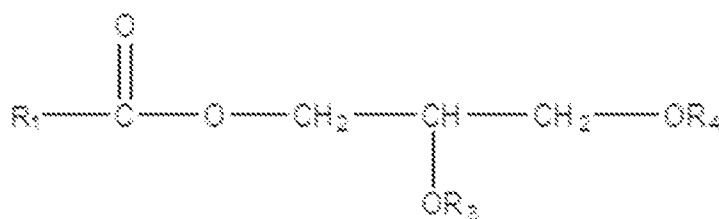
$$[X]_m R_5$$

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;

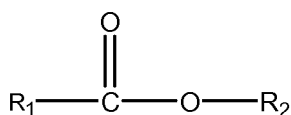
R_5 = a C1 to C6 linear, branched, or cyclic hydrocarbyl moiety or a five-membered cyclic heteroaromatic moiety or six-membered cyclic aromatic or heteroaromatic moiety optionally substituted with hydroxyl groups; wherein each carbon atom in R_5 individually comprises no more than one hydroxyl group 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; and
 wherein the second or optional additional chamber contains a peroxide source,

such that when one or more barriers between the chambers breaks, e.g., upon squeezing the first chamber, the low viscosity liquid solution mixes with the peroxide source and the acyl donor substrate, and the enzyme having perhydrolytic activity catalyzes a reaction between the peroxide released by the peroxide source and the acyl donor substrate to form a peracid; and

the package has an opening means, for example a scored region, cap or plug to allow opening of the package, to provide an outlet through which the mixture can be dispensed.

1.1. Package 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.

Package 1 or 1.1 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.

1.2. Any of the foregoing packages 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.

1.3. Any of the foregoing packages wherein the enzyme having perhydrolytic activity has affinity for an oral tissue and comprises an amino acid sequence selected from SEQ ID NO: 2 and an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO: 2.

1.4. Any of the foregoing packages wherein the deformable material is plastic or aluminum.

1.5. Any of the foregoing packages wherein the low viscosity liquid solution has a viscosity sufficiently low to ensure efficient mixing with the contents of the second chamber, e.g., below 5,000 cps, e.g. below 500 cps.

1.6. Any of the foregoing packages wherein the low viscosity liquid solution comprises a buffer.

1.7. Any of the foregoing packages wherein the acyl donor substrate is selected from (i) one or more 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) one or more hydrolysable and acceptable esters thereof (e.g. mono-, di-, and tri-glycerides and acylated saccharides) and (iii) mixtures thereof.
- 1.8. Any of the foregoing packages wherein the acyl donor substrate is selected from 1,2,3-triacetoxyp propane (sometimes referred to herein as triacetin or glycerin triacetate) and acylated saccharides, e.g. acetylated saccharides.
- 1.9. Any of the foregoing packages comprising an acyl donor substrate which comprises an ester compound having solubility in water of at least 5 ppm at 25 °C.
- 1.10. Any of the foregoing packages wherein the peroxide source is selected from solid peroxides and solid peroxide donors and mixtures thereof, e.g., selected from peroxide salts or complexes (e.g., such as peroxyphosphate, peroxy carbonate, perborate, peroxy silicate, 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; metal peroxides e.g. zinc peroxide and calcium peroxide; for example a solid peroxide selected from urea peroxide, polyvinylpyrrolidone-hydrogen peroxide complexes, sodium percarbonate, sodium perborate, and metal peroxides e.g. zinc peroxide and calcium peroxide.
- 1.11. The foregoing package wherein the peroxide source is urea peroxide.
- 1.12. The foregoing package wherein the peroxide source comprises a hydrogen peroxide-polyvinylpyrrolidone complex.
- 1.13. Any of the foregoing packages wherein the ingredients of the chambers are present in amounts sufficient to provide, upon mixing, a bleaching agent in an amount and concentration effective to whiten teeth.
- 1.14. Any of the foregoing packages wherein the second chamber contains a gellant in powder form.
- 1.15. The foregoing package wherein the gellant is selected from carbomer gellants (e.g., Carbopol 971P), polysaccharide gums, such as xanthan gum, modified food starches, animal or fish-based gelatin, and silicas.
- 1.16. The foregoing package wherein the gellant is a carbomer gellant.
- 1.17. Any of the foregoing packages wherein the second chamber contains a gellant in powder form in a relative amount to provide a viscosity of 100,000 to 150,000 cps, e.g., about

125,000 cps, upon mixing with the contents of the first chamber, e.g., wherein the gellant is present in amounts of from 5% to 50% by weight of the final mixture of the contents of the first and second chambers.

- 1.18. Any of the foregoing packages wherein the first chamber contains a low viscosity aqueous solution comprising an enzyme having perhydrolytic activity and a buffer, and the second chamber contains a gellant, a peroxide source, and an acetyl-containing compound, all in powder form, such that when the frangible barrier is broken and the contents of the two chambers allowed to mix, the peroxide and the acetyl containing compound can react, the reaction being catalyzed by the protein having perhydrolase activity, to form peracetic acid, in an extrudable gel formed by the liquid and the gellant, which extrudable gel can then be extruded and applied to the teeth, e.g., using a tray or strip, for sufficient time, e.g., 10-30 minutes, to allow the teeth to bleach.
- 1.19. Any of the foregoing packages which further comprises an applicator device such as a dental tray or strip for applying a mixture of the contents of the first and second chambers to the teeth.
- 1.20. The foregoing package wherein, when the mixture is dispensed, the opening from the second chamber is directly attached to a tray so that the mixture is extruded into the tray.
2. Composition 2, being a multi-part oral care composition comprising a first part which is physically separated from the second part during storage and combined with the second part just prior to use, e.g., within 10 minutes of use, wherein the first part comprises an enzyme having perhydrolytic activity as described for any of the foregoing packages, and second part comprises a peroxide source and a carboxy donor selected from carboxylic acids and acyl compounds, wherein the peroxide source and the carboxy donor react in the presence of the perhydrolase to form a peracid, e.g., a peroxide source and a carboxy donor as described for any of the foregoing packages, e.g.,
 - 2.1. The foregoing composition wherein the carboxy donor is selected from C₂₋₁₈ carboxylic acids (e.g., acetic acid), and hydrolysable and acceptable esters thereof (e.g. mono-, di-, and tri-glycerides) and mixtures thereof
 - 2.2. The foregoing composition wherein the carboxy donor is 1,2,3-triacetoxyp propane (sometimes referred to herein as triacetin or glycerin triacetate).

- 2.3. Any of the foregoing compositions wherein the peroxide source is a solid peroxide selected from urea peroxide, polyvinylpyrrolidone-hydrogen peroxide complexes, sodium percarbonate, sodium perborate, and metal peroxides e.g. zinc peroxide and calcium peroxide.
 - 2.4. Any of the foregoing compositions wherein the peroxide source is urea peroxide.
 - 2.5. Any of the foregoing compositions wherein the peroxide source comprises a hydrogen peroxide-polyvinylpyrrolidone complex.
 - 2.6. Any of the foregoing compositions when packaged in a package as hereinbefore described, e.g. Package 1 et seq.
3. A method (Method 3) of whitening teeth comprising activating a two part oral care composition as hereinbefore described, by combining the two parts, and applying an effective amount of the mixture thus obtained to the teeth, e.g., using an applicator, for example a dental tray or a strip, for a sufficient time, e.g., at least 10 minutes, for example 10-30 minutes, to whiten the teeth.

[00142] Peroxycarboxylic acids (“peracids”) are known as effective antimicrobial and bleaching agents. U.S. Patent 5,302,375 to Viscio, D., discloses oral compositions for whitening comprising peracetic acid dissolved in a vehicle, wherein the peracetic acid is generated within the vehicle in situ by combining water, acetylsalicylic acid, and a water soluble alkali metal percarbonate. U.S. Patent 5,279,816 to Church *et al.* discloses the use of a composition comprising peracetic acid to whiten stained or discolored teeth. U.S. Patents 6,221,341 and 7,189,385 to Montgomery, R., disclose peroxy acid tooth-whitening compositions suitable for use in a method to whiten teeth. More specifically, a peracetic acid composition may be produced by combining a hydrogen peroxide precursor, an acetic acid ester of glycerin, and water to generate, via chemical perhydrolysis, peracetic acid.

[00143] Enzymatic perhydrolysis is not described in these references. U.S. Patent Application Publication No. 2009-0311198 to Concar *et al.* discloses an oral composition comprising a *M. smegmatis* enzyme having perhydrolytic activity to bleach teeth.

[00144] Many hydrolases and esterases, for example, lipases, serine hydrolases and carbohydrate esterases, catalyze perhydrolysis, the reversible formation of peracids from carboxylic acids and hydrogen peroxide. Perhydrolases, esterases, and lipases generally contain a catalytic triad consisting of a serine (Ser), a glutamate (Glu) or aspartate (Asp), and a histidine

(His). Many perhydrolases (e.g. metal-free haloperoxidases) contain a Ser-His-Asp catalytic triad and catalyze the reversible formation of peracid from hydrogen peroxide and carboxylic acids. Without being bound by theory, it is believed that perhydrolysis takes place with an esterase-like mechanism in which a carboxylic acid reacts with the active site serine to form an acyl enzyme intermediate, which then reacts with hydrogen peroxide to form a peracid.

[00145] Numerous perhydrolases have been described in the art. The inclusion of specific variant subtilisin Carlsberg proteases having perhydrolytic activity in a body care product is disclosed in U.S. Patent 7,510,859 to Wieland *et al.* Perhydrolytic enzymes beyond the specific variant proteases are not described nor are there any working examples demonstrating the enzymatic production of peracid as a personal care benefit agent. U.S. Patent Application Publication Nos. 2008-0176783 A1; 2008-0176299 A1; 2009-0005590 A1; and 2010-0041752 A1 to DiCosimo *et al.* disclose enzymes structurally classified as members of the CE-7 family of carbohydrate esterases (i.e., cephalosporin C deacetylases [CAHs] and acetyl xylan esterases [AXEs]) that are characterized by significant perhydrolytic activity for converting carboxylic acid ester substrates (in the presence of a suitable source of peroxygen, such as hydrogen peroxide) into peroxy-carboxylic acids at concentrations sufficient for use as a disinfectant and/or a bleaching agent. Some members of the CE-7 family of carbohydrate esterases have been demonstrated to have perhydrolytic activity sufficient to produce 4000 – 5000 ppm peracetic acid from acetyl esters of alcohols, diols, and glycerols in 1 minute and up to 9000 ppm between 5 minutes and 30 minutes once the reaction components were mixed (DiCosimo *et al.*, U.S. 2009-0005590 A1). U.S. Patent application publication No. 2010-0087529 A1 describes variant CE-7 enzymes having improved perhydrolytic activity.

[00146] In one embodiment, the invention uses a perhydrolase which contains the catalytic domain of a member of the carbohydrate esterase family 7 having perhydrolytic activity (“CE-7 perhydrolase”). Although the CE-7 perhydrolases have exceptional perhydrolytic activity, their use in cosmetic personal care products has not been disclosed prior to the aforementioned provisional application.

[00147] Acyl donors in the present invention are selected from (i) one or more C₂₋₁₈ carboxylic acid esters, e.g. C₂₋₆ carboxylic acid esters, including lower linear or branched alkyl carboxylic acids, optionally substituted with hydroxy and/or C₁₋₄ alkoxy and (ii) 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.

[00148] The acyl donors or other materials 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.

[00149] As noted above, the invention may comprise gellants, for example carbomer gellants (e.g., Carbopol 971P), polysaccharide gums, such as xanthan gum, modified food starches, animal or fish-based gelatin, and silicas. Adhesive gel formulations for use with tooth whitening agents are known in the art, e.g. as described in US Patents 7,862,801; 5,746,598; 6,730,316; 7,128,899. The gellant is useful to thicken whitening solutions to a point where they will not run out of a dental tray or away from the teeth to soft tissue areas. This allows the bleaching agent to stay in contact with the teeth for extended periods of time and protects soft tissues. The use of a dental tray and a viscous bleach allows a low concentration bleach to effectively whiten a person's teeth over a 1-2 week period of time with minimal risk to the patient. Gellants for this use should be selected and adjusted to provide a viscosity upon application of 100,000 to 150,000 cps, e.g., about 125,000 cps,

[00150] In a particular embodiment, the package or multi-part composition as hereinbefore described comprises a carbomer gellant, for example a modified polyacrylic acid hydrophilic polymer such as CARBOPOL® manufactured by Lubrizol. Carbomers are capable of forming viscous gels at concentrations above as little as 5% by weight.

[00151] All ingredients for use in the formulations described herein should be orally acceptable. By “orally acceptable” as the term is used herein is meant an ingredient which is present in the formulation as described in an amount and form which does not render the formulation unsafe for use in the oral cavity.

[00152] In some embodiments, 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.

[00153] In some embodiments, 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.

[00154] In some embodiments, 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.

[00155] In some embodiments, the immobilized enzyme is adsorbed to the insoluble material, trapped in insoluble beads, covalently bonded to the insoluble material through a chemical reaction, attached by binding domain of the peptide having affinity for the insoluble material, or entrapped in an insoluble matrix.

[00156] Some embodiments provide a method of whitening the teeth or treating gingivitis, dental plaque or halitosis, comprising preparing a liquid comprising whitening agent in accordance with the method of the preceding claim, and administering the liquid to the oral cavity, e.g., by rinsing the mouth with the liquid for a period of 15 seconds to one minute and then expectorating the liquid.

[00157] In some embodiments, the product delivers a bleaching agent in a mouthwash, wherein the bleaching agent is a peracid produced by enzyme catalyzed reaction of hydrogen peroxide and triacetin. In some embodiments, two compositions – one comprising hydrogen peroxide and the other comprising triacetin - are kept in the mouthwash bottle (first compartment). Some embodiments comprise - on top of the bottle - a second compartment that is connected, e.g., using insert or screw type joints. In some embodiments, the second compartment is, e.g. a flow through cartridge, which contains an enzyme having perhydrolytic activity immobilized onto the surfaces of carried materials, such as hydroxyapatite or cellulose particles. In some embodiments, the second compartment serves as the activation component of the mouth wash.

[00158] In some embodiments, the hydrogen peroxide and triacetin are separated from the enzyme having perhydrolytic activity. In some embodiments, during use, the mixture flows through the cartridge and comes in contact with the enzyme having perhydrolytic activity on the surfaces, and the reaction is catalyzed to produce a peracid quickly. In some embodiments - after use - the mixture is separated again from the enzyme.

[00159] 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 entirety. In the event of a conflict in a definition in the present disclosure and that of a cited reference, the present disclosure controls.

[00160] 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

Example 1

[00161] In a two-chambered package, 1.0 mL of pH 7 phosphate buffer containing 0.04 mg perhydrolase enzyme is stored separately from a multi-component powder. The multi-component powder is illustrated in Tables 1A, 1B, and 1C, and comprises the encapsulated triacetin & flavor, granular urea peroxide, and a carbomer gellant. The ratio of well-blended powders, 1A:1B:1C, in this example is 92.3:1.7:6. The two chambers are separated with a water impermeable heat-sealed barrier which is less strong than the outer seals around the package (see e.g. Figure 1). To prepare for use, the consumer presses on the buffer/enzyme chamber, which breaks the frangible internal seal and pushes the buffer/enzyme into the powder chamber. The powders rapidly mix with the liquid, dissolving the peroxide source, the starch with adsorbed triacetin & flavor, and, more slowly, hydrating the gellant. After several seconds of mixing these components, the gel has effectively formed, and is ready to be applied to a tray. Approximately 0.5 grams of the newly-formed gel is applied to both an upper and lower delivery device, yielding a dose of 4.3 mg urea peroxide (equivalent to 1.5 mg hydrogen peroxide), 10 mg triacetin, and 0.01 mg hydrolase enzyme.

[00162] Opening a hole in the package, via a pre-scored opening (see Figure 1), the user can apply the gel to a tray, and then wear the tray for 20-30 minutes. Alternatively, the gel can be applied to a flexible strip such as a non-porous flexible polyethylene or a slowly dissolvable film.

TABLE 1A – Encapsulated triacetin

Ingredient	Weight %
Starch (CAPSUL®, National Starch)	94.6
Triacetin	4.3
Flavor	1.1
Total	100

TABLE 1B – Peroxide

Ingredient	Weight %
Urea peroxide granules, 5-10 microns	100

TABLE 1C – Solid Gellant

Ingredient	Weight %
Carbomer gellant (CARBOPOL® 971P, Lubrizol)	100

Example 2

[00163] An exemplary perhydrolytic enzyme was immobilized on a solid permeable matrix. The matrix was loaded into a syringe and a solution comprising hydrogen peroxide and triacetin were pushed through the matrix to generate and dispense peracetic acid (PAA).

[00164] The prototype enzyme matrix was prepared as follows: 0.1 g of hydroxyapatite powder was incubated with 1500 microliters of 5 micromolar enzyme having a perhydrolytic region and a hydroxyapatite binding domain, in 10 mM phosphate buffer pH 7.2 for 1 hour at 37°C. The powder was then washed 3x with 1 ml of 10 mM phosphate buffer, each time spinning down, pipetting out liquid, resuspending in buffer, and repeating. The powder was then resuspended in 500 microliters of 10 mM phosphate buffer and loaded into a 3 ml syringe with a 25 m syringe filter tip (5 micron membrane), and excess liquid was dispensed through the filter.

[00165] 500 microliters of reaction buffer comprising 100 mM phosphate buffer, 100 mM triacetan, and 100 mM hydrogen peroxide was loaded into the syringe, dispensed through the filter, and collected. 90 microliters of product was collected, then the reaction was stopped with 40 microliters of 1.3M H₃PO₄. The resulting mixture was then diluted 1:10 in phosphate buffer

and added to detection reagent, incubated 10 minutes and read at A_{405} nm. The proportion of PAA generated was measured. The procedure was then repeated without enzyme, as a control. [00166] Approximately 900 ppm of PAA was generated by this method, compared to 32 ppm without the perhydrolase enzyme. The reactants were in contact with the immobilized enzyme for less than 60 seconds:

Table 2A

Sample	PAA (ppm)	Standard Deviation
Enzyme	903.818	122.922
No Enzyme	32.438	2.414

[00167] The experiment was repeated, three times with enzyme, three times without; allowing no more than 15 seconds contact with enzyme. PAA was produced consistently at levels of ca. 300-350 ppm with enzyme, and about 65 ppm without. The results are described below in Table 2B.

Table 2B

Sample	PAA (ppm)	Standard Deviation
Enzyme 1	363.380	74.462
Enzyme 2	356.309	43.188
Enzyme 3	308.960	55.096
No Enzyme 1	66.066	2.663
No Enzyme 2	63.299	0.533
No Enzyme 3	65.759	0.533

[00168] Similar results were obtained using a larger (10 ml) syringe:

Table 2C

Sample	PAA (ppm)	Standard Deviation
Enzyme	333.864	65.987
No Enzyme	62.223	1.399

[00169] The reaction in the presence of the immobilized enzyme thus proceeds reproducibly, rapidly and efficiently, to provide levels of PAA which are many times the levels needed to kill bacteria, and would be sufficient to whiten teeth.

CLAIMS

1. A package comprising a deformable material configured to form at least two sealed chambers, the package comprising:

- a first chamber;
- a second chamber; and
- optionally additional chambers,

wherein the chambers are separated by one or more barriers which are frangible or tearable, wherein the first chamber contains a low viscosity liquid solution comprising 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

the second chamber comprises at least one acyl donor substrate, said substrate selected from the group consisting of:

- i) esters having the structure



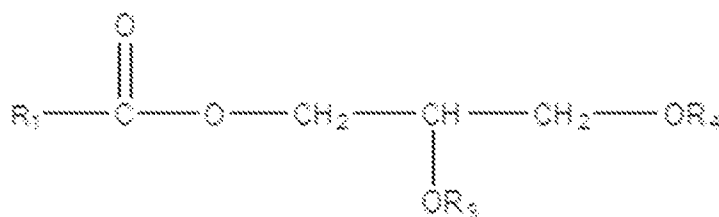
wherein X = an ester group of the formula $R_6C(O)O$

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;

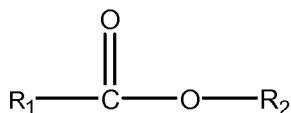
M is an integer ranging from 1 to the number of carbon atoms in R_5 ; and
wherein said esters have solubility in water of at least 5 ppm at 25 °C;

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



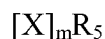
wherein R_1 is a C1 to C7 straight chain or branched chain alkyl optionally substituted with an hydroxyl or a C1 to C4 alkoxy group and R_2 is a C1 to C10 straight chain or branched chain alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, heteroaryl, $(\text{CH}_2\text{CH}_2\text{O})_n$, or $(\text{CH}_2\text{CH}(\text{CH}_3)-\text{O})_n\text{H}$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

iv) acetylated saccharides selected from the group consisting of acetylated monosaccharides, acetylated disaccharides, and acetylated polysaccharide; and

wherein the second or optional additional chamber contains a peroxide source, such that when one or more barriers between the chambers breaks upon squeezing the first chamber, the low viscosity liquid solution mixes with the peroxide source and the acyl donor substrate, and the enzyme having perhydrolytic activity catalyzes a reaction between the peroxide released by the peroxide source and the acyl donor substrate to form a peracid; and the package has an opening means, for example a scored region, cap or plug to allow opening of the package, to provide an outlet through which the mixture can be dispensed.

2. The package of 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 package of claim 1 or 2 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.
4. Any of the foregoing packages wherein the binding domain having affinity for an oral tissue comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 178-197.
5. Any of the foregoing packages wherein the enzyme having perhydrolytic activity has affinity for an oral tissue and comprises an amino acid sequence selected from SEQ ID NO: 2, and an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO: 2.
6. The package of any of the foregoing claims wherein the material in the second chamber is in the form of a powder.
7. The package of any of the foregoing claims wherein the peroxide source is a solid peroxide source selected from urea peroxide, a polyvinylpyrrolidone-hydrogen peroxide complex, sodium percarbonate, sodium perborate, and metal peroxides e.g. zinc peroxide and calcium peroxide.
8. The package according to any of the foregoing claims wherein the second chamber contains a gellant, such that upon mixing and formation of the peracid, an extrudable gel is formed by the liquid and the gellant, comprising the peracid, which extrudable gel can then be extruded and applied to a tooth surface, for sufficient time to whiten a tooth.

9. The package according to any of the foregoing claims, wherein the gellant is in powder form.
10. The package according to claim 9, wherein the gellant is selected from a carbomer gellant, a polysaccharide gum, a modified food starch, an animal or fish-based gelatin, a silica; and a combination of two or more thereof.
11. A package according to any of the foregoing claims wherein
 - a. the low viscosity aqueous solution further comprises a buffer, and
 - b. the second chamber contains a gellant, a peroxide source, and an acetyl-containing compound, all in powder form,
 such that when the frangible barrier is compromised and the contents of the two chambers allowed to mix, the peroxide and the acetyl containing compound react in the presence of the enzyme having perhydrolytic activity to form peracetic acid, in an extrudable gel formed by the liquid and the gellant.
12. A multi-part oral care composition comprising a first part which is physically separated from a second part during storage and combined with a second part just prior to use, wherein the first part comprises 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
 the second part comprises a peroxide source and 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 $R_6C(O)O$

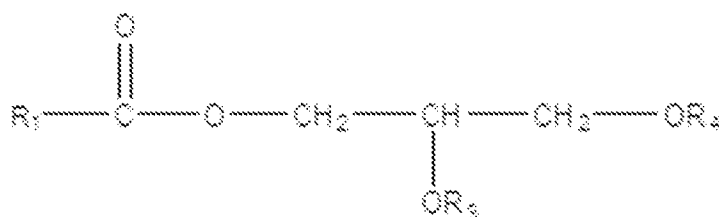
R_6 = C1 to C7 linear, branched or cyclic hydrocarbyl moiety, optionally substituted with hydroxyl groups or C1 to C4 alkoxy groups, wherein R_6 optionally comprises one or more ether linkages for R_6 = C2 to C7;

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

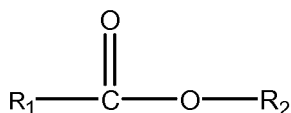
wherein said esters have solubility in water of at least 5 ppm at 25 °C;

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 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; and wherein the peroxide source and the at least one acyl donor substrate react in the presence of the enzyme having perhydrolytic activity to form a peracid.

13. The oral care composition according to claim 12 wherein the peroxide source is a solid peroxide source selected from urea peroxide, polyvinylpyrrolidone-hydrogen peroxide complexes, sodium percarbonate, sodium perborate, and metal peroxides.

14. The oral care composition according to claim 12 or claim 13 wherein the peroxide source is urea peroxide or a polyvinylpyrrolidone-hydrogen peroxide complex.

15. A method of whitening teeth comprising

- a. activating a package according to claim 1 or a multi-part oral care composition according to claim 12 by combining the materials in the different chambers or parts respectively; and
- b. applying an effective amount of the mixture thus obtained to the teeth for a sufficient time to whiten the teeth.