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(54) **Title**: POLY-ALPHA-1,3-1,6-GLUCANS FOR VISCOSITY MODIFICATION

(57) **Abstract**: Compositions are disclosed herein comprising poly alpha-1,3-1,6-glucan with a weight average degree of polymerization (DPw) of at least 1000. This glucan polymer comprises at least 30% alpha-1,3 linkages and at least 30% alpha-1,6 linkages. Further disclosed are glucosyltransferase enzymes that synthesize poly alpha-1,3-1,6-glucan. Ether derivatives of poly alpha-1,3-1,6-glucan and methods of using such derivatives as viscosity modifiers are also disclosed.

TITLE

POLY-ALPHA-1 ,3-1 ,6-GLUCANS FOR VISCOSITY MODIFICATION

This application claims the benefit of U.S. Provisional Application Nos. 61/939,802 (filed February 14, 2014) and 62/014,293 (filed June 19, 2014), both of which are incorporated herein by reference in their entireties.

FIELD OF INVENTION

This invention is in the field of polysaccharides and polysaccharide derivatives. Specifically, this invention pertains to certain poly alpha-1,3-1,6-glucans, glucosyltransferase enzymes that synthesize these glucans, ethers of these glucans, and use of such ethers as viscosity modifiers.

BACKGROUND

Driven by a desire to find new structural polysaccharides using enzymatic syntheses or genetic engineering of microorganisms, researchers have discovered polysaccharides that are biodegradable and can be made economically from renewably sourced feedstocks. One such polysaccharide is poly alpha-1,3-glucan, a glucan polymer characterized by having alpha-1,3-glycosidic linkages.

Poly alpha-1,3-glucan has been isolated by contacting an aqueous solution of sucrose with a glucosyltransferase (gtf) enzyme isolated from *Streptococcus salivarius* (Simpson et al., *Microbiology* 141:1451-1460, 1995). U.S. Patent 7,000,000 disclosed the preparation of a polysaccharide fiber using an *S. salivarius* gtfJ enzyme. At least 50% of the hexose units within the polymer of this fiber were linked via alpha-1,3-glycosidic linkages. The disclosed polymer formed a liquid crystalline solution when it was dissolved above a critical concentration in a solvent or in a mixture comprising a solvent. From this solution continuous, strong, cotton-like fibers, highly suitable for use in textiles, were spun and used.

Development of new glucan polysaccharides and derivatives thereof is desirable given their potential utility in various applications. It is also desirable to identify glucosyltransferase enzymes that can synthesize new glucan

polysaccharides, especially those with mixed glycosidic linkages and high molecular weight.

SUMMARY OF INVENTION

In one embodiment, the invention concerns a composition comprising poly alpha-1,3-1,6-glucan, wherein (i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,3 linkages, (ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,6 linkages, (iii) the poly alpha-1,3-1,6-glucan has a weight average degree of polymerization (DP_w) of at least 1000; and (iv) the alpha-1,3 linkages and alpha-1,6 linkages of the poly alpha-1,3-1,6-glucan do not consecutively alternate with each other.

In a second embodiment, at least 60% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,6 linkages.

In a third embodiment, the DP_w of the poly alpha-1,3-1,6-glucan is at least 10000.

In a fourth embodiment, the poly alpha-1,3-1,6-glucan is a product of a glucosyltransferase enzyme comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

In a fifth embodiment, the invention concerns a composition comprising a poly alpha-1,3-1,6-glucan ether compound, wherein (i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,3 linkages, (ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,6 linkages, (iii) the poly alpha-1,3-1,6-glucan ether compound has a weight average degree of polymerization (DP_w) of at least 1000; (iv) the alpha-1,3 linkages and alpha-1,6 linkages of the poly alpha-1,3-1,6-glucan ether compound do not consecutively alternate with each other, and (v) the poly alpha-1,3-1,6-glucan ether compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0.

In a sixth embodiment, at least 60% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,6 linkages.

In a seventh embodiment, at least one organic group is selected from the group consisting of carboxy alkyl group, hydroxy alkyl group, and alkyl group. The poly alpha-1,3-1,6-glucan ether compound in this embodiment may contain one type of organic group, or two or more types of organic group. At least one organic group is selected from the group consisting of carboxymethyl, hydroxypropyl, dihydroxypropyl, hydroxyethyl, methyl, and ethyl group, for example.

In an eighth embodiment, at least one organic group is a positively charged organic group.

In a ninth embodiment, the poly alpha-1,3-1,6-glucan from which the poly alpha-1,3-1,6-glucan ether compound is derived is a product of a glucosyltransferase enzyme comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

In a tenth embodiment, the composition can be a hydrocolloid or aqueous solution having a viscosity of at least about 10 cPs. The hydrocolloid or aqueous solution is in the form of a personal care product, pharmaceutical product, food product, household product, or industrial product in an eleventh embodiment.

In a twelfth embodiment, the invention concerns a method of producing a poly alpha-1,3-1,6-glucan ether compound. This method comprises contacting poly alpha-1,3-1,6-glucan in a reaction under alkaline conditions with at least one etherification agent comprising an organic group, wherein at least one organic group is etherified to the poly alpha-1,3-1,6-glucan thereby producing a poly alpha-1,3-1,6-glucan ether compound. Further, (i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,3 linkages, (ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,6 linkages, (iii) the poly alpha-1,3-1,6-glucan has a weight average degree of polymerization (DP_w) of at least 1000, (iv) the alpha-1,3 linkages and alpha-1,6 linkages of the poly alpha-1,3-1,6-glucan do not consecutively alternate with each other, and (v) the poly alpha-1,3-1,6-glucan ether compound has a degree of substitution (DoS)

with at least one organic group of about 0.05 to about 3.0. A poly alpha-1,3-1,6-glucan ether compound produced by this method can optionally be isolated.

In a thirteenth embodiment, the alkaline conditions of the reaction comprise an alkali hydroxide solution.

In a fourteenth embodiment, the invention concerns a method for increasing the viscosity of an aqueous composition. This method comprises contacting one or more poly alpha-1,3-1,6-glucan ether compounds with the aqueous composition, wherein (i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,3 linkages, (ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,6 linkages, (iii) the poly alpha-1,3-1,6-glucan ether compound has a weight average degree of polymerization (DP_w) of at least 1000; (iv) the alpha-1,3 linkages and alpha-1,6 linkages of the poly alpha-1,3-1,6-glucan ether compound do not consecutively alternate with each other; and the compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0. The contacting step in this method results in increasing the viscosity of the aqueous composition.

In a fifteenth embodiment, the invention concerns a method of treating a material. This method comprises contacting a material with an aqueous composition comprising a poly alpha-1,3-1,6-glucan ether compound as disclosed herein. The poly alpha-1,3-1,6-glucan ether compound adsorbs to the surface of the material in certain embodiments of this method.

BRIEF DESCRIPTION OF THE SEQUENCES

Table 1. Summary of Nucleic Acid and Protein SEQ ID Numbers

Description	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.
"4297 gtf", *Streptococcus oralis.* DNA codon-optimized for expression in *E. coli*. The first 228 amino acids of the protein are deleted compared to GENBANK Identification No. 7684297; a start methionine is included.	1	2 (1348 aa)
"3298 gtf", *Streptococcus* sp. C150. The first 209	3	4 (1242 aa)

amino acids of the protein are deleted compared to GENBANK Identification No. 322373298; a start methionine is included.		
"0544 gtf", *Streptococcus mutans.* DNA codon-optimized for expression in *E. coli*. The first 164 amino acids of the protein are deleted compared to GENBANK Identification No. 290580544; a start methionine is included.	5	6 (1313 aa)
"5618 gtf", *Streptococcus sanguinis.* DNA codon-optimized for expression in *E. coli*. The first 223 amino acids of the protein are deleted compared to GENBANK Identification No. 328945618; a start methionine is included.	7	8 (1348 aa)
"2379 gtf", *Streptococcus salivarius.* DNA codon-optimized for expression in *E. coli*. The first 203 amino acids of the protein are deleted compared to GENBANK Identification No. 662379; a start methionine is included.	9	10 (1247 aa)

DETAILED DESCRIPTION OF THE INVENTION

The disclosures of all cited patent and non-patent literature are incorporated herein by reference in their entirety.

As used herein, the term "invention" or "disclosed invention" is not meant to be limiting, but applies generally to any of the inventions defined in the claims or described herein. These terms are used interchangeably herein.

The term "glucan" herein refers to a polysaccharide of D-glucose monomers that are linked by glycosidic linkages.

The terms "glycosidic linkage" and "glycosidic bond" are used interchangeably herein and refer to the type of covalent bond that joins a carbohydrate molecule to another carbohydrate molecule. The term "alpha-1,3-glycosidic linkage" as used herein refers to the type of covalent bond that joins alpha-D-glucose molecules to each other through carbons 1 and 3 on adjacent alpha-D-glucose rings. The term "alpha-1,6-glycosidic linkage" as used herein refers to the type of covalent bond that joins alpha-D-glucose molecules to each other through carbons 1 and 6 on adjacent alpha-D-glucose rings. Herein, "alpha-D-glucose" will be referred to as "glucose." All glycosidic linkages disclosed herein are alpha-glycosidic linkages, except where otherwise noted.

The glycosidic linkage profile of a poly alpha-1,3-1,6-glucan herein can be determined using any method known in the art. For example, a linkage profile can be determined using methods that use nuclear magnetic resonance (NMR) spectroscopy (e.g., ^{13}C NMR or ^{1}H NMR). These and other methods that can be used are disclosed in Food Carbohydrates: Chemistry, Physical Properties, and Applications (S. W. Cui, Ed., Chapter 3, S. W. Cui, Structural Analysis of Polysaccharides, Taylor & Francis Group LLC, Boca Raton, FL, 2005), which is incorporated herein by reference.

The terms "poly alpha-1,3-1,6-glucan", "alpha-1,3-1,6-glucan polymer", and "poly (alpha-1,3)(alpha-1,6) glucan" are used interchangeably herein (note that the order of the linkage denotations "1,3" and "1,6" in these terms is of no moment). Poly alpha-1,3-1,6-glucan herein is a polymer comprising glucose monomeric units linked together by glycosidic linkages (i.e., glucosidic linkages), wherein at least about 30% of the glycosidic linkages are alpha-1,3-glycosidic linkages, and at least about 30% of the glycosidic linkages are alpha-1,6-glycosidic linkages. Poly alpha-1,3-1,6-glucan is a type of polysaccharide containing a mixed glycosidic linkage content. The meaning of the term poly alpha-1,3-1,6-glucan in certain embodiments herein excludes "alternan," which is a glucan containing alpha-1,3 linkages and alpha-1,6 linkages that consecutively alternate with each other (U.S. Pat. No. 5702942, U.S. Pat. Appl. Publ. No. 2006/0127328). Alpha-1,3 and alpha-1,6 linkages that "consecutively alternate" with each other can be visually represented by ...G-1,3-G-1,6-G-1,3-G-1,6-G-1,3-G-1,6-G-1,3-G-..., for example, where G represents glucose.

Poly alpha-1,3-1,6-glucan herein, for example, can be produced by a glucosyltransferase enzyme comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10. Such production can be from a gtf reaction herein.

The term "sucrose" herein refers to a non-reducing disaccharide composed of an alpha-D-glucose molecule and a beta-D-fructose molecule linked by an alpha-1,2-glycosidic bond. Sucrose is known commonly as table sugar.

The "molecular weight" of a poly alpha-1,3-1,6-glucan or poly alpha-1,3-1,6-glucan ether compound herein can be represented as number-average molecular weight (M_n) or as weight-average molecular weight (M_w). Alternatively, molecular weight can be represented as Daltons, grams/mole, DP_w (weight average degree of polymerization), or DP_n (number average degree of polymerization). Various means are known in the art for calculating these molecular weight measurements such as with high-pressure liquid chromatography (HPLC), size exclusion chromatography (SEC), or gel permeation chromatography (GPC).

The terms "glucosyltransferase enzyme", "gtf enzyme", "gtf enzyme catalyst", "gtf", and "glucansucrase" are used interchangeably herein. The activity of a gtf enzyme herein catalyzes the reaction of the substrate sucrose to make the products poly alpha-1,3-1,6-glucan and fructose. Other products (byproducts) of a gtf reaction can include glucose (where glucose is hydrolyzed from the glucosyl-gtf enzyme intermediate complex), various soluble oligosaccharides (e.g., DP2-DP7), and leucrose (where glucose of the glucosyl-gtf enzyme intermediate complex is linked to fructose). Leucrose is a disaccharide composed of glucose and fructose linked by an alpha-1,5 linkage. Wild type forms of glucosyltransferase enzymes generally contain (in the N-terminal to C-terminal direction) a signal peptide, a variable domain, a catalytic domain, and a glucan-binding domain. A gtf herein is classified under the glycoside hydrolase family 70 (GH70) according to the CAZy (Carbohydrate-Active EnZymes) database (Cantarel et al., *Nucleic Acids Res.* 37:D233-238, 2009).

The terms "glucosyltransferase catalytic domain" and "catalytic domain" are used interchangeably herein and refer to the domain of a glucosyltransferase enzyme that provides poly alpha-1,3-1,6-glucan-producing activity to the glucosyltransferase enzyme.

The terms "gtf reaction" and "enzymatic reaction" are used interchangeably herein and refer to a reaction that is performed by a glucosyltransferase enzyme. A "gtf reaction solution" as used herein generally

refers to a solution comprising at least one active glucosyltransferase enzyme in a solution comprising sucrose and water, and optionally other components. It is in a gtf reaction solution where the step of contacting water, sucrose and a glucosyltransferase enzyme is performed. The term "under suitable gtf reaction conditions" as used herein, refers to gtf reaction conditions that support conversion of sucrose to poly alpha-1,3-1,6-glucan via glucosyltransferase enzyme activity. A gtf reaction herein is not naturally occurring.

The terms "percent by volume", "volume percent", "vol %" and "v/v %" are used interchangeably herein. The percent by volume of a solute in a solution can be determined using the formula: [(volume of solute)/(volume of solution)] x 100%.

The terms "percent by weight", "weight percentage (wt %)" and "weight-weight percentage (% w/w)" are used interchangeably herein. Percent by weight refers to the percentage of a material on a mass basis as it is comprised in a composition, mixture, or solution.

The terms "increased", "enhanced" and "improved" are used interchangeably herein. These terms refer to a greater quantity or activity such as a quantity or activity slightly greater than the original quantity or activity, or a quantity or activity in large excess compared to the original quantity or activity, and including all quantities or activities in between. Alternatively, these terms may refer to, for example, a quantity or activity that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20% more than the quantity or activity for which the increased quantity or activity is being compared.

The terms "polynucleotide", "polynucleotide sequence", and "nucleic acid sequence" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of DNA or RNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof.

The term “gene” as used herein refers to a polynucleotide sequence that expresses a protein, and which may refer to the coding region alone or may include regulatory sequences upstream and/or downstream to the coding region (e.g., 5' untranslated regions upstream of the transcription start site of the coding region). A gene that is “native” or “endogenous” refers to a gene as found in nature with its own regulatory sequences; this gene is located in its natural location in the genome of an organism. “Chimeric gene” refers to any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. A “foreign” or “heterologous” gene refers to a gene that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, native genes introduced into a new location within the native host, or chimeric genes. Polynucleotide sequences in certain embodiments disclosed herein are heterologous. A “transgene” is a gene that has been introduced into the genome by a transformation procedure. A “codon-optimized gene” is a gene having its frequency of codon usage designed to mimic the frequency of preferred codon usage of particular host cell.

The term “recombinant” or “heterologous” as used herein refers to an artificial combination of two otherwise separate segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques. The terms “recombinant”, “transgenic”, “transformed”, “engineered” or “modified for exogenous gene expression” are used interchangeably herein.

The term “transformation” as used herein refers to the transfer of a nucleic acid molecule into a host organism. The nucleic acid molecule may be a plasmid that replicates autonomously, or it may integrate into the genome of the host organism. Host organisms containing a transformed nucleic acid fragment(s) are “transgenic”, “recombinant”, or “transformed”, and can be referred to as “transformants”.

A native amino acid sequence or polynucleotide sequence is naturally occurring, whereas a non-native amino acid sequence or polynucleotide sequence does not occur in nature.

"Coding sequence" as used herein refers to a DNA sequence that codes for a specific amino acid sequence. "Regulatory sequences" as used herein refer to nucleotide sequences located upstream of the coding sequence's transcription start site, 5' untranslated regions and 3' non-coding regions, and which may influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, enhancers, silencers, 5' untranslated leader sequence, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites, stem-loop structures and other elements involved in regulation of gene expression.

The terms "sequence identity" or "identity" as used herein with respect to polynucleotide or polypeptide sequences refer to the nucleic acid bases or amino acid residues in two sequences that are the same when aligned for maximum correspondence over a specified comparison window. Thus, "percentage of sequence identity" or "percent identity" refers to the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the results by 100 to yield the percentage of sequence identity.

The Basic Local Alignment Search Tool (BLAST) algorithm, which is available online at the National Center for Biotechnology Information (NCBI) website, may be used, for example, to measure percent identity between or among two or more of the polynucleotide sequences (BLASTN algorithm) or

polypeptide sequences (BLASTP algorithm) disclosed herein. Alternatively, percent identity between sequences may be performed using a Clustal algorithm (e.g., ClustalW or ClustalV). For multiple alignments using a Clustal method of alignment, the default values may correspond to GAP PENALTY=10 and GAP LENGTH PENALTY=10. Default parameters for pairwise alignments and calculation of percent identity of protein sequences using a Clustal method may be KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids, these parameters may be KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. Alternatively still, percent identity between sequences may be performed using an EMBOSS algorithm (e.g., needle) with parameters such as GAP OPEN=10, GAP EXTEND=0.5, END GAP PENALTY=false, END GAP OPEN=10, END GAP EXTEND=0.5 using a BLOSUM matrix (e.g., BLOSUM62).

Various polypeptide amino acid sequences and polynucleotide sequences are disclosed herein as features of certain embodiments. Variants of these sequences that are at least about 70-85%, 85-90%, or 90%-95% identical to the sequences disclosed herein can be used. Alternatively, a variant amino acid sequence or polynucleotide sequence can have at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with a sequence disclosed herein. The variant amino acid sequence or polynucleotide sequence may have the same function/activity of the disclosed sequence, or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the function/activity of the disclosed sequence.

The term “isolated” as used in certain embodiments refers to any cellular component that has been completely or partially purified from its native source (e.g., an isolated polynucleotide or polypeptide molecule). In some instances, an isolated polynucleotide or polypeptide molecule is part of a greater composition, buffer system or reagent mix. For example, an isolated polynucleotide or polypeptide molecule can be comprised within a cell or organism in a

heterologous manner. Another example is an isolated glucosyltransferase enzyme.

The terms "poly alpha-1,3-1,6-glucan ether compound", "poly alpha-1,3-1,6-glucan ether", and "poly alpha-1,3-1,6-glucan ether derivative" are used interchangeably herein. A poly alpha-1,3-1,6-glucan ether compound herein is a poly alpha-1,3-1,6-glucan that has been etherified with one or more organic groups such that the compound has a degree of substitution (DoS) with one or more organic groups of about 0.05 to about 3.0. Such etherification occurs at one or more hydroxyl groups of at least 30% of the glucose monomeric units of the poly alpha-1,3-1,6-glucan.

A poly alpha-1,3-1,6-glucan ether compound is termed an "ether" herein by virtue of comprising the substructure -C_G-O-C-, where "-C_G-" represents a carbon atom of a glucose monomeric unit of a poly alpha-1,3-1,6-glucan ether compound (where such carbon atom was bonded to a hydroxyl group [-OH] in the poly alpha-1,3-1,6-glucan precursor of the ether), and where "-C-" is a carbon atom of the organic group. Thus, for example, with regard to a glucose monomeric unit (G) involved in -1,3-G-1,3- within an ether herein, C_G atoms 2, 4 and/or 6 of the glucose (G) may independently be linked to an OH group or be in ether linkage to an organic group. Similarly, for example, with regard to a glucose monomeric unit (G) involved in -1,3-G-1,6- within an ether herein, C_G atoms 2, 4 and/or 6 of the glucose (G) may independently be linked to an OH group or be in ether linkage to an organic group. Also, for example, with regard to a glucose monomeric unit (G) involved in -1,6-G-1,6- within an ether herein, C_G atoms 2, 3 and/or 4 of the glucose (G) may independently be linked to an OH group or be in ether linkage to an organic group. Similarly, for example, with regard to a glucose monomeric unit (G) involved in -1,6-G-1,3- within an ether herein, C_G atoms 2, 3 and/or 4 of the glucose (G) may independently be linked to an OH group or be in ether linkage to an organic group.

It would be understood that a "glucose" monomeric unit of a poly alpha-1,3-1,6-glucan ether compound herein typically has one or more organic groups

in ether linkage. Thus, such a glucose monomeric unit can also be referred to as an etherized glucose monomeric unit.

Poly alpha-1,3-1,6-glucan ether compounds disclosed herein are synthetic, man-made compounds. Likewise, compositions comprising poly alpha-1,3-1,6-glucan (e.g., isolated poly alpha-1,3-1,6-glucan) are synthetic, man-made compounds.

An "organic group" group as used herein can refer to a chain of one or more carbons that (i) has the formula $-C_nH_{2n+1}$ (i.e., an alkyl group, which is completely saturated) or (ii) is mostly saturated but has one or more hydrogens substituted with another atom or functional group (i.e., a "substituted alkyl group"). Such substitution may be with one or more hydroxyl groups, oxygen atoms (thereby forming an aldehyde or ketone group), carboxyl groups, or other alkyl groups. Thus, as examples, an organic group herein can be an alkyl group, carboxy alkyl group, or hydroxy alkyl group. An organic group herein may thus be uncharged or anionic (an example of an anionic organic group is a carboxy alkyl group).

A "carboxy alkyl" group herein refers to a substituted alkyl group in which one or more hydrogen atoms of the alkyl group are substituted with a carboxyl group. A "hydroxy alkyl" group herein refers to a substituted alkyl group in which one or more hydrogen atoms of the alkyl group are substituted with a hydroxyl group.

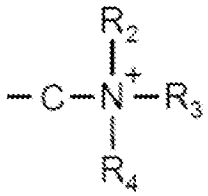
An "organic group" can alternatively refer to a "positively charged organic group". A positively charged organic group as used herein refers to a chain of one or more carbons ("carbon chain") that has one or more hydrogens substituted with another atom or functional group (i.e., a "substituted alkyl group"), where one or more of the substitutions is with a positively charged group. Where a positively charged organic group has a substitution in addition to a substitution with a positively charged group, such additional substitution may be with one or more hydroxyl groups, oxygen atoms (thereby forming an aldehyde or ketone group), alkyl groups, and/or additional positively charged

groups. A positively charged organic group has a net positive charge since it comprises one or more positively charged groups.

The terms "positively charged group", "positively charged ionic group" and "cationic group" are used interchangeably herein. A positively charged group comprises a cation (a positively charged ion). Examples of positively charged groups include substituted ammonium groups, carbocation groups and acyl cation groups.

A composition that is "positively charged" herein typically has more protons than electrons and is repelled from other positively charged substances, but attracted to negatively charged substances.

The terms "substituted ammonium group", "substituted ammonium ion" and "substituted ammonium cation" are used interchangeably herein. A substituted ammonium group herein comprises structure I:



(I).

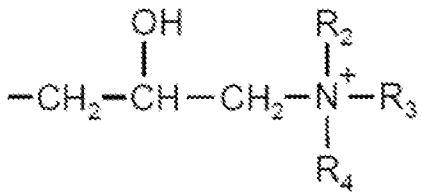
R_2, R_3 and R_4 in structure I each independently represent a hydrogen atom or an alkyl, aryl, cycloalkyl, aralkyl, or alkaryl group. The carbon atom (C) in structure I is part of the chain of one or more carbons ("carbon chain") of the positively charged organic group. The carbon atom is either directly ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan, or is part of a chain of two or more carbon atoms ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan. The carbon atom in structure I can be $-CH_2-$, -CH- (where a H is substituted with another group such as a hydroxy group), or -C- (where both H's are substituted).

A substituted ammonium group can be a "primary ammonium group", "secondary ammonium group", "tertiary ammonium group", or "quaternary ammonium" group, depending on the composition of R_2, R_3 and R_4 in structure I. A primary ammonium group herein refers to structure I in which each of R_2, R_3 and R_4 is a hydrogen atom (i.e., $-C-NH_3^+$). A secondary ammonium group herein

refers to structure I in which each of R_2 and R_3 is a hydrogen atom and R_4 is an alkyl, aryl, or cycloalkyl group. A tertiary ammonium group herein refers to structure I in which R_2 is a hydrogen atom and each of R_3 and R_4 is an alkyl, aryl, or cycloalkyl group. A quaternary ammonium group herein refers to structure I in which each of R_2, R_3 and R_4 is an alkyl, aryl, or cycloalkyl group (i.e., none of R_2, R_3 and R_4 is a hydrogen atom).

A quaternary ammonium poly alpha-1,3-1,6-glucan ether herein can comprise a trialkyl ammonium group (where each of R_2, R_3 and R_4 is an alkyl group), for example. A trimethylammonium group is an example of a trialkyl ammonium group, where each of R_2, R_3 and R_4 is a methyl group. It would be understood that a fourth member (i.e., R_1) implied by "quaternary" in this nomenclature is the chain of one or more carbons of the positively charged organic group that is ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan.

An example of a quaternary ammonium poly alpha-1,3-1,6-glucan ether compound is trimethylammonium hydroxypropyl poly alpha-1,3-1,6-glucan. The positively charged organic group of this ether compound can be represented as structure II:



(II), where each of R_2, R_3 and R_4 is a methyl group. Structure II is an example of a quaternary ammonium hydroxypropyl group.

A "halide" herein refers to a compound comprising one or more halogen atoms (e.g., fluorine, chlorine, bromine, iodine). A halide herein can refer to a compound comprising one or more halide groups such as fluoride, chloride, bromide, or iodide. A halide group may serve as a reactive group of an etherification agent.

The terms "reaction", "reaction composition", and "etherification reaction" are used interchangeably herein and refer to a reaction comprising at least poly

alpha-1,3-1,6-glucan and an etherification agent. These components are typically mixed (e.g., resulting in a slurry) and/or dissolved in a solvent (organic and/or aqueous) comprising alkali hydroxide. A reaction is placed under suitable conditions (e.g., time, temperature) for the etherification agent to etherify one or more hydroxyl groups of the glucose units of poly alpha-1,3-1,6-glucan with an organic group, thereby yielding a poly alpha-1,3-1,6-glucan ether compound.

The term "alkaline conditions" herein refers to a solution or mixture pH of at least 11 or 12. Alkaline conditions can be prepared by any means known in the art, such as by dissolving an alkali hydroxide in a solution or mixture.

The terms "etherification agent" and "alkylation agent" are used interchangeably herein. An etherification agent herein refers to an agent that can be used to etherify one or more hydroxyl groups of one or more glucose units of poly alpha-1,3-1,6-glucan with an organic group. An etherification agent thus comprises an organic group.

The term "poly alpha-1,3-1,6-glucan slurry" herein refers to an aqueous mixture comprising the components of a glucosyltransferase enzymatic reaction such as poly alpha-1,3-1,6-glucan, sucrose, one or more glucosyltransferase enzymes, glucose and fructose. This composition is a slurry since the poly alpha-1,3-1,6-glucan is not dissolved therein.

The term "poly alpha-1,3-1,6-glucan wet cake" herein refers to poly alpha-1,3-1,6-glucan that has been separated from a slurry and washed with water or an aqueous solution. Poly alpha-1,3-1,6-glucan is not completely dried when preparing a wet cake.

The term "degree of substitution" (DoS) as used herein refers to the average number of hydroxyl groups substituted in each monomeric unit (glucose) of a poly alpha-1,3-1,6-glucan ether compound. Since there are at most three hydroxyl groups in a glucose monomeric unit in a poly alpha-1,3-1,6-glucan herein (which is believed to be linear or branched), the degree of substitution in a poly alpha-1,3-1,6-glucan ether compound herein can be no higher than 3.

The term "molar substitution" (M.S.) as used herein refers to the moles of an organic group per monomeric unit of a poly alpha-1,3-1,6-glucan ether

compound. Alternatively, M.S. can refer to the average moles of etherification agent used to react with each monomeric unit in poly alpha-1,3-1,6-glucan (M.S. can thus describe the degree of derivatization with an etherification agent). It is noted that the M.S. value for poly alpha-1,3-1,6-glucan may have no upper limit. For example, when an organic group containing a hydroxyl group (e.g., hydroxyethyl or hydroxypropyl) has been etherified to poly alpha-1,3-1,6-glucan, the hydroxyl group of the organic group may undergo further reaction, thereby coupling more of the organic group to the poly alpha-1,3-1,6-glucan.

The term "crosslink" herein refers to a chemical bond, atom, or group of atoms that connects two adjacent atoms in one or more polymer molecules. It should be understood that, in a composition comprising crosslinked poly alpha-1,3-1,6-glucan ether, crosslinks can be between at least two poly alpha-1,3-1,6-glucan ether molecules (i.e., intermolecular crosslinks); there can also be intramolecular crosslinking. A "crosslinking agent" as used herein is an atom or compound that can create crosslinks.

An "aqueous composition" herein refers to a solution or mixture in which the solvent is at least about 20 wt% water, for example, and which comprises poly alpha-1,3-1,6-glucan and/or a poly alpha-1,3-1,6-glucan ether compound. Examples of aqueous compositions herein are aqueous solutions and hydrocolloids.

The terms "hydrocolloid" and "hydrogel" are used interchangeably herein. A hydrocolloid refers to a colloid system in which water is the dispersion medium. A "colloid" herein refers to a substance that is microscopically dispersed throughout another substance. Therefore, a hydrocolloid herein can also refer to a dispersion, emulsion, mixture, or solution of poly alpha-1,3-1,6-glucan and/or one or more poly alpha-1,3-1,6-glucan ether compounds in water or aqueous solution.

The term "aqueous solution" herein refers to a solution in which the solvent is water. Poly alpha-1,3-1,6-glucan and/or one or more poly alpha-1,3-1,6-glucan ether compounds herein can be dispersed, mixed, and/or dissolved in

an aqueous solution. An aqueous solution can serve as the dispersion medium of a hydrocolloid herein.

The terms “dispersant” and “dispersion agent” are used interchangeably herein to refer to a material that promotes the formation and stabilization of a dispersion of one substance in another. A ‘dispersion” herein refers to an aqueous composition comprising one or more particles (e.g., any ingredient of a personal care product, pharmaceutical product, food product, household product, or industrial product disclosed herein) that are scattered, or uniformly scattered, throughout the aqueous composition. It is believed that poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compounds can act as dispersants in aqueous compositions disclosed herein.

The term “viscosity” as used herein refers to the measure of the extent to which a fluid or an aqueous composition such as a hydrocolloid resists a force tending to cause it to flow. Various units of viscosity that can be used herein include centipoise (cPs) and Pascal-second (Pa·s). A centipoise is one one-hundredth of a poise; one poise is equal to 0.100 $kg \cdot m^{-1} \cdot s^{-1}$. Thus, the terms “viscosity modifier” and “viscosity-modifying agent” as used herein refer to anything that can alter/modify the viscosity of a fluid or aqueous composition.

The term “shear thinning behavior” as used herein refers to a decrease in the viscosity of the hydrocolloid or aqueous solution as shear rate increases. The term “shear thickening behavior” as used herein refers to an increase in the viscosity of the hydrocolloid or aqueous solution as shear rate increases. “Shear rate” herein refers to the rate at which a progressive shearing deformation is applied to the hydrocolloid or aqueous solution. A shearing deformation can be applied rotationally.

The term “contacting” as used herein with respect to methods of increasing the viscosity of an aqueous composition refers to any action that results in bringing together an aqueous composition with a poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compound. Contacting can be performed by any means known in the art, such as dissolving, mixing, shaking, or homogenization, for example.

The terms “fabric”, “textile”, and “cloth” are used interchangeably herein to refer to a woven material having a network of natural and/or artificial fibers. Such fibers can be thread or yarn, for example.

A “fabric care composition” herein is any composition suitable for treating fabric in some manner. Examples of such a composition include laundry detergents and fabric softeners.

The terms “heavy duty detergent” and “all-purpose detergent” are used interchangeably herein to refer to a detergent useful for regular washing of white and colored textiles at any temperature. The terms “low duty detergent” or “fine fabric detergent” are used interchangeably herein to refer to a detergent useful for the care of delicate fabrics such as viscose, wool, silk, microfiber or other fabric requiring special care. “Special care” can include conditions of using excess water, low agitation, and/or no bleach, for example.

An “oral care composition” herein is any composition suitable for treating an soft or hard surface in the oral cavity such as dental (teeth) and/or gum surfaces.

The term “adsorption” herein refers to the adhesion of a compound (e.g., poly alpha-1,3-1,6-glucan ether) to the surface of a material.

The terms “cellulase” and “cellulase enzyme” are used interchangeably herein to refer to an enzyme that hydrolyzes beta-1,4-D-glucosidic linkages in cellulose, thereby partially or completely degrading cellulose. Cellulase can alternatively be referred to as “beta-1,4-glucanase”, for example, and can have endocellulase activity (EC 3.2.1.4), exocellulase activity (EC 3.2.1.91), or cellobiase activity (EC 3.2.1.21). A cellulase in certain embodiments herein can also hydrolyze beta-1,4-D-glucosidic linkages in cellulose ether derivatives such as carboxymethyl cellulose. “Cellulose” refers to an insoluble polysaccharide having a linear chain of beta-1,4-linked D-glucose monomeric units.

Development of new glucan polysaccharides and derivatives thereof is desirable given their potential utility in various applications. It is also desirable to identify glucosyltransferase enzymes that can synthesize new glucan

polysaccharides, especially those with mixed glycosidic linkages and high molecular weight.

Embodiments of the disclosed invention concern a reaction solution comprising water, sucrose, and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-1,6-glucan. The glucosyltransferase enzyme comprises an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10. Significantly, these enzymes can synthesize poly alpha-1,3-1,6-glucan that can be derivatized into ethers having enhanced viscosity modification qualities.

Regarding poly alpha-1,3-1,6-glucan produced in a reaction solution herein:

(i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,6 linkages, and

(iii) the poly alpha-1,3-1,6-glucan has a weight average degree of polymerization (DP_w) of at least 1000.

At least 30% of the glycosidic linkages of poly alpha-1,3-1,6-glucan synthesized by a glucosyltransferase enzyme herein are alpha-1,3 linkages, and at least 30% of the glycosidic linkages are alpha-1,6 linkages. Alternatively, the percentage of alpha-1,3 linkages can be at least 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, or 64%. Alternatively still, the percentage of alpha-1,6 linkages can be at least 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, or 69%.

A poly alpha-1,3-1,6-glucan synthesized by a glucosyltransferase enzyme herein can have any one the aforementioned percentages of alpha-1,3 linkages and any one of the aforementioned percentages of alpha-1,6 linkages, just so

long that the total of the percentages is not greater than 100%. For example, the poly alpha-1,3-1,6-glucan can have (i) any one of 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, or 40% (30%-40%) alpha-1,3 linkages and (ii) any one of 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, or 69% (60%-69%) alpha-1,6 linkages, just so long that the total of the percentages is not greater than 100%. Non-limiting examples include poly alpha-1,3-1,6-glucan with 31% alpha-1,3 linkages and 67% alpha-1,6 linkages. Other examples of alpha-1,3 and alpha-1,6 linkage profiles are provided in Table 2. In certain embodiments, at least 60% of the glycosidic linkages of poly alpha-1,3-1,6-glucan produced in a gtf reaction solution herein are alpha-1,6 linkages.

Poly alpha-1,3-1,6-glucan synthesized by a glucosyltransferase enzyme herein can have, for example, less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of glycosidic linkages other than alpha-1,3 and alpha-1,6. In another embodiment, poly alpha-1,3-1,6-glucan only has alpha-1,3 and alpha-1,6 linkages.

The backbone of a poly alpha-1,3-1,6-glucan synthesized by a glucosyltransferase enzyme herein can be linear/unbranched. Alternatively, there can be branches in the poly alpha-1,3-1,6-glucan. A poly alpha-1,3-1,6-glucan in certain embodiments can thus have no branch points or less than about 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% branch points as a percent of the glycosidic linkages in the polymer.

In certain embodiments of the disclosed invention, a glucosyltransferase enzyme can synthesize poly alpha-1,3-1,6-glucan comprising alpha-1,3 linkages and alpha-1,6 linkages that do not consecutively alternate with each other. For the following discussion, consider that ...G-1,3-G-1,6-G-1,3-G-1,6-G-1,3-G-... (where G represents glucose) represents a stretch of six glucose monomeric units linked by consecutively alternating alpha-1,3 linkages and alpha-1,6 linkages. Alternatively, poly alpha-1,3-1,6-glucan synthesized by a glucosyltransferase enzyme herein can comprise, for example, less than 2, 3, 4,

5, 6, 7, 8, 9, 10, or more glucose monomeric units that are linked consecutively with alternating alpha-1,3 and alpha-1,6 linkages.

The molecular weight of poly alpha-1,3-1,6-glucan synthesized by a glucosyltransferase enzyme herein can be measured as DP_w (weight average degree of polymerization) or DP_n (number average degree of polymerization). Alternatively, molecular weight can be measured in Daltons or grams/mole. It may also be useful to refer to the number-average molecular weight (M_n) or weight-average molecular weight (M_w) of the poly alpha-1,3-1,6-glucan.

Poly alpha-1,3-1,6-glucan synthesized by a glucosyltransferase enzyme herein can have a DP_w of at least about 1000. For example, the DP_w of the poly alpha-1,3-1,6-glucan can be at least about 10000. Alternatively, the DP_w can be at least about 1000 to about 15000. Alternatively still, the DP_w can be at least about 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 11000, 12000, 13000, 14000, or 15000 (or any integer between 1000 and 15000), for example. Given that poly alpha-1,3-1,6-glucan herein has a DP_w of at least about 1000, such a glucan polymer is typically water-insoluble.

In certain embodiments of the disclosed gtf reaction solution, poly alpha-1,3-1,6-glucan can have an M_w of at least about 50000, 100000, 200000, 300000, 400000, 500000, 600000, 700000, 800000, 900000, 1000000, 1100000, 1200000, 1300000, 1400000, 1500000, or 1600000 (or any integer between 50000 and 1600000), for example. Alternatively, poly alpha-1,3-1,6-glucan can have an M_w of at least about 4000, 5000, 10000, 20000, 30000, or 40000, for example.

A glucosyltransferase enzyme herein may be obtained from any microbial source, such as a bacteria or fungus. Examples of bacterial glucosyltransferase enzymes are those derived from a *Streptococcus* species, *Leuconostoc* species or *Lactobacillus* species. Examples of *Streptococcus* species include *S. salivarius*, *S. sobrinus*, *S. dentirousetti*, *S. downei*, *S. mutans*, *S. oralis*, *S. gallolyticus* and *S. sanguinis*. Examples of *Leuconostoc* species include *L. mesenteroides*, *L. amelibiosum*, *L. argentinum*, *L. carnosum*, *L. citreum*, *L. cremoris*, *L. dextranicum* and *L. fructosum*. Examples of *Lactobacillus* species

include *L. acidophilus*, *L. delbrueckii*, *L. helveticus*, *L. salivarius*, *L. casei*, *L. curvatus*, *L. plantarum*, *L. sakei*, *L. brevis*, *L. buchneri*, *L. fermentum* and *L. reuteri*.

A glucosyltransferase enzyme herein can comprise, or consist of, an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10, wherein the glucosyltransferase enzyme has activity. Alternatively, a glucosyltransferase enzyme can comprise, or consist of, an amino acid sequence that is at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:4, SEQ ID NO:20, SEQ ID NO:28, or SEQ ID NO:30, wherein the glucosyltransferase enzyme has activity. Alternatively still, a glucosyltransferase enzyme can comprise, or consist of, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

Given that certain amino acids share similar structural and/or charge features with each other (i.e., conserved), one or more amino acids of the disclosed gtf enzyme sequences may be substituted with a conserved amino acid residue ("conservative amino acid substitution") as follows:

1. The following small aliphatic, nonpolar or slightly polar residues can substitute for each other: Ala (A), Ser (S), Thr (T), Pro (P), Gly (G);
2. The following polar, negatively charged residues and their amides can substitute for each other: Asp (D), Asn (N), Glu (E), Gln (Q);
3. The following polar, positively charged residues can substitute for each other: His (H), Arg (R), Lys (K);
4. The following aliphatic, nonpolar residues can substitute for each other: Ala (A), Leu (L), Ile (I), Val (V), Cys (C), Met (M); and
5. The following large aromatic residues can substitute for each other: Phe (F), Tyr (Y), Trp (W).

Examples of glucosyltransferase enzymes for use in a gtf reaction solution may be any of the amino acid sequences disclosed herein and that further include 1-300 (or any integer there between) residues on the N-terminus and/or C-terminus. Such additional residues may be from a corresponding wild type

sequence from which the glucosyltransferase enzyme is derived, or may be another sequence such as an epitope tag (at either N- or C-terminus) or a heterologous signal peptide (at N-terminus), for example.

The amino acid sequence of a glucosyltransferase enzyme herein can be encoded by the polynucleotide sequence provided in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, for example. Alternatively, such an amino acid sequence can be encoded by a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9.

One or more different glucosyltransferase enzymes may be used to practice the disclosed invention. The glucosyltransferase enzyme does not have, or has very little (less than 1%), alternansucrase activity, for example.

A glucosyltransferase enzyme herein can be primer-independent or primer-dependent. Primer-independent glucosyltransferase enzymes do not require the presence of a primer to perform glucan synthesis. A primer-dependent glucosyltransferase enzyme requires the presence of an initiating molecule in the reaction solution to act as a primer for the enzyme during glucan polymer synthesis. The term “primer” as used herein refers to any molecule that can act as the initiator for a glucosyltransferase enzyme. Oligosaccharides and polysaccharides can serve a primers, for example. Primers that can be used in certain embodiments include dextran and other carbohydrate-based primers, such as hydrolyzed glucan, for example. U.S. Appl. Publ. No. 2013/0244287, which is incorporated herein by reference, discloses preparation of hydrolyzed glucan using poly alpha-1,3-glucan as the starting material. Dextran for use as a primer can be dextran T10 (i.e., dextran having a molecular weight of 10 kD), for example. Alternatively, dextran primer can have a molecular weight of about 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, or 25 kD, for example.

A glucosyltransferase enzyme of the disclosed invention may be produced by any means known in the art. For example, the glucosyltransferase enzyme

may be produced recombinantly in a heterologous expression system, such as a microbial heterologous expression system. Examples of heterologous expression systems include bacterial (e.g., *E. coli* such as TOP10 or MG1655; *Bacillus* sp.) and eukaryotic (e.g., yeasts such as *Pichia* sp. and *Saccharomyces* sp.) expression systems.

A glucosyltransferase enzyme described herein may be used in any purification state (e.g., pure or non-pure). For example, the glucosyltransferase enzyme may be purified and/or isolated prior to its use. Examples of glucosyltransferase enzymes that are non-pure include those in the form of a cell lysate. A cell lysate or extract may be prepared from a bacteria (e.g., *E. coli*) used to heterologously express the enzyme. For example, the bacteria may be subjected to disruption using a French pressure cell. In alternative embodiments, bacteria may be homogenized with a homogenizer (e.g., APV, Rannie, Gaulin). A glucosyltransferase enzyme is typically soluble in these types of preparations. A bacterial cell lysate, extract, or homogenate herein may be used at about 0.15-0.3% (v/v) in a reaction solution for producing poly alpha-1,3-1,6-glucan from sucrose.

A heterologous gene expression system in certain embodiments may be one that is designed for protein secretion. The glucosyltransferase enzyme comprises a signal peptide (signal sequence) in such embodiments. The signal peptide may be either its native signal peptide or a heterologous signal peptide.

The activity of a glucosyltransferase enzyme herein can be determined using any method known in the art. For example, glucosyltransferase enzyme activity can be determined by measuring the production of reducing sugars (fructose and glucose) in a reaction solution containing sucrose (~50 g/L), dextran T10 (~1 mg/mL) and potassium phosphate buffer (~pH 6.5, 50 mM), where the solution is held at ~22-25 °C for ~24-30 hours. The reducing sugars can be measured by adding 0.01 mL of the reaction solution to a mixture containing ~1 N NaOH and ~0.1% triphenyltetrazolium chloride and then monitoring the increase in absorbance at OD_{480nm} for ~five minutes.

The temperature of a gtf reaction solution herein can be controlled, if desired. In certain embodiments, the temperature is between about 5 °C to about 50 °C. The temperature in certain other embodiments is between about 20 °C to about 40 °C. Alternatively, the temperature may be about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 °C.

The temperature of a gtf reaction solution herein may be maintained using various means known in the art. For example, the temperature can be maintained by placing the vessel containing the reaction solution in an air or water bath incubator set at the desired temperature.

The initial concentration of sucrose in a gtf reaction solution herein can be about 20 g/L to about 400 g/L, for example. Alternatively, the initial concentration of sucrose can be about 75 g/L to about 175 g/L, or from about 50 g/L to about 150 g/L. Alternatively still, the initial concentration of sucrose can be about 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, or 160 g/L (or any integer between 40 and 160 g/L), for example. "Initial concentration of sucrose" refers to the sucrose concentration in a gtf reaction solution just after all the reaction solution components have been added (water, sucrose, gtf enzyme).

Any grade of sucrose can be used in a reaction solution disclosed herein. For example, the sucrose can be highly pure (≥ 99.5%), have a purity of at least 99.0%, or be reagent grade sucrose. Sucrose for use herein may be derived from any renewable sugar source such as sugar cane, sugar beets, cassava, sweet sorghum, or corn. The sucrose can be provided in any form such as crystalline form or non-crystalline form (e.g., syrup or cane juice).

The pH of a gtf reaction solution in certain embodiments can be between about 4.0 to about 8.0. Alternatively, the pH can be about 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, or 8.0. The pH can be adjusted or controlled by the addition or incorporation of a suitable buffer, including but not limited to: phosphate, tris, citrate, or a combination thereof. Buffer concentration in a gtf reaction solution can be from 0 mM to about 100 mM, or about 10, 20, or 50 mM, for example.

The disclosed invention also concerns a method for producing poly alpha-1,3-1,6-glucan comprising the step of contacting at least water, sucrose, and a

glucosyltransferase enzyme that synthesizes poly alpha-1,3-1,6-glucan. The glucosyltransferase enzyme comprises an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10. Poly alpha-1,3-1,6-glucan is produced in the contacting step. This poly alpha-1,3-1,6-glucan can optionally be isolated.

The contacting step in a method herein of producing poly alpha-1,3-1,6-glucan can comprise providing a gtf reaction solution comprising water, sucrose and any glucosyltransferase enzyme disclosed herein. It would be understood that, as the glucosyltransferase enzyme synthesizes poly alpha-1,3-1,6-glucan, the reaction solution typically becomes a reaction mixture given that insoluble poly alpha-1,3-1,6-glucan falls out of solution as indicated by clouding of the reaction. The contacting step of the disclosed method can be performed in any number of ways. For example, the desired amount of sucrose can first be dissolved in water (optionally, other components may also be added at this stage of preparation, such as buffer components), followed by addition of the glucosyltransferase enzyme. The solution may be kept still, or agitated via stirring or orbital shaking, for example. The reaction can be, and typically is, cell-free.

Completion of a gtf reaction in certain embodiments can be determined visually (e.g., no more accumulation of precipitated poly alpha-1,3-1,6-glucan) and/or by measuring the amount of sucrose left in the solution (residual sucrose), where a percent sucrose consumption of over about 90% can indicate reaction completion. Typically, a reaction of the disclosed process can take about 12, 18, 24, 30, 36, 48, 60, 72, 84, or 96 hours to complete. Reaction time may depend, for example, on certain parameters such as the amount of sucrose and glucosyltransferase enzyme used in the reaction.

The yield of poly alpha-1,3-1,6-glucan produced in a gtf reaction in certain embodiments herein can be at least about 4%, 5%, 6%, 7%, or 8%, based on the weight of the sucrose used in the reaction solution.

Poly alpha-1,3-1,6-glucan produced in the disclosed method may optionally be isolated. For example, insoluble poly alpha-1,3-1,6-glucan may be

separated by centrifugation or filtration. In doing so, the poly alpha-1,3-1,6-glucan is separated from the rest of the reaction solution, which may comprise water, fructose and certain byproducts (e.g., leucrose, soluble oligosaccharides). This solution may also comprise glucose monomer and residual sucrose.

The linkage profile and/or molecular weight of poly alpha-1,3-1,6-glucan produced in a gtf reaction herein can be any of those disclosed above. For example, (i) at least 30% of the glycosidic linkages are alpha-1,3 linkages, (ii) at least 30% of the glycosidic linkages are alpha-1,6 linkages, and (iii) the poly alpha-1,3-1,6-glucan has a DP_w of at least 1000. Poly alpha-1,3-1,6-glucan produced in a gtf reaction can have at least 60% alpha-1,6 linkages, and/or have a DP_w of at least about 10000, for example.

Embodiments of the disclosed invention concern a composition comprising poly alpha-1,3-1,6-glucan, wherein:

(i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,6 linkages,

(iii) the poly alpha-1,3-1,6-glucan has a weight average degree of polymerization (DP_w) of at least 1000; and

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of the poly alpha-1,3-1,6-glucan do not consecutively alternate with each other.

Significantly, poly alpha-1,3-1,6-glucan disclosed herein can be derivatized into ethers having enhanced viscosity modification qualities.

At least 30% of the glycosidic linkages of poly alpha-1,3-1,6-glucan disclosed herein are alpha-1,3 linkages, and at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,6 linkages. Alternatively, the percentage of alpha-1,3 linkages in poly alpha-1,3-1,6-glucan herein can be at least 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%,

57%, 58%, 59%, 60%, 61%, 62%, 63%, or 64%. Alternatively still, the percentage of alpha-1,6 linkages in poly alpha-1,3-1,6-glucan herein can be at least 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, or 69%.

A poly alpha-1,3-1,6-glucan of the invention can have any one the aforementioned percentages of alpha-1,3 linkages and any one of the aforementioned percentages of alpha-1,6 linkages, just so long that the total of the percentages is not greater than 100%. For example, poly alpha-1,3-1,6-glucan herein can have (i) any one of 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, or 40% (30%-40%) alpha-1,3 linkages and (ii) any one of 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, or 69% (60%-69%) alpha-1,6 linkages, just so long that the total of the percentages is not greater than 100%. Non-limiting examples include poly alpha-1,3-1,6-glucan with 31% alpha-1,3 linkages and 67% alpha-1,6 linkages. Other examples of alpha-1,3 and alpha-1,6 linkage profiles are provided in Table 2. In certain embodiments, at least 60% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,6 linkages.

A poly alpha-1,3-1,6-glucan of the invention can have, for example, less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of glycosidic linkages other than alpha-1,3 and alpha-1,6. In another embodiment, a poly alpha-1,3-1,6-glucan only has alpha-1,3 and alpha-1,6 linkages.

The backbone of a poly alpha-1,3-1,6-glucan disclosed herein can be linear/unbranched. Alternatively, there can be branches in the poly alpha-1,3-1,6-glucan. A poly alpha-1,3-1,6-glucan in certain embodiments can thus have no branch points or less than about 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% branch points as a percent of the glycosidic linkages in the polymer.

The alpha-1,3 linkages and alpha-1,6 linkages of a poly alpha-1,3-1,6-glucan in the disclosed composition do not consecutively alternate with each

other. For the following discussion, consider that ...G-1,3-G-1,6-G-1,3-G-1,6-G-1,3-G-... (where G represents glucose) represents a stretch of six glucose monomeric units linked by consecutively alternating alpha-1,3 linkages and alpha-1,6 linkages. Poly alpha-1,3-1,6-glucan in certain embodiments herein comprises less than 2, 3, 4, 5, 6, 7, 8, 9, 10, or more glucose monomeric units that are linked consecutively with alternating alpha-1,3 and alpha-1,6 linkages.

The molecular weight of a poly alpha-1,3-1,6-glucan disclosed herein can be measured as DP_w (weight average degree of polymerization) or DP_n (number average degree of polymerization). Alternatively, molecular weight can be measured in Daltons or grams/mole. It may also be useful to refer to the number-average molecular weight (M_n) or weight-average molecular weight (M_w) of the poly alpha-1,3-1,6-glucan.

A poly alpha-1,3-1,6-glucan herein can have a DP_w of at least about 1000. For example, the DP_w of the poly alpha-1,3-1,6-glucan can be at least about 10000. Alternatively, the DP_w can be at least about 1000 to about 15000. Alternatively still, the DP_w can be at least about 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 11000, 12000, 13000, 14000, or 15000 (or any integer between 1000 and 15000), for example. Given that a poly alpha-1,3-1,6-glucan herein can have a DP_w of at least about 1000, such a glucan polymer is typically water-insoluble.

A poly alpha-1,3-1,6-glucan herein can have an M_w of at least about 50000, 100000, 200000, 300000, 400000, 500000, 600000, 700000, 800000, 900000, 1000000, 1100000, 1200000, 1300000, 1400000, 1500000, or 1600000 (or any integer between 50000 and 1600000), for example. The M_w in certain embodiments is at least about 1000000. Alternatively, poly alpha-1,3-1,6-glucan can have an M_w of at least about 4000, 5000, 10000, 20000, 30000, or 40000, for example.

A poly alpha-1,3-1,6-glucan herein can comprise at least 20 glucose monomeric units, for example. Alternatively, the number of glucose monomeric units can be at least 25, 50, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, or 9000 (or any integer between 10 and 9000), for example.

Poly alpha-1,3-1,6-glucan herein can be produced, for example, using a glucosyltransferase enzyme comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10. Alternatively, the glucosyltransferase enzyme can comprise an amino acid sequence that is at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, or 100% identical to, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10. Production of poly alpha-1,3-1,6-glucan of the disclosed invention can be accomplished with a gtf reaction as disclosed herein, for example.

Poly alpha-1,3-1,6-glucan herein can be provided in the form of a powder when dry, or a paste, colloid or other dispersion when wet, for example. A composition comprising poly alpha-1,3-1,6-glucan in certain embodiments is one in which the constituent poly alpha-1,3-1,6-glucan behaves as a thickening agent. It is believed that poly alpha-1,3-1,6-glucan herein is suitable as a thickening agent, which is a substance that absorbs liquids such as water and swells upon such absorption. Swelling of poly alpha-1,3-1,6-glucan in a liquid can yield a slurry or colloid, for example.

A composition comprising poly alpha-1,3-1,6-glucan may be in the form of a personal care product, pharmaceutical product, food product, household product, or industrial product, such as any of those products disclosed below for the application of ether derivatives of poly alpha-1,3-1,6-glucan. The amount of poly alpha-1,3-1,6-glucan in the composition can be, for example, about 0.1-10 wt%, 0.1-5 wt%, 0.1-4 wt%, 0.1-3 wt%, 0.1-2 wt%, or 0.1-1 wt%, or an amount that provides the desired degree of thickening or dispersion to the composition.

Embodiments of the disclosed invention concern a composition comprising a poly alpha-1,3-1,6-glucan ether compound, wherein:

(i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,6 linkages,

(iii) the poly alpha-1,3-1,6-glucan ether compound has a weight average degree of polymerization (DP_w) of at least 1000;

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of the poly alpha-1,3-1,6-glucan ether compound do not consecutively alternate with each other, and

(v) the poly alpha-1,3-1,6-glucan ether compound has a degree of substitution (DoS) with an organic group of about 0.05 to about 3.0.

Significantly, a poly alpha-1,3-1,6-glucan ether compound disclosed herein has enhanced viscosity modification qualities such as the ability to viscosify an aqueous composition at low concentration. Also, a poly alpha-1,3-1,6-glucan ether compound herein can have a relatively low DoS and still be an effective viscosity modifier. It is believed that the viscosity modification effect of the disclosed poly alpha-1,3-1,6-glucan ether compounds is often coupled with a rheology modification effect. It is further believed that, by contacting a hydrocolloid or aqueous solution herein with a surface (e.g., fabric surface), one or more poly alpha-1,3-1,6-glucan ether compounds adsorb to the surface. These features of rheology modification and surface adsorption by poly alpha-1,3-1,6-glucan ether compounds may be useful in applications such as fabric care.

At least 30% of the glycosidic linkages of a poly alpha-1,3-1,6-glucan ether compound disclosed herein are alpha-1,3 linkages, and at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,6 linkages. Alternatively, the percentage of alpha-1,3 linkages in a poly alpha-1,3-1,6-glucan ether compound herein can be at least 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, or 64%. Alternatively still, the percentage of alpha-1,6 linkages in a poly alpha-1,3-1,6-glucan ether compound herein can be at least 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, or 69%.

A poly alpha-1,3-1,6-glucan ether compound of the invention can have any one the aforementioned percentages of alpha-1,3 linkages and any one of the aforementioned percentages of alpha-1,6 linkages, just so long that the total of the percentages is not greater than 100%. For example, the poly alpha-1,3-1,6-glucan ether compound can have (i) any one of 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, or 40% (30%-40%) alpha-1,3 linkages and (ii) any one of 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, or 69% (60%-69%) alpha-1,6 linkages, just so long that the total of the percentages is not greater than 100%. Non-limiting examples include poly alpha-1,3-1,6-glucan ether compounds with 31% alpha-1,3 linkages and 67% alpha-1,6 linkages. Other examples of alpha-1,3 and alpha-1,6 linkage profiles of certain poly alpha-1,3-1,6-glucan ether compounds herein are provided in Table 2, which discloses linkage profiles of isolated poly alpha-1,3-1,6-glucan that can be used to prepare the disclosed ethers. In certain embodiments, at least 60% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,6 linkages.

A poly alpha-1,3-1,6-glucan ether compound of the invention can have, for example, less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of glycosidic linkages other than alpha-1,3 and alpha-1,6. In another embodiment, a poly alpha-1,3-1,6-glucan ether compound only has alpha-1,3 and alpha-1,6 linkages.

The backbone of a poly alpha-1,3-1,6-glucan ether compound disclosed herein can be linear/unbranched. Alternatively, there can be branches in the poly alpha-1,3-1,6-glucan ether compound. A poly alpha-1,3-1,6-glucan ether compound in certain embodiments can thus have no branch points or less than about 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% branch points as a percent of the glycosidic linkages in the polymer.

The alpha-1,3 linkages and alpha-1,6 linkages of a poly alpha-1,3-1,6-glucan ether compound disclosed herein do not consecutively alternate with each other. For the following discussion, consider that ...G-1,3-G-1,6-G-1,3-G-1,6-G-1,3-G-... (where G represents etherized glucose) represents a stretch of six glucose monomeric units linked by consecutively alternating alpha-1,3 linkages

and alpha-1,6 linkages. Poly alpha-1,3-1,6-glucan ether compounds in certain embodiments herein comprise less than 2, 3, 4, 5, 6, 7, 8, 9, 10, or more glucose monomeric units that are linked consecutively with alternating alpha-1,3 and alpha-1,6 linkages.

The molecular weight of a poly alpha-1,3-1,6-glucan ether compound disclosed herein can be measured as DP_w (weight average degree of polymerization) or DP_n (number average degree of polymerization). Alternatively, molecular weight can be measured in Daltons or grams/mole. It may also be useful to refer to the number-average molecular weight (M_n) or weight-average molecular weight (M_w) of the poly alpha-1,3-1,6-glucan ether compound.

A poly alpha-1,3-1,6-glucan ether compound herein can have a DP_w of at least about 1000. For example, the DP_w of the poly alpha-1,3-1,6-glucan ether compound can be at least about 10000. Alternatively, the DP_w can be at least about 1000 to about 15000. Alternatively still, the DP_w can be at least about 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 11000, 12000, 13000, 14000, or 15000 (or any integer between 1000 and 15000), for example.

A poly alpha-1,3-1,6-glucan ether compound herein can have an M_w of at least about 50000, 100000, 200000, 300000, 400000, 500000, 600000, 700000, 800000, 900000, 1000000, 1100000, 1200000, 1300000, 1400000, 1500000, or 1600000 (or any integer between 50000 and 1600000), for example. The M_w in certain embodiments is at least about 1000000. Alternatively, poly alpha-1,3-1,6-glucan can have an M_w of at least about 4000, 5000, 10000, 20000, 30000, or 40000, for example.

A poly alpha-1,3-1,6-glucan ether compound herein can comprise at least 20 glucose monomeric units (most of such units typically contain ether-linked organic groups), for example. Alternatively, the number of glucose monomeric units can be at least 25, 50, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, or 9000 (or any integer between 10 and 9000), for example.

Poly alpha-1,3-1,6-glucan ether compounds of the invention have a DoS with an organic group of about 0.05 to about 3.0. In certain embodiments, the DoS of a poly alpha-1,3-1,6-glucan ether compound can be about 0.3 to 1.0.

The DoS can alternatively be at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3.0.

The percentage of glucose monomeric units of a poly alpha-1,3-1,6-glucan ether compound herein that are ether-linked to an organic group (i.e., where one or more hydroxyl groups of a glucose monomeric unit have been etherified with an organic group) can vary depending on the degree to which a poly alpha-1,3-1,6-glucan is etherified with an organic group in an etherification reaction. This percentage can be at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (or any integer value between 30% and 100%), for example.

It would be understood that, depending on the glycosidic linkages with which a glucose monomeric unit of an ether compound is involved (e.g., -1,6-G-1,3-), certain carbon atoms of the glucose monomeric unit may independently be linked to an OH group or be in ether linkage to an organic group.

An organic group herein may be an alkyl group such as a methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, or decyl group, for example.

Alternatively, an organic group may be a substituted alkyl group in which there is a substitution on one or more carbons of the alkyl group. The substitution(s) may be one or more hydroxyl, aldehyde, ketone, and/or carboxyl groups. For example, a substituted alkyl group may be a hydroxy alkyl group, dihydroxy alkyl group, or carboxy alkyl group.

Examples of suitable hydroxy alkyl groups are hydroxymethyl ($-CH_2OH$), hydroxyethyl (e.g., $-CH_2CH_2OH$, $-CH(OH)CH_3$), hydroxypropyl (e.g., $-CH_2CH_2CH_2OH$, $-CH_2CH(OH)CH_3$, $-CH(OH)CH_2CH_3$), hydroxybutyl and hydroxypentyl groups. Other examples include dihydroxy alkyl groups (diols) such as dihydroxymethyl, dihydroxyethyl (e.g., $-CH(OH)CH_2OH$), dihydroxypropyl (e.g., $-CH_2CH(OH)CH_2OH$, $-CH(OH)CH(OH)CH_3$), dihydroxybutyl and dihydroxypentyl groups.

Examples of suitable carboxy alkyl groups are carboxymethyl ($-CH_2COOH$), carboxyethyl (e.g., $-CH_2CH_2COOH$, $-CH(COOH)CH_3$),

carboxypropyl (e.g., -$CH_2CH_2CH_2COOH$, -$CH_2CH(COOH)CH_3$, -$CH(COOH)CH_2CH_3$), carboxybutyl and carboxypentyl groups.

Alternatively still, one or more carbons of an alkyl group can have a substitution(s) with another alkyl group. Examples of such substituent alkyl groups are methyl, ethyl and propyl groups. To illustrate, an organic group can be -$CH(CH_3)CH_2CH_3$ or -$CH_2CH(CH_3)CH_3$, for example, which are both propyl groups having a methyl substitution.

As should be clear from the above examples of various substituted alkyl groups, a substitution (e.g., hydroxy or carboxy group) on an alkyl group in certain embodiments may be bonded to the terminal carbon atom of the alkyl group, where the terminal carbon group is opposite the terminus that is in ether linkage to a glucose monomeric unit in a poly alpha-1,3-1,6-glucan ether compound. An example of this terminal substitution is the hydroxypropyl group -$CH_2CH_2CH_2OH$. Alternatively, a substitution may be on an internal carbon atom of an alkyl group. An example on an internal substitution is the hydroxypropyl group -$CH_2CH(OH)CH_3$. An alkyl group can have one or more substitutions, which may be the same (e.g., two hydroxyl groups [dihydroxy]) or different (e.g., a hydroxyl group and a carboxyl group).

Poly alpha-1,3-1,6-glucan ether compounds in certain embodiments disclosed herein may contain one type of organic group. Examples of such compounds contain a carboxy alkyl group as the organic group (carboxyalkyl poly alpha-1,3-1,6-glucan, generically speaking). A specific non-limiting example of such a compound is carboxymethyl poly alpha-1,3-1,6-glucan.

Alternatively, poly alpha-1,3-1,6-glucan ether compounds disclosed herein can contain two or more different types of organic groups. Examples of such compounds contain (i) two different alkyl groups as organic groups, (ii) an alkyl group and a hydroxy alkyl group as organic groups (alkyl hydroxyalkyl poly alpha-1,3-1,6-glucan, generically speaking), (iii) an alkyl group and a carboxy alkyl group as organic groups (alkyl carboxyalkyl poly alpha-1,3-1,6-glucan, generically speaking), (iv) a hydroxy alkyl group and a carboxy alkyl group as organic groups (hydroxyalkyl carboxyalkyl poly alpha-1,3-1,6-glucan, generically

speaking), (v) two different hydroxy alkyl groups as organic groups, or (vi) two different carboxy alkyl groups as organic groups. Specific non-limiting examples of such compounds include ethyl hydroxyethyl poly alpha-1,3-1,6-glucan, hydroxyalkyl methyl poly alpha-1,3-1,6-glucan, carboxymethyl hydroxyethyl poly alpha-1,3-1,6-glucan, and carboxymethyl hydroxypropyl poly alpha-1,3-1,6-glucan.

An organic group herein can alternatively be a positively charged organic group. As defined above, a positively charged organic group comprises a chain of one or more carbons having one or more hydrogens substituted with another atom or functional group, where one or more of the substitutions is with a positively charged group.

A positively charged group may be a substituted ammonium group, for example. Examples of substituted ammonium groups are primary, secondary, tertiary and quaternary ammonium groups. Structure I depicts a primary, secondary, tertiary or quaternary ammonium group, depending on the composition of R_2, R_3 and R_4 in structure I. Each of R_2, R_3 and R_4 in structure I independently represent a hydrogen atom or an alkyl, aryl, cycloalkyl, aralkyl, or alkaryl group. Alternatively, each of R_2, R_3 and R_4 in can independently represent a hydrogen atom or an alkyl group. An alkyl group can be a methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, or decyl group, for example. Where two or three of R_2, R_3 and R_4 are an alkyl group, they can be the same or different alkyl groups.

A “primary ammonium poly alpha-1,3-1,6-glucan ether compound” herein can comprise a positively charged organic group having an ammonium group. In this example, the positively charged organic group comprises structure I in which each of R_2, R_3 and R_4 is a hydrogen atom. A non-limiting example of such a positively charged organic group is represented by structure II when each of R_2, R_3 and R_4 is a hydrogen atom. An example of a primary ammonium poly alpha-1,3-1,6-glucan ether compound can be represented in shorthand as ammonium poly alpha-1,3-1,6-glucan ether. It would be understood that a first member (i.e., R_1) implied by “primary” in the above nomenclature is the chain of one or more

carbons of the positively charged organic group that is ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan.

A "secondary ammonium poly alpha-1,3-1,6-glucan ether compound" herein can comprise a positively charged organic group having a monoalkylammonium group, for example. In this example, the positively charged organic group comprises structure I in which each of R_2 and R_3 is a hydrogen atom and R_4 is an alkyl group. A non-limiting example of such a positively charged organic group is represented by structure II when each of R_2 and R_3 is a hydrogen atom and R_4 is an alkyl group. An example of a secondary ammonium poly alpha-1,3-1,6-glucan ether compound can be represented in shorthand herein as monoalkylammonium poly alpha-1,3-1,6-glucan ether (e.g., monomethyl-, monoethyl-, monopropyl-, monobutyl-, monopentyl-, monohexyl-, monoheptyl-, monooctyl-, monononyl- or monodecyl- ammonium poly alpha-1,3-1,6-glucan ether). It would be understood that a second member (i.e., R_1) implied by "secondary" in the above nomenclature is the chain of one or more carbons of the positively charged organic group that is ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan.

A "tertiary ammonium poly alpha-1,3-1,6-glucan ether compound" herein can comprise a positively charged organic group having a dialkylammonium group, for example. In this example, the positively charged organic group comprises structure I in which R_2 is a hydrogen atom and each of R_3 and R_4 is an alkyl group. A non-limiting example of such a positively charged organic group is represented by structure II when R_2 is a hydrogen atom and each of R_3 and R_4 is an alkyl group. An example of a tertiary ammonium poly alpha-1,3-1,6-glucan ether compound can be represented in shorthand as dialkylammonium poly alpha-1,3-1,6-glucan ether (e.g., dimethyl-, diethyl-, dipropyl-, dibutyl-, dipentyl-, dihexyl-, diheptyl-, dioctyl-, dinonyl- or didecyl- ammonium poly alpha-1,3-1,6-glucan ether). It would be understood that a third member (i.e., R_1) implied by "tertiary" in the above nomenclature is the chain of one or more carbons of the positively charged organic group that is ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan.

A "quaternary ammonium poly alpha-1,3-1,6-glucan ether compound" herein can comprise a positively charged organic group having a trialkylammonium group, for example. In this example, the positively charged organic group comprises structure I in which each of R_2, R_3 and R_4 is an alkyl group. A non-limiting example of such a positively charged organic group is represented by structure II when each of R_2, R_3 and R_4 is an alkyl group. An example of a quaternary ammonium poly alpha-1,3-1,6-glucan ether compound can be represented in shorthand as trialkylammonium poly alpha-1,3-1,6-glucan ether (e.g., trimethyl-, triethyl-, tripropyl-, tributyl-, tripentyl-, trihexyl-, triheptyl-, trioctyl-, trinonyl- or tridecyl- ammonium poly alpha-1,3-1,6-glucan ether). It would be understood that a fourth member (i.e., R_1) implied by "quaternary" in the above nomenclature is the chain of one or more carbons of the positively charged organic group that is ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan.

Additional non-limiting examples of substituted ammonium groups that can serve as a positively charged group herein are represented in structure I when each of R_2, R_3 and R_4 independently represent a hydrogen atom; an alkyl group such as a methyl, ethyl, or propyl group; an aryl group such as a phenyl or naphthyl group; an aralkyl group such as a benzyl group; an alkaryl group; or a cycloalkyl group. Each of R_2, R_3 and R_4 may further comprise an amino group or a hydroxyl group, for example.

The nitrogen atom in a substituted ammonium group represented by structure I is bonded to a chain of one or more carbons as comprised in a positively charged organic group. This chain of one or more carbons ("carbon chain") is ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan, and may have one or more substitutions in addition to the substitution with the nitrogen atom of the substituted ammonium group. There can be 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbons, for example, in a carbon chain. To illustrate, the carbon chain of structure II is 3 carbon atoms in length.

Examples of a carbon chain of a positively charged organic group that do not have a substitution in addition to the substitution with a positively charged

group include -CH_2-, -CH_2CH_2-, -$CH_2CH_2CH_2$-, -$CH_2CH_2CH_2CH_2$- and -$CH_2CH_2CH_2CH_2CH_2$-. In each of these examples, the first carbon atom of the chain is ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan, and the last carbon atom of the chain is linked to a positively charged group. Where the positively charged group is a substituted ammonium group, the last carbon atom of the chain in each of these examples is represented by the C in structure I.

Where a carbon chain of a positively charged organic group has a substitution in addition to a substitution with a positively charged group, such additional substitution may be with one or more hydroxyl groups, oxygen atoms (thereby forming an aldehyde or ketone group), alkyl groups (e.g., methyl, ethyl, propyl, butyl), and/or additional positively charged groups. A positively charged group is typically bonded to the terminal carbon atom of the carbon chain.

Examples of a carbon chain of a positively charged organic group having one or more substitutions with a hydroxyl group include hydroxyalkyl (e.g., hydroxyethyl, hydroxypropyl, hydroxybutyl, hydroxypentyl) groups and dihydroxyalkyl (e.g., dihydroxyethyl, dihydroxypropyl, dihydroxybutyl, dihydroxypentyl) groups. Examples of hydroxyalkyl and dihydroxyalkyl (diol) carbon chains include -CH(OH)-, -CH(OH)CH_2-, -$C(OH)_2CH_2$-, -$CH_2CH(OH)CH_2$-, -CH(OH)CH_2CH_2-, -CH(OH)CH(OH)CH_2-, -$CH_2CH_2CH(OH)CH_2$-, -$CH_2CH(OH)CH_2CH_2$-, -CH(OH)$CH_2CH_2CH_2$-, -$CH_2CH(OH)CH(OH)CH_2$-, -CH(OH)CH(OH)CH_2CH_2- and -CH(OH)$CH_2CH(OH)CH_2$-. In each of these examples, the first carbon atom of the chain is ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan, and the last carbon atom of the chain is linked to a positively charged group. Where the positively charged group is a substituted ammonium group, the last carbon atom of the chain in each of these examples is represented by the C in structure I.

Examples of a carbon chain of a positively charged organic group having one or more substitutions with an alkyl group include chains with one or more substituent methyl, ethyl and/or propyl groups. Examples of methylalkyl groups include -CH(CH_3)CH_2CH_2- and -$CH_2CH(CH_3)CH_2$-, which are both propyl groups having a methyl substitution. In each of these examples, the first carbon atom of

the chain is ether-linked to a glucose monomer of poly alpha-1,3-1,6-glucan, and the last carbon atom of the chain is linked to a positively charged group. Where the positively charged group is a substituted ammonium group, the last carbon atom of the chain in each of these examples is represented by the C in structure I.

Poly alpha-1,3-1,6-glucan ether compounds in certain embodiments disclosed herein may contain one type of positively charged organic group. For example, one or more positively charged organic groups ether-linked to the glucose monomer of poly alpha-1,3-1,6-glucan may be trimethylammonium hydroxypropyl groups (structure II). Alternatively, poly alpha-1,3-1,6-glucan ether compounds disclosed herein can contain two or more different types of positively charged organic groups.

Poly alpha-1,3-1,6-glucan ether compounds herein can comprise at least one nonionic organic group and at least one anionic group, for example. As another example, poly alpha-1,3-1,6-glucan ether compounds herein can comprise at least one nonionic organic group and at least one positively charged organic group.

Poly alpha-1,3-1,6-glucan ether compounds may be derived from any poly alpha-1,3-1,6-glucan disclosed herein. For example, a poly alpha-1,3-1,6-glucan ether compound of the invention can be produced by ether-derivatizing poly alpha-1,3-1,6-glucan using an etherification reaction as disclosed herein.

In certain embodiments of the disclosed invention, the poly alpha-1,3-1,6-glucan from which a poly alpha-1,3-1,6-glucan ether compound is derived is a product of a glucosyltransferase enzyme comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10. Alternatively, the glucosyltransferase enzyme can comprise an amino acid sequence that is at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, or 100% identical to, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

In certain embodiments of the disclosed invention, a composition comprising a poly alpha-1,3-1,6-glucan ether compound can be a hydrocolloid or aqueous solution having a viscosity of at least about 10 cPs. Alternatively, such a hydrocolloid or aqueous solution has a viscosity of at least about 100, 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 3000, 3500, or 4000 cPs (or any integer between 100 and 4000 cPs), for example.

Viscosity can be measured with the hydrocolloid or aqueous solution at any temperature between about 3 °C to about 110 °C (or any integer between 3 and 110 °C). Alternatively, viscosity can be measured at a temperature between about 4 °C to 30 °C, or about 20 °C to 25 °C. Viscosity can be measured at atmospheric pressure (about 760 torr) or any other higher or lower pressure.

The viscosity of a hydrocolloid or aqueous solution disclosed herein can be measured using a viscometer or rheometer, or using any other means known in the art. It would be understood by those skilled in the art that a viscometer or rheometer can be used to measure the viscosity of those hydrocolloids and aqueous solutions of the invention that exhibit shear thinning behavior or shear thickening behavior (i.e., liquids with viscosities that vary with flow conditions). The viscosity of such embodiments can be measured at a rotational shear rate of about 10 to 1000 rpm (revolutions per minute) (or any integer between 10 and 1000 rpm), for example. Alternatively, viscosity can be measured at a rotational shear rate of about 10, 60, 150, 250, or 600 rpm.

The pH of a hydrocolloid or aqueous solution disclosed herein can be between about 2.0 to about 12.0. Alternatively, pH can be about 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0; or between 5.0 to about 12.0; or between about 4.0 and 8.0; or between about 5.0 and 8.0.

An aqueous composition herein such as a hydrocolloid or aqueous solution can comprise a solvent having at least about 20 wt% water. In other embodiments, a solvent is at least about 30, 40, 50, 60, 70, 80, 90, or 100 wt% water (or any integer value between 20 and 100 wt%), for example.

A poly alpha-1,3-1,6-glucan ether compound disclosed herein can be present in a hydrocolloid or aqueous solution at a weight percentage (wt%) of at

least about 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.2%, 1.4%, 1.6%, 1.8%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, for example.

A hydrocolloid or aqueous solution herein can comprise other components in addition to one or more poly alpha-1,3-1,6-glucan ether compounds. For example, the hydrocolloid or aqueous solution can comprise one or more salts such as a sodium salt (e.g., NaCl, Na_2SO_4). Other non-limiting examples of salts include those having (i) an aluminum, ammonium, barium, calcium, chromium (II or III), copper (I or II), iron (II or III), hydrogen, lead (II), lithium, magnesium, manganese (II or III), mercury (I or II), potassium, silver, sodium strontium, tin (II or IV), or zinc cation, and (ii) an acetate, borate, bromate, bromide, carbonate, chlorate, chloride, chlorite, chromate, cyanamide, cyanide, dichromate, dihydrogen phosphate, ferricyanide, ferrocyanide, fluoride, hydrogen carbonate, hydrogen phosphate, hydrogen sulfate, hydrogen sulfide, hydrogen sulfite, hydride, hydroxide, hypochlorite, iodate, iodide, nitrate, nitride, nitrite, oxalate, oxide, perchlorate, permanganate, peroxide, phosphate, phosphide, phosphite, silicate, stannate, stannite, sulfate, sulfide, sulfite, tartrate, or thiocyanate anion. Thus, any salt having a cation from (i) above and an anion from (ii) above can be in a hydrocolloid or aqueous solution, for example. A salt can be present in a hydrocolloid or aqueous solution at a wt% of about .01% to about 10.00% (or any hundredth increment between .01% and 10.00%), for example.

Those skilled in the art would understand that in certain embodiments of the invention, a poly alpha-1,3-1,6-glucan ether compound can be in an anionic form in a hydrocolloid or aqueous solution. Examples may include those poly alpha-1,3-1,6-glucan ether compounds having an organic group comprising an alkyl group substituted with a carboxyl group. Carboxyl (COOH) groups in a carboxyalkyl poly alpha-1,3-1,6-glucan ether compound can convert to carboxylate (COO^-) groups in aqueous conditions. Such anionic groups can interact with salt cations such as any of those listed above in (i) (e.g., potassium, sodium, or lithium cation). Thus, a poly alpha-1,3-1,6-glucan ether compound

can be a sodium carboxyalkyl poly alpha-1,3-1,6-glucan ether (e.g., sodium carboxymethyl poly alpha-1,3-1,6-glucan), potassium carboxyalkyl poly alpha-1,3-1,6-glucan ether (e.g., potassium carboxymethyl poly alpha-1,3-1,6-glucan), or lithium carboxyalkyl poly alpha-1,3-1,6-glucan ether (e.g., lithium carboxymethyl poly alpha-1,3-1,6-glucan), for example.

In alternative embodiments, a composition comprising a poly alpha-1,3-1,6-glucan ether compound herein can be non-aqueous (e.g., a dry composition). Examples of such embodiments include powders, granules, microcapsules, flakes, or any other form of particulate matter. Other examples include larger compositions such as pellets, bars, kernels, beads, tablets, sticks, or other agglomerates. A non-aqueous or dry composition herein typically has less than 3, 2, 1, 0.5, or 0.1 wt% water comprised therein.

A poly alpha-1,3-1,6-glucan ether compound comprised in certain embodiments of the disclosed composition may be crosslinked using any means known in the art. Such crosslinks may be borate crosslinks, where the borate is from any boron-containing compound (e.g., boric acid, diborates, tetraborates, pentaborates, polymeric compounds such as Polybor®, polymeric compounds of boric acid, alkali borates), for example. Alternatively, crosslinks can be provided with polyvalent metals such as titanium or zirconium. Titanium crosslinks may be provided, for example, using titanium IV-containing compounds such as titanium ammonium lactate, titanium triethanolamine, titanium acetylacetonate, and polyhydroxy complexes of titanium. Zirconium crosslinks can be provided using zirconium IV-containing compounds such as zirconium lactate, zirconium carbonate, zirconium acetylacetonate, zirconium triethanolamine, zirconium diisopropylamine lactate and polyhydroxy complexes of zirconium, for example. Alternatively still, crosslinks can be provided with any crosslinking agent described in U.S. Patent Nos. 4462917, 4464270, 4477360 and 4799550, which are all incorporated herein by reference. A crosslinking agent (e.g., borate) may be present in an aqueous composition herein at a concentration of about 0.2% to 20 wt%, or about 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 wt%, for example.

It is believed that a poly alpha-1,3-1,6-glucan ether compound disclosed herein that is crosslinked typically has a higher viscosity in an aqueous solution compared to its non-crosslinked counterpart. In addition, it is believed that a crosslinked poly alpha-1,3-1,6-glucan ether compound can have increased shear thickening behavior compared to its non-crosslinked counterpart.

A composition herein may optionally contain one or more active enzymes. Non-limiting examples of suitable enzymes include proteases, cellulases, hemicellulases, peroxidases, lipolytic enzymes (e.g., metallolipolytic enzymes), xylanases, lipases, phospholipases, esterases (e.g., arylesterase, polyesterase), perhydrolases, cutinases, pectinases, pectate lyases, mannanases, keratinases, reductases, oxidases (e.g., choline oxidase), phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, beta-glucanases, arabinosidases, hyaluronidases, chondroitinases, laccases, metalloproteinases, amadoriases, glucoamylases, arabinofuranosidases, phytases, isomerases, transferases and amylases. If an enzyme(s) is included, it may be comprised in a composition herein at about 0.0001-0.1 wt% (e.g., 0.01-0.03 wt%) active enzyme (e.g., calculated as pure enzyme protein), for example.

A cellulase herein can have endocellulase activity (EC 3.2.1.4), exocellulase activity (EC 3.2.1.91), or cellobiase activity (EC 3.2.1.21). A cellulase herein is an "active cellulase" having activity under suitable conditions for maintaining cellulase activity; it is within the skill of the art to determine such suitable conditions. Besides being able to degrade cellulose, a cellulase in certain embodiments can also degrade cellulose ether derivatives such as carboxymethyl cellulose. Examples of cellulose ether derivatives which are expected to not be stable to cellulase are disclosed in U.S. Patent Nos. 7012053, 7056880, 6579840, 7534759 and 7576048.

A cellulase herein may be derived from any microbial source, such as a bacteria or fungus. Chemically-modified cellulases or protein-engineered mutant cellulases are included. Suitable cellulases include, but are not limited to, cellulases from the genera *Bacillus*, *Pseudomonas*, *Streptomyces*, *Trichoderma*, *Humicola*, *Fusarium*, *Thielavia* and *Acremonium*. As other examples, a cellulase

may be derived from *Humicola insolens*, *Myceliophthora thermophila* or *Fusarium oxysporum*; these and other cellulases are disclosed in U.S. Patent Nos. 4435307, 5648263, 5691178, 5776757 and 7604974, which are all incorporated herein by reference. Exemplary *Trichoderma reesei* cellulases are disclosed in U.S. Patent Nos. 4689297, 5814501, 5324649, and International Patent Appl. Publ. Nos. WO92/06221 and WO92/06165, all of which are incorporated herein by reference. Exemplary *Bacillus* cellulases are disclosed in U.S. Patent No. 6562612, which is incorporated herein by reference. A cellulase, such as any of the foregoing, preferably is in a mature form lacking an N-terminal signal peptide. Commercially available cellulases useful herein include CELLUZYME® and CAREZYME® (Novozymes A/S); CLAZINASE® and PURADAX® HA (DuPont Industrial Biosciences), and KAC-500(B)® (Kao Corporation).

Alternatively, a cellulase herein may be produced by any means known in the art, such as described in U.S. Patent Nos. 4435307, 5776757 and 7604974, which are incorporated herein by reference. For example, a cellulase may be produced recombinantly in a heterologous expression system, such as a microbial or fungal heterologous expression system. Examples of heterologous expression systems include bacterial (e.g., *E. coli*, *Bacillus* sp.) and eukaryotic systems. Eukaryotic systems can employ yeast (e.g., *Pichia* sp., *Saccharomyces* sp.) or fungal (e.g., *Trichoderma* sp. such as *T. reesei*, *Aspergillus* species such as *A. niger*) expression systems, for example.

One or more cellulases can be directly added as an ingredient when preparing a composition disclosed herein. Alternatively, one or more cellulases can be indirectly (inadvertently) provided in the disclosed composition. For example, cellulase can be provided in a composition herein by virtue of being present in a non-cellulase enzyme preparation used for preparing a composition. Cellulase in compositions in which cellulase is indirectly provided thereto can be present at about 0.1-10 ppb (e.g., less than 1 ppm), for example. A contemplated benefit of a composition herein, by virtue of employing a poly alpha-1,3-1,6-glucan ether compound instead of a cellulose ether compound, is that non-cellulase enzyme preparations that might have background cellulase

activity can be used without concern that the desired effects of the glucan ether will be negated by the background cellulase activity.

A cellulase in certain embodiments can be thermostable. Cellulase thermostability refers to the ability of the enzyme to retain activity after exposure to an elevated temperature (e.g. about 60-70 °C) for a period of time (e.g., about 30-60 minutes). The thermostability of a cellulase can be measured by its half-life (t1/2) given in minutes, hours, or days, during which time period half the cellulase activity is lost under defined conditions.

A cellulase in certain embodiments can be stable to a wide range of pH values (e.g. neutral or alkaline pH such as pH of ~7.0 to ~11.0). Such enzymes can remain stable for a predetermined period of time (e.g., at least about 15 min., 30 min., or 1 hour) under such pH conditions.

At least one, two, or more cellulases may be included in the composition. The total amount of cellulase in a composition herein typically is an amount that is suitable for the purpose of using cellulase in the composition (an "effective amount"). For example, an effective amount of cellulase in a composition intended for improving the feel and/or appearance of a cellulose-containing fabric is an amount that produces measurable improvements in the feel of the fabric (e.g., improving fabric smoothness and/or appearance, removing pills and fibrils which tend to reduce fabric appearance sharpness). As another example, an effective amount of cellulase in a fabric stonewashing composition herein is that amount which will provide the desired effect (e.g., to produce a worn and faded look in seams and on fabric panels). The amount of cellulase in a composition herein can also depend on the process parameters in which the composition is employed (e.g., equipment, temperature, time, and the like) and cellulase activity, for example. The effective concentration of cellulase in an aqueous composition in which a fabric is treated can be readily determined by a skilled artisan. In fabric care processes, cellulase can be present in an aqueous composition (e.g., wash liquor) in which a fabric is treated in a concentration that is minimally about 0.01-0.1 ppm total cellulase protein, or about 0.1-10 ppb total cellulase protein

(e.g., less than 1 ppm), to maximally about 100, 200, 500, 1000, 2000, 3000, 4000, or 5000 ppm total cellulase protein, for example.

Poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ethers herein are mostly or completely stable (resistant) to being degraded by cellulase. For example, the percent degradation of a poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compound by one or more cellulases is less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or is 0%. Such percent degradation can be determined, for example, by comparing the molecular weight of polymer before and after treatment with a cellulase for a period of time (e.g., ~24 hours).

Hydrocolloids and aqueous solutions in certain embodiments of the invention are believed to have either shear thinning behavior or shear thickening behavior. Shear thinning behavior is observed as a decrease in viscosity of the hydrocolloid or aqueous solution as shear rate increases, whereas shear thickening behavior is observed as an increase in viscosity of the hydrocolloid or aqueous solution as shear rate increases. Modification of the shear thinning behavior or shear thickening behavior of an aqueous solution herein is due to the admixture of a poly alpha-1,3-1,6-glucan ether to the aqueous composition. Thus, one or more poly alpha-1,3-1,6-glucan ether compounds of the invention can be added to an aqueous composition to modify its rheological profile (i.e., the flow properties of the aqueous liquid, solution, or mixture are modified). Also, one or more poly alpha-1,3-1,6-glucan ether compounds of the invention can be added to an aqueous composition to modify its viscosity.

The rheological properties of hydrocolloids and aqueous solutions of the invention can be observed by measuring viscosity over an increasing rotational shear rate (e.g., from about 10 rpm to about 250 rpm). For example, shear thinning behavior of a hydrocolloid or aqueous solution disclosed herein can be observed as a decrease in viscosity (cPs) by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% (or any integer between 5% and 95%) as the rotational shear rate

increases from about 10 rpm to 60 rpm, 10 rpm to 150 rpm, 10 rpm to 250 rpm, 60 rpm to 150 rpm, 60 rpm to 250 rpm, or 150 rpm to 250 rpm. As another example, shear thickening behavior of a hydrocolloid or aqueous solution disclosed herein can be observed as an increase in viscosity (cPs) by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, or 200% (or any integer between 5% and 200%) as the rotational shear rate increases from about 10 rpm to 60 rpm, 10 rpm to 150 rpm, 10 rpm to 250 rpm, 60 rpm to 150 rpm, 60 rpm to 250 rpm, or 150 rpm to 250 rpm.

A hydrocolloid or aqueous solution disclosed herein can be in the form of, and/or comprised in, a personal care product, pharmaceutical product, food product, household product, or industrial product. Poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compounds disclosed herein can be used as thickening agents and/or dispersion agents in each of these products. Such a thickening agent may be used in conjunction with one or more other types of thickening agents if desired, such as those disclosed in U.S. Patent No. 8541041, the disclosure of which is incorporated herein by reference in its entirety.

Personal care products herein are not particularly limited and include, for example, skin care compositions, cosmetic compositions, antifungal compositions, and antibacterial compositions. Personal care products herein may be in the form of, for example, lotions, creams, pastes, balms, ointments, pomades, gels, liquids, combinations of these and the like. The personal care products disclosed herein can include at least one active ingredient, if desired. An active ingredient is generally recognized as an ingredient that causes an intended pharmacological effect.

In certain embodiments, a skin care product can be applied to skin for addressing skin damage related to a lack of moisture. A skin care product may also be used to address the visual appearance of skin (e.g., reduce the appearance of flaky, cracked, and/or red skin) and/or the tactile feel of the skin

(e.g., reduce roughness and/or dryness of the skin while improved the softness and subtleness of the skin). A skin care product typically may include at least one active ingredient for the treatment or prevention of skin ailments, providing a cosmetic effect, or for providing a moisturizing benefit to skin, such as zinc oxide, petrolatum, white petrolatum, mineral oil, cod liver oil, lanolin, dimethicone, hard fat, vitamin A, allantoin, calamine, kaolin, glycerin, or colloidal oatmeal, and combinations of these. A skin care product may include one or more natural moisturizing factors such as ceramides, hyaluronic acid, glycerin, squalane, amino acids, cholesterol, fatty acids, triglycerides, phospholipids, glycosphingolipids, urea, linoleic acid, glycosaminoglycans, mucopolysaccharide, sodium lactate, or sodium pyrrolidone carboxylate, for example. Other ingredients that may be included in a skin care product include, without limitation, glycerides, apricot kernel oil, canola oil, squalane, squalene, coconut oil, corn oil, jojoba oil, jojoba wax, lecithin, olive oil, safflower oil, sesame oil, shea butter, soybean oil, sweet almond oil, sunflower oil, tea tree oil, shea butter, palm oil, cholesterol, cholesterol esters, wax esters, fatty acids, and orange oil.

A personal care product herein can also be in the form of makeup, lipstick, mascara, rouge, foundation, blush, eyeliner, lip liner, lip gloss, other cosmetics, sunscreen, sun block, nail polish, mousse, hair spray, styling gel, nail conditioner, bath gel, shower gel, body wash, face wash, shampoo, hair conditioner (leave-in or rinse-out), cream rinse, hair dye, hair coloring product, hair shine product, hair serum, hair anti-frizz product, hair split-end repair product, lip balm, skin conditioner, cold cream, moisturizer, body spray, soap, body scrub, exfoliant, astringent, scruffing lotion, depilatory, permanent waving solution, antidandruff formulation, antiperspirant composition, deodorant, shaving product, pre-shaving product, after-shaving product, cleanser, skin gel, rinse, dentifrice composition, toothpaste, or mouthwash, for example.

A pharmaceutical product herein can be in the form of an emulsion, liquid, elixir, gel, suspension, solution, cream, or ointment, for example. Also, a pharmaceutical product herein can be in the form of any of the personal care products disclosed herein, such as an antibacterial or antifungal composition. A

pharmaceutical product can further comprise one or more pharmaceutically acceptable carriers, diluents, and/or pharmaceutically acceptable salts. A poly alpha-1,3-1,6-glucan ether compound disclosed herein can also be used in capsules, encapsulants, tablet coatings, and as an excipients for medicaments and drugs.

Non-limiting examples of food products herein include vegetable, meat, and soy patties; reformed seafood; reformed cheese sticks; cream soups; gravies and sauces; salad dressing; mayonnaise; onion rings; jams, jellies, and syrups; pie filling; potato products such as French fries and extruded fries; batters for fried foods, pancakes/waffles and cakes; pet foods; beverages; frozen desserts; ice cream; cultured dairy products such as cottage cheese, yogurt, cheeses, and sour creams; cake icing and glazes; whipped topping; leavened and unleavened baked goods; and the like.

Poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compounds, hydrocolloids and aqueous compositions disclosed herein can be used to provide one or more of the following physical properties to a food product (or any personal care product, pharmaceutical product, or industrial product): thickening, freeze/thaw stability, lubricity, moisture retention and release, texture, consistency, shape retention, emulsification, binding, suspension, dispersion, and gelation, for example. Poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compounds disclosed herein can typically be used in a food product at a level of about 0.01 to about 5 wt%, for example.

A poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compound disclosed herein can be comprised in a foodstuff or any other ingestible material (e.g., enteral pharmaceutical preparation) in an amount that provides the desired degree of thickening and/or dispersion. For example, the concentration or amount of a poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compound in a product, on a weight basis, can be about 0.1-3 wt%, 0.1-4 wt%, 0.1-5 wt%, or 0.1-10 wt%.

A household and/or industrial product herein can be in the form of drywall tape-joint compounds; mortars; grouts; cement plasters; spray plasters; cement

stucco; adhesives; pastes; wall/ceiling texturizers; binders and processing aids for tape casting, extrusion forming, injection molding and ceramics; spray adherents and suspending/dispersing aids for pesticides, herbicides, and fertilizers; fabric care products such as fabric softeners and laundry detergents; hard surface cleaners; air fresheners; polymer emulsions; gels such as water-based gels; surfactant solutions; paints such as water-based paints; protective coatings; adhesives; sealants and caulks; inks such as water-based ink; metal-working fluids; emulsion-based metal cleaning fluids used in electroplating, phosphatizing, galvanizing and/or general metal cleaning operations; hydraulic fluids (e.g., those used for fracking in downhole operations); and aqueous mineral slurries, for example.

Poly alpha-1,3-1,6-glucan and/or a poly alpha-1,3-1,6-glucan ether compound disclosed herein can be comprised in a personal care product, pharmaceutical product, household product, or industrial product in an amount that provides a desired degree of thickening or dispersion, for example. Examples of a concentration or amount of a poly alpha-1,3-1,6-glucan ether compound in a product, on a weight basis, can be about 0.1-3 wt%, 1-2 wt%, 1.5-2.5 wt%, 2.0 wt%, 0.1-4 wt%, 0.1-5 wt%, or 0.1-10 wt%.

Compositions disclosed herein can be in the form of a fabric care composition. A fabric care composition herein can be used for hand wash, machine wash and/or other purposes such as soaking and/or pretreatment of fabrics, for example. A fabric care composition may take the form of, for example, a laundry detergent; fabric conditioner; any wash-, rinse-, or dryer-added product; unit dose or spray. Fabric care compositions in a liquid form may be in the form of an aqueous composition as disclosed herein. In other aspects, a fabric care composition can be in a dry form such as a granular detergent or dryer-added fabric softener sheet. Other non-limiting examples of fabric care compositions herein include: granular or powder-form all-purpose or heavy-duty washing agents; liquid, gel or paste-form all-purpose or heavy-duty washing agents; liquid or dry fine-fabric (e.g. delicates) detergents; cleaning auxiliaries

such as bleach additives, “stain-stick”, or pre-treatments; substrate-laden products such as dry and wetted wipes, pads, or sponges; sprays and mists.

A detergent composition herein may be in any useful form, e.g., as powders, granules, pastes, bars, unit dose, or liquid. A liquid detergent may be aqueous, typically containing up to about 70 wt% of water and 0 wt% to about 30 wt% of organic solvent. It may also be in the form of a compact gel type containing only about 30 wt% water.

A detergent composition herein typically comprises one or more surfactants, wherein the surfactant is selected from nonionic surfactants, anionic surfactants, cationic surfactants, ampholytic surfactants, zwitterionic surfactants, semi-polar nonionic surfactants and mixtures thereof. In some embodiments, the surfactant is present at a level of from about 0.1% to about 60%, while in alternative embodiments the level is from about 1% to about 50%, while in still further embodiments the level is from about 5% to about 40%, by weight of the detergent composition. A detergent will usually contain 0 wt% to about 50 wt% of an anionic surfactant such as linear alkylbenzenesulfonate (LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkanesulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid, or soap. In addition, a detergent composition may optionally contain 0 wt% to about 40 wt% of a nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, or polyhydroxy alkyl fatty acid amide (as described for example in WO92/06154, which is incorporated herein by reference).

A detergent composition herein typically comprises one or more detergent builders or builder systems. In some embodiments incorporating at least one builder, the cleaning compositions comprise at least about 1%, from about 3% to about 60%, or even from about 5% to about 40%, builder by weight of the composition. Builders include, but are not limited to, alkali metal, ammonium and alkanolammonium salts of polyphosphates, alkali metal silicates, alkaline

earth and alkali metal carbonates, aluminosilicates, polycarboxylate compounds, ether hydroxypolycarboxylates, copolymers of maleic anhydride with ethylene or vinyl methyl ether, 1, 3, 5-trihydroxy benzene-2, 4, 6-trisulphonic acid, and carboxymethyloxysuccinic acid, various alkali metal, ammonium and substituted ammonium salts of polyacetic acids such as ethylenediamine tetraacetic acid and nitrilotriacetic acid, as well as polycarboxylates such as mellitic acid, succinic acid, citric acid, oxydisuccinic acid, polymaleic acid, benzene 1,3,5-tricarboxylic acid, carboxymethyloxysuccinic acid, and soluble salts thereof. Indeed, it is contemplated that any suitable builder will find use in various embodiments of the present invention. Examples of a detergent builder or complexing agent include zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g., SKS-6 from Hoechst). A detergent may also be unbuilt, i.e., essentially free of detergent builder.

In some embodiments, builders form water-soluble hardness ion complexes (e.g., sequestering builders), such as citrates and polyphosphates (e.g., sodium tripolyphosphate and sodium tripolyphospate hexahydrate, potassium tripolyphosphate, and mixed sodium and potassium tripolyphosphate, etc.). It is contemplated that any suitable builder will find use in the present invention, including those known in the art (See, e.g., EP2100949).

In some embodiments, builders for use herein include phosphate builders and non-phosphate builders. In some embodiments, the builder is a phosphate builder. In some embodiments, the builder is a non-phosphate builder. If present, builders are used in a level of from 0.1% to 80%, or from 5% to 60%, or from 10% to 50%, by weight of the composition. In some embodiments, the product comprises a mixture of phosphate and non-phosphate builders. Suitable phosphate builders include mono-phosphates, di-phosphates, tri-polyphosphates or oligomeric-poylphosphates, including the alkali metal salts of these compounds, including the sodium salts. In some embodiments, a builder can be sodium tripolyphosphate (STPP). Additionally, the composition can comprise

carbonate and/or citrate, preferably citrate that helps to achieve a neutral pH composition. Other suitable non-phosphate builders include homopolymers and copolymers of polycarboxylic acids and their partially or completely neutralized salts, monomeric polycarboxylic acids and hydroxycarboxylic acids and their salts. In some embodiments, salts of the above mentioned compounds include ammonium and/or alkali metal salts, i.e., lithium, sodium, and potassium salts, including sodium salts. Suitable polycarboxylic acids include acyclic, alicyclic, hetero-cyclic and aromatic carboxylic acids, wherein in some embodiments, they can contain at least two carboxyl groups which are in each case separated from one another by, in some instances, no more than two carbon atoms.

A detergent composition herein can comprise at least one chelating agent. Suitable chelating agents include, but are not limited to copper, iron and/or manganese chelating agents and mixtures thereof. In embodiments in which at least one chelating agent is used, the composition comprises from about 0.1% to about 15%, or even from about 3.0% to about 10%, chelating agent by weight of the composition.

A detergent composition herein can comprise at least one deposition aid. Suitable deposition aids include, but are not limited to, polyethylene glycol, polypropylene glycol, polycarboxylate, soil release polymers such as polytelephthalic acid, clays such as kaolinite, montmorillonite, atapulgite, illite, bentonite, halloysite, and mixtures thereof.

A detergent composition herein can comprise one or more dye transfer inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof. Additional dye transfer inhibiting agents include manganese phthalocyanine, peroxidases, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles and/or mixtures thereof; chelating agents examples of which include ethylene-diamine-tetraacetic acid (EDTA); diethylene triamine penta methylene phosphonic acid (DTPMP);

hydroxy-ethane diphosphonic acid (HEDP); ethylenediamine N,N'-disuccinic acid (EDDS); methyl glycine diacetic acid (MGDA); diethylene triamine penta acetic acid (DTPA); propylene diamine tetracetic acid (PDT A); 2-hydroxypyridine-N-oxide (HPNO); or methyl glycine diacetic acid (MGDA); glutamic acid N,N-diacetic acid (N,N-dicarboxymethyl glutamic acid tetrasodium salt (GLDA); nitrilotriacetic acid (NTA); 4,5-dihydroxy-m-benzenedisulfonic acid; citric acid and any salts thereof; N-hydroxyethylethylenediaminetri-acetic acid (HEDTA), triethylenetetraaminehexaacetic acid (TTHA), N-hydroxyethyliminodiacetic acid (HEIDA), dihydroxyethylglycine (DHEG), ethylenediaminetetrapropionic acid (EDTP) and derivatives thereof, which can be used alone or in combination with any of the above. In embodiments in which at least one dye transfer inhibiting agent is used, a composition herein may comprise from about 0.0001% to about 10%, from about 0.01% to about 5%, or even from about 0.1% to about 3%, by weight of the composition.

A detergent composition herein can comprise silicates. In some of these embodiments, sodium silicates (e.g., sodium disilicate, sodium metasilicate, and/or crystalline phyllosilicates) find use. In some embodiments, silicates are present at a level of from about 1% to about 20% by weight of the composition. In some embodiments, silicates are present at a level of from about 5% to about 15% by weight of the composition.

A detergent composition herein can comprise dispersants. Suitable water-soluble organic materials include, but are not limited to the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

A detergent composition herein may additionally comprise one or more enzymes. Examples of enzymes include proteases, cellulases, hemicellulases, peroxidases, lipolytic enzymes (e.g., metallolipolytic enzymes), xylanases, lipases, phospholipases, esterases (e.g., arylesterase, polyesterase), perhydrolases, cutinases, pectinases, pectate lyases, mannanases, keratinases, reductases, oxidases (e.g., choline oxidase, phenoloxidase), phenoloxidases,

lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, beta-glucanases, arabinosidases, hyaluronidases, chondroitinases, laccases, metalloproteinases, amadoriases, glucoamylases, alpha-amylases, beta-amylases, galactosidases, galactanases, catalases, carageenases, hyaluronidases, keratinases, lactases, ligninases, peroxidases, phosphatases, polygalacturonases, pullulanases, rhamnogalactouronases, tannases, transglutaminases, xyloglucanases, xylosidases, metalloproteases, arabinofuranosidases, phytases, isomerases, transferases and/or amylases in any combination.

Any cellulase disclosed above is contemplated for use in the disclosed detergent compositions. Suitable cellulases include, but are not limited to Humicola insolens cellulases (See e.g., U.S. Pat. No. 4435307). Exemplary cellulases contemplated for use herein are those having color care benefit for a textile. Examples of cellulases that provide a color care benefit are disclosed in EP0495257, EP0531372, EP531315, WO96/11262, WO96/29397, WO94/07998; WO98/12307; WO95/24471, WO98/08940, and U.S. Patent Nos. 5457046, 5686593 and 5763254,all of which are incorporated herein by reference. Examples of commercially available cellulases useful in a detergent include CELLUSOFT®, CELLUCLEAN®, CELLUZYME®, and CAREZYME® (Novo Nordisk A/S and Novozymes A/S); CLAZINASE®, PURADAX HA®, and REVITALENZ™ (DuPont Industrial Biosciences); BIOTOUCH® (AB Enzymes); and KAC-500(B)™ (Kao Corporation). Additional cellulases are disclosed in, e.g., US7595182, US8569033, US7138263, US3844890, US4435307, US4435307, and GB2095275.

In some embodiments of the present invention, the detergent compositions of the present invention can comprise one or more enzymes, each at a level from about 0.00001 % to about 10% by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In some other embodiments of the present invention, the detergent compositions also comprise each enzyme at a level of about 0.0001 % to about 10%, about 0.001%

to about 5%, about 0.001% to about 2%, about 0.005% to about 0.5%, enzyme by weight of the composition.

Suitable proteases include those of animal, vegetable or microbial origin. In some embodiments, microbial proteases are used. In some embodiments, chemically or genetically modified mutants are included. In some embodiments, the protease is a serine protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases include subtilisins, especially those derived from Bacillus (e.g., subtilisin, lentus, amyloliquefaciens, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168). Additional examples include those mutant proteases described in U.S. Pat. Nos. RE34606, 5955340, 5700676, 6312936 and 6482628, all of which are incorporated herein by reference. Additional protease examples include, but are not limited to, trypsin (e.g., of porcine or bovine origin), and the Fusarium protease described in WO89/06270. In some embodiments, commercially available protease enzymes include, but are not limited to, MAXATASE®, MAXACAL™, MAXAPEM™, OPTICLEAN®, OPTIMASE®, PROPERASE®, PURAFECT®, PURAFECT® OXP, PURAMAX™, EXCELLASE™, PREFERENZ™ proteases (e.g. P100, P110, P280), EFFECTENZ™ proteases (e.g. P1000, P1050, P2000), EXCELLENZ™ proteases (e.g. P1000), ULTIMASE®, and PURAFAST™ (Genencor); ALCALASE®, SAVINASE®, PRIMASE®, DURAZYM™, POLARZYME®, OVOZYME®, KANNASE®, LIQUANASE®, NEUTRASE®, RELASE® and ESPERASE® (Novozymes); BLAP™ and BLAP™ variants (Henkel Kommanditgesellschaft auf Aktien, Duesseldorf, Germany), and KAP (B. alkalophilus subtilisin; Kao Corp., Tokyo, Japan). Various proteases are described in WO95/23221, WO92/21760, WO09/149200, WO09/149144, WO09/149145, WO11/072099, WO10/056640, WO10/056653, WO11/140364, WO12/151534, U.S. Pat. Publ. No. 2008/0090747, and U.S. Pat. Nos. 5801039, 5340735, 5500364, 5855625, RE34606, 5955340, 5700676, 6312936, 6482628, 8530219, and various other patents. In some further embodiments, neutral metalloproteases find use in the present invention, including but not limited to, the neutral metalloproteases described in WO1999014341, WO1999033960,

WO1999014342, WO1999034003, WO2007044993, WO2009058303 and WO2009058661, all of which are incorporated herein by reference. Exemplary metalloproteases include nprE, the recombinant form of neutral metalloprotease expressed in Bacillus subtilis (See e.g., WO07/044993), and PMN, the purified neutral metalloprotease from Bacillus amyloliquefaciens.

Suitable mannanases include, but are not limited to, those of bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. Various mannanases are known which find use in the present invention (See, e.g., U.S. Pat. Nos. 6566114, 6602842, and 6440991, all of which are incorporated herein by reference). Commercially available mannanases that find use in the present invention include, but are not limited to MANNASTAR®, PURABRITE™, and MANNAWAY®.

Suitable lipases include those of bacterial or fungal origin. Chemically modified, proteolytically modified, or protein engineered mutants are included. Examples of useful lipases include those from the genera *Humicola* (e.g., *H. lanuginosa*, EP258068 and EP305216; *H. insolens*, WO96/13580), *Pseudomonas* (e.g., *P. alcaligenes* or *P. pseudoalcaligenes*, EP218272; *P. cepacia*, EP331376; *P. stutzeri*, GB1372034; *P. fluorescens* and *Pseudomonas* sp. strain SD 705, WO95/06720 and WO96/27002; *P. wisconsinensis*, WO96/12012); and *Bacillus* (e.g., *B. subtilis*, Dartois et al., *Biochemica et Biophysica Acta* 1131:253-360; *B. stearothermophilus*, JP64/744992; *B. pumilus*, WO91/16422). Furthermore, a number of cloned lipases find use in some embodiments of the present invention, including but not limited to, Penicillium camembertii lipase (See, Yamaguchi et al., Gene 103:61-67 [1991]), Geotricum candidum lipase (See, Schimada et al., J. Biochem., 106:383-388 [1989]), and various Rhizopus lipases such as R. delemar lipase (See, Hass et al., Gene 109:117-113 [1991]), a R. niveus lipase (Kugimiya et al., Biosci. Biotech. Biochem. 56:716-719 [1992]) and R. oryzae lipase. Additional lipases useful herein include, for example, those disclosed in WO92/05249, WO94/01541, WO95/35381, WO96/00292, WO95/30744, WO94/25578, WO95/14783, WO95/22615, WO97/04079, WO97/07202, EP407225 and EP260105. Other

types of lipase polypeptide enzymes such as cutinases also find use in some embodiments of the present invention, including but not limited to, cutinase derived from Pseudomonas mendocina (See, WO88/09367), and cutinase derived from Fusarium solani pisi (See, WO90/09446). Examples of certain commercially available lipase enzymes useful herein include M1 LIPASE™, LUMA FAST™, and LIPOMAX™ (Genencor); LIPEX®, LIPOLASE® and LIPOLASE® ULTRA (Novozymes); and LIPASE P™ "Amano" (Amano Pharmaceutical Co. Ltd., Japan).

Suitable polyesterases include, for example, those disclosed in WO01/34899, WO01/14629 and U.S. Patent No. 6933140.

A detergent composition herein can also comprise 2,6-beta-D-fructan hydrolase, which is effective for removal/cleaning of certain biofilms present on household and/or industrial textiles/laundry.

Suitable amylases include, but are not limited to those of bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. Amylases that find use in the present invention, include, but are not limited to, alpha-amylases obtained from B. licheniformis (See e.g., GB1296839). Additional suitable amylases include those disclosed in W09510603, WO9526397, WO9623874, WO9623873, WO9741213, WO9919467, WO0060060, WO0029560, WO9923211, WO9946399, WO0060058, WO0060059, WO9942567, WO0114532, WO02092797, WO0166712, WO0188107, WO0196537, WO0210355, WO9402597, WO0231124, WO9943793, WO9943794, WO2004113551, WO2005001064, WO2005003311, WO0164852, WO2006063594, WO2006066594, WO2006066596, WO2006012899, WO2008092919, WO2008000825, WO2005018336, WO2005066338, WO2009140504, WO2005019443, WO2010091221, WO2010088447, WO0134784, WO2006012902, WO2006031554, WO2006136161, WO2008101894, WO2010059413, WO2011098531, WO2011080352, WO2011080353, WO2011080354, WO2011082425, WO2011082429, WO2011076123, WO2011087836, WO2011076897, WO94183314, WO9535382, WO9909183, WO9826078,

WO9902702, WO9743424, WO9929876, WO9100353, WO9605295, WO9630481, WO9710342, WO2008088493, WO2009149419, WO2009061381, WO2009100102, WO2010104675, WO2010117511, and WO2010115021, all of which are incorporated herein by reference.

Suitable amylases include, for example, commercially available amylases such as STAINZYME®, STAINZYME PLUS®, NATALASE®, DURAMYL®, TERMAMYL®, TERMAMYL ULTRA®, FUNGAMYL® and BAN™ (Novo Nordisk A/S and Novozymes A/S); RAPIDASE®, POWERASE®, PURASTAR® and PREFERENZ™ (DuPont Industrial Biosciences).

Suitable peroxidases/oxidases contemplated for use in the compositions include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of peroxidases useful herein include those from the genus *Coprinus* (e.g., *C. cinereus*, WO93/24618, WO95/10602, and WO98/15257), as well as those referenced in WO2005056782, WO2007106293, WO2008063400, WO2008106214, and WO2008106215. Commercially available peroxidases useful herein include, for example, GUARDZYME™ (Novo Nordisk A/S and Novozymes A/S).

In some embodiments, peroxidases are used in combination with hydrogen peroxide or a source thereof (e.g., a percarbonate, perborate or persulfate) in the compositions of the present invention. In some alternative embodiments, oxidases are used in combination with oxygen. Both types of enzymes are used for "solution bleaching" (i.e., to prevent transfer of a textile dye from a dyed fabric to another fabric when the fabrics are washed together in a wash liquor), preferably together with an enhancing agent (See e.g., WO94/12621 and WO95/01426). Suitable peroxidases/oxidases include, but are not limited to, those of plant, bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments.

Enzymes that may be comprised in a detergent composition herein may be stabilized using conventional stabilizing agents, e.g., a polyol such as propylene glycol or glycerol; a sugar or sugar alcohol; lactic acid; boric acid or a boric acid derivative (e.g., an aromatic borate ester).

A detergent composition herein may contain about 1 wt% to about 65 wt% of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g., SKS-6 from Hoechst). A detergent may also be unbuilt, i.e., essentially free of detergent builder.

A detergent composition in certain embodiments may comprise one or more other types of polymers in addition to a poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compound. Examples of other types of polymers useful herein include carboxymethyl cellulose (CMC), poly(vinylpyrrolidone) (PVP), polyethylene glycol (PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

A detergent composition herein may contain a bleaching system. For example, a bleaching system can comprise an H_2O_2 source such as perborate or percarbonate, which may be combined with a peracid-forming bleach activator such as tetraacetylethylenediamine (TAED) or nonanoyloxybenzenesulfonate (NOBS). Alternatively, a bleaching system may comprise peroxyacids (e.g., amide, imide, or sulfone type peroxyacids). Alternatively still, a bleaching system can be an enzymatic bleaching system comprising perhydrolase, for example, such as the system described in WO2005/056783.

A detergent composition herein may also contain conventional detergent ingredients such as fabric conditioners, clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil redeposition agents, dyes, bactericides, tarnish inhibiters, optical brighteners, or perfumes. The pH of a detergent composition herein (measured in aqueous solution at use concentration) is usually neutral or alkaline (e.g., pH of about 7.0 to about 11.0).

Particular forms of detergent compositions that can be adapted for purposes disclosed herein are disclosed in, for example, US20090209445A1, US20100081598A1, US7001878B2, EP1504994B1, WO2001085888A2,

WO2003089562A1, WO2009098659A1, WO2009098660A1, WO2009112992A1, WO2009124160A1, WO2009152031A1, WO2010059483A1, WO2010088112A1, WO2010090915A1, WO2010135238A1, WO2011094687A1, WO2011094690A1, WO2011127102A1, WO2011163428A1, WO2008000567A1, WO2006045391A1, WO2006007911A1, WO2012027404A1, EP1740690B1, WO2012059336A1, US6730646B1, WO2008087426A1, WO2010116139A1, and WO2012104613A1, all of which are incorporated herein by reference.

Laundry detergent compositions herein can optionally be heavy duty (all purpose) laundry detergent compositions. Exemplary heavy duty laundry detergent compositions comprise a detersive surfactant (10%-40% wt/wt), including an anionic detersive surfactant (selected from a group of linear or branched or random chain, substituted or unsubstituted alkyl sulphates, alkyl sulphonates, alkyl alkoxylated sulphate, alkyl phosphates, alkyl phosphonates, alkyl carboxylates, and/or mixtures thereof), and optionally non-ionic surfactant (selected from a group of linear or branched or random chain, substituted or unsubstituted alkyl alkoxylated alcohol, e.g., C8-C18 alkyl ethoxylated alcohols and/or C6-C12 alkyl phenol alkoxylates), where the weight ratio of anionic detersive surfactant (with a hydrophilic index (HIc) of from 6.0 to 9) to non-ionic detersive surfactant is greater than 1:1. Suitable detersive surfactants also include cationic detersive surfactants (selected from a group of alkyl pyridinium compounds, alkyl quaternary ammonium compounds, alkyl quaternary phosphonium compounds, alkyl ternary sulphonium compounds, and/or mixtures thereof); zwitterionic and/or amphoteric detersive surfactants (selected from a group of alkanolamine sulpho-betaines); ampholytic surfactants; semi-polar non-ionic surfactants and mixtures thereof.

A detergent herein such as a heavy duty laundry detergent composition may optionally include, a surfactancy boosting polymer consisting of amphiphilic alkoxylated grease cleaning polymers (selected from a group of alkoxylated polymers having branched hydrophilic and hydrophobic properties, such as alkoxylated polyalkylenimines in the range of 0.05 wt% - 10 wt%) and/or random graft polymers (typically comprising of hydrophilic backbone comprising

monomers selected from the group consisting of: unsaturated C1-C6 carboxylic acids, ethers, alcohols, aldehydes, ketones, esters, sugar units, alkoxy units, maleic anhydride, saturated polyalcohols such as glycerol, and mixtures thereof; and hydrophobic side chain(s) selected from the group consisting of: C4-C25 alkyl group, polypropylene, polybutylene, vinyl ester of a saturated C1-C6 mono-carboxylic acid, C1-C6 alkyl ester of acrylic or methacrylic acid, and mixtures thereof.

A detergent herein such as a heavy duty laundry detergent composition may optionally include additional polymers such as soil release polymers (include anionically end-capped polyesters, for example SRP1, polymers comprising at least one monomer unit selected from saccharide, dicarboxylic acid, polyol and combinations thereof, in random or block configuration, ethylene terephthalate-based polymers and co-polymers thereof in random or block configuration, for example REPEL-O-TEX SF, SF-2 AND SRP6, TEXCARE SRA100, SRA300, SRN100, SRN170, SRN240, SRN300 AND SRN325, MARLOQUEST SL), anti-redeposition polymers (0.1 wt% to 10 wt%), include carboxylate polymers, such as polymers comprising at least one monomer selected from acrylic acid, maleic acid (or maleic anhydride), fumaric acid, itaconic acid, aconitic acid, mesaconic acid, citraconic acid, methylenemalonic acid, and any mixture thereof, vinylpyrrolidone homopolymer, and/or polyethylene glycol, molecular weight in the range of from 500 to 100,000 Da); and polymeric carboxylate (such as maleate/acrylate random copolymer or polyacrylate homopolymer).

A detergent herein such as a heavy duty laundry detergent composition may optionally further include saturated or unsaturated fatty acids, preferably saturated or unsaturated C12-C24 fatty acids (0 wt% to 10 wt%); deposition aids in addition to a poly alpha-1,3-1,6-glucan ether compound disclosed herein (examples for which include polysaccharides, cellulosic polymers, poly diallyl dimethyl ammonium halides (DADMAC), and co-polymers of DAD MAC with vinyl pyrrolidone, acrylamides, imidazoles, imidazolinium halides, and mixtures thereof, in random or block configuration, cationic guar gum, cationic starch, cationic polyacylamides, and mixtures thereof.

A detergent herein such as a heavy duty laundry detergent composition may optionally further include dye transfer inhibiting agents, examples of which include manganese phthalocyanine, peroxidases, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles and/or mixtures thereof; chelating agents, examples of which include ethylene-diamine-tetraacetic acid (EDTA), diethylene triamine penta methylene phosphonic acid (DTPMP), hydroxy-ethane diphosphonic acid (HEDP), ethylenediamine N,N'-disuccinic acid (EDDS), methyl glycine diacetic acid (MGDA), diethylene triamine penta acetic acid (DTPA), propylene diamine tetracetic acid (PDTA), 2-hydroxypyridine-N-oxide (HPNO), or methyl glycine diacetic acid (MGDA), glutamic acid N,N-diacetic acid (N,N-dicarboxymethyl glutamic acid tetrasodium salt (GLDA), nitrilotriacetic acid (NTA), 4,5-dihydroxy-m-benzenedisulfonic acid, citric acid and any salts thereof, N-hydroxyethylethylenediaminetriacetic acid (HEDTA), triethylenetetraaminehexaacetic acid (TTHA), N-hydroxyethyliminodiacetic acid (HEIDA), dihydroxyethylglycine (DHEG), ethylenediaminetetrapropionic acid (EDTP), and derivatives thereof.

A detergent herein such as a heavy duty laundry detergent composition may optionally include silicone or fatty-acid based suds suppressors; hueing dyes, calcium and magnesium cations, visual signaling ingredients, anti-foam (0.001 wt% to about 4.0 wt%), and/or a structurant/thickener (0.01 wt% to 5 wt%) selected from the group consisting of diglycerides and triglycerides, ethylene glycol distearate, microcrystalline cellulose, microfiber cellulose, biopolymers, xanthan gum, gellan gum, and mixtures thereof). Such structurant/thickener would be in addition to the one or more poly alpha-1,3-1,6-glucan compounds comprised in the detergent. A structurant can also be referred to as a structural agent.

A detergent herein can be in the form of a heavy duty dry/solid laundry detergent composition, for example. Such a detergent may include: (i) a detersive surfactant, such as any anionic detersive surfactant disclosed herein, any non-ionic detersive surfactant disclosed herein, any cationic detersive

surfactant disclosed herein, any zwitterionic and/or amphoteric detersive surfactant disclosed herein, any ampholytic surfactant, any semi-polar non-ionic surfactant, and mixtures thereof; (ii) a builder, such as any phosphate-free builder (e.g., zeolite builders in the range of 0 wt% to less than 10 wt%), any phosphate builder (e.g., sodium tri-polyphosphate in the range of 0 wt% to less than 10 wt%), citric acid, citrate salts and nitrilotriacetic acid, any silicate salt (e.g., sodium or potassium silicate or sodium meta-silicate in the range of 0 wt% to less than 10 wt%); any carbonate salt (e.g., sodium carbonate and/or sodium bicarbonate in the range of 0 wt% to less than 80 wt%), and mixtures thereof; (iii) a bleaching agent, such as any photobleach (e.g., sulfonated zinc phthalocyanines, sulfonated aluminum phthalocyanines, xanthenes dyes, and mixtures thereof), any hydrophobic or hydrophilic bleach activator (e.g., dodecanoyl oxybenzene sulfonate, decanoyl oxybenzene sulfonate, decanoyl oxybenzoic acid or salts thereof, 3,5,5-trimethy hexanoyl oxybenzene sulfonate, tetraacetyl ethylene diamine-TAED, nonanoyloxybenzene sulfonate-NOBS, nitrile quats, and mixtures thereof), any source of hydrogen peroxide (e.g., inorganic perhydrate salts, examples of which include mono or tetra hydrate sodium salt of perborate, percarbonate, persulfate, perphosphate, or persilicate), any preformed hydrophilic and/or hydrophobic peracids (e.g., percarboxylic acids and salts, percarbonic acids and salts, perimidic acids and salts, peroxymonosulfuric acids and salts, and mixtures thereof); and/or (iv) any other components such as a bleach catalyst (e.g., imine bleach boosters examples of which include iminium cations and polyions, iminium zwitterions, modified amines, modified amine oxides, N-sulphonyl imines, N-phosphonyl imines, N-acyl imines, thiadiazole dioxides, perfluoroimines, cyclic sugar ketones, and mixtures thereof), and a metal-containing bleach catalyst (e.g., copper, iron, titanium, ruthenium, tungsten, molybdenum, or manganese cations along with an auxiliary metal cations such as zinc or aluminum and a sequestrate such as EDTA, ethylenediaminetetra(methylenephosphonic acid).

Compositions disclosed herein can be in the form of a dishwashing detergent composition. Examples of dishwashing detergents include automatic

dishwashing detergents (typically used in dishwasher machines) and hand-washing dish detergents. A dishwashing detergent composition can be in any dry or liquid/aqueous form as disclosed herein, for example. Components that may be included in certain embodiments of a dishwashing detergent composition include, for example, one or more of a phosphate; oxygen- or chlorine-based bleaching agent; non-ionic surfactant; alkaline salt (e.g., metasilicates, alkali metal hydroxides, sodium carbonate); any active enzyme disclosed herein; anti-corrosion agent (e.g., sodium silicate); anti-foaming agent; additives to slow down the removal of glaze and patterns from ceramics; perfume; anti-caking agent (in granular detergent); starch (in tablet-based detergents); gelling agent (in liquid/gel based detergents); and/or sand (powdered detergents).

Dishwashing detergents such as an automatic dishwasher detergent or liquid dishwashing detergent can comprise (i) a non-ionic surfactant, including any ethoxylated non-ionic surfactant, alcohol alkoxylated surfactant, epoxy-capped poly(oxyalkylated) alcohol, or amine oxide surfactant present in an amount from 0 to 10 wt%; (ii) a builder, in the range of about 5-60 wt%, including any phosphate builder (e.g., mono-phosphates, di-phosphates, tri-polyphosphates, other oligomeric-polyphosphates, sodium tripolyphosphate-STPP), any phosphate-free builder (e.g., amino acid-based compounds including methyl-glycine-diacetic acid [MGDA] and salts or derivatives thereof, glutamic-N,N-diacetic acid [GLDA] and salts or derivatives thereof, iminodisuccinic acid (IDS) and salts or derivatives thereof, carboxy methyl inulin and salts or derivatives thereof, nitrilotriacetic acid [NTA], diethylene triamine penta acetic acid [DTPA], B-alaninediacetic acid [B-ADA] and salts thereof), homopolymers and copolymers of poly-carboxylic acids and partially or completely neutralized salts thereof, monomeric polycarboxylic acids and hydroxycarboxylic acids and salts thereof in the range of 0.5 wt% to 50 wt%, or sulfonated/carboxylated polymers in the range of about 0.1 wt% to about 50 wt%; (iii) a drying aid in the range of about 0.1 wt% to about 10 wt% (e.g., polyesters, especially anionic polyesters, optionally together with further monomers with 3 to 6 functionalities – typically acid, alcohol or ester functionalities which are conducive to

polycondensation, polycarbonate-, polyurethane- and/or polyurea-polyorganosiloxane compounds or precursor compounds thereof, particularly of the reactive cyclic carbonate and urea type); (iv) a silicate in the range from about 1 wt% to about 20 wt% (e.g., sodium or potassium silicates such as sodium disilicate, sodium meta-silicate and crystalline phyllosilicates); (v) an inorganic bleach (e.g., perhydrate salts such as perborate, percarbonate, perphosphate, persulfate and persilicate salts) and/or an organic bleach (e.g., organic peroxyacids such as diacyl- and tetraacylperoxides, especially diperoxydodecanedioic acid, diperoxytetradecanedioic acid, and diperoxyhexadecanedioic acid); (vi) a bleach activator (e.g., organic peracid precursors in the range from about 0.1 wt% to about 10 wt%) and/or bleach catalyst (e.g., manganese triazacyclononane and related complexes; Co, Cu, Mn, and Fe bispyridylamine and related complexes; and pentamine acetate cobalt(III) and related complexes); (vii) a metal care agent in the range from about 0.1 wt% to 5 wt% (e.g., benzatriazoles, metal salts and complexes, and/or silicates); and/or (viii) any active enzyme disclosed herein in the range from about 0.01 to 5.0 mg of active enzyme per gram of automatic dishwashing detergent composition, and an enzyme stabilizer component (e.g., oligosaccharides, polysaccharides, and inorganic divalent metal salts).

Various examples of detergent formulations comprising at least one poly alpha-1,3-1,6-glucan ether compound (e.g., a carboxyalkyl poly alpha-1,3-1,6-glucan ether such as carboxymethyl poly alpha-1,3-1,6-glucan) are disclosed below (1-19):

1) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising: linear alkylbenzenesulfonate (calculated as acid) at about 7-12 wt%; alcohol ethoxysulfate (e.g., C12-18 alcohol, 1-2 ethylene oxide [EO]) or alkyl sulfate (e.g., C16-18) at about 1-4 wt%; alcohol ethoxylate (e.g., C14-15 alcohol) at about 5-9 wt%; sodium carbonate at about 14-20 wt%; soluble silicate (e.g., Na_2O $2SiO_2$) at about 2-6 wt%; zeolite (e.g., $NaAlSiO_4$) at about 15-22 wt%; sodium sulfate at about 0-6 wt%; sodium citrate/citric acid at about 0-15 wt%; sodium perborate at about 11-18 wt%; TAED

at about 2-6 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; other polymers (e.g., maleic/acrylic acid copolymer, PVP, PEG) at about 0-3 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., suds suppressors, perfumes, optical brightener, photobleach) at about 0-5 wt%.

2) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising: linear alkylbenzenesulfonate (calculated as acid) at about 6-11 wt%; alcohol ethoxysulfate (e.g., C12-18 alcohol, 1-2 EO) or alkyl sulfate (e.g., C16-18) at about 1-3 wt%; alcohol ethoxylate (e.g., C14-15 alcohol) at about 5-9 wt%; sodium carbonate at about 15-21 wt%; soluble silicate (e.g., Na_2O $2SiO_2$) at about 1-4 wt%; zeolite (e.g., $NaAlSiO_4$) at about 24-34 wt%; sodium sulfate at about 4-10 wt%; sodium citrate/citric acid at about 0-15 wt%; sodium perborate at about 11-18 wt%; TAED at about 2-6 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; other polymers (e.g., maleic/acrylic acid copolymer, PVP, PEG) at about 1-6 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., suds suppressors, perfumes, optical brightener, photobleach) at about 0-5 wt%.

3) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising: linear alkylbenzenesulfonate (calculated as acid) at about 5-9 wt%; alcohol ethoxysulfate (e.g., C12-18 alcohol, 7 EO) at about 7-14 wt%; soap as fatty acid (e.g., C16-22 fatty acid) at about 1-3 wt%; sodium carbonate at about 10-17 wt%; soluble silicate (e.g., Na_2O $2SiO_2$) at about 3-9 wt%; zeolite (e.g., $NaAlSiO_4$) at about 23-33 wt%; sodium sulfate at about 0-4 wt%; sodium perborate at about 8-16 wt%; TAED at about 2-8 wt%; phosphonate (e.g., EDTMPA) at about 0-1 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; other polymers (e.g., maleic/acrylic acid copolymer, PVP, PEG) at about 0-3 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., suds suppressors, perfumes, optical brightener) at about 0-5 wt%.

4) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising: linear alkylbenzenesulfonate (calculated

as acid) at about 8-12 wt%; alcohol ethoxylate (e.g., C12-18 alcohol, 7 EO) at about 10-25 wt%; sodium carbonate at about 14-22 wt%; soluble silicate (e.g., Na_2O $2SiO_2$) at about 1-5 wt%; zeolite (e.g., $NaAlSiO_4$) at about 25-35 wt%; sodium sulfate at about 0-10 wt%; sodium perborate at about 8-16 wt%; TAED at about 2-8 wt%; phosphonate (e.g., EDTMPA) at about 0-1 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; other polymers (e.g., maleic/acrylic acid copolymer, PVP, PEG) at about 1-3 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., suds suppressors, perfumes) at about 0-5 wt%.

5) An aqueous liquid detergent composition comprising: linear alkylbenzenesulfonate (calculated as acid) at about 15-21 wt%; alcohol ethoxylate (e.g., C12-18 alcohol, 7 EO; or C12-15 alcohol, 5 EO) at about 12-18 wt%; soap as fatty acid (e.g., oleic acid) at about 3-13 wt%; alkenylsuccinic acid (C12-14) at about 0-13 wt%; aminoethanol at about 8-18 wt%; citric acid at about 2-8 wt%; phosphonate at about 0-3 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; other polymers (e.g., PVP, PEG) at about 0-3 wt%; borate at about 0-2 wt%; ethanol at about 0-3 wt%; propylene glycol at about 8-14 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., dispersants, suds suppressors, perfume, optical brightener) at about 0-5 wt%.

6) An aqueous structured liquid detergent composition comprising: linear alkylbenzenesulfonate (calculated as acid) at about 15-21 wt%; alcohol ethoxylate (e.g., C12-18 alcohol, 7 EO; or C12-15 alcohol, 5 EO) at about 3-9 wt%; soap as fatty acid (e.g., oleic acid) at about 3-10 wt%; zeolite (e.g., $NaAlSiO_4$) at about 14-22 wt%; potassium citrate about 9-18 wt%; borate at about 0-2 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; other polymers (e.g., PVP, PEG) at about 0-3 wt%; ethanol at about 0-3 wt%; anchoring polymers (e.g., lauryl methacrylate/acrylic acid copolymer, molar ratio 25:1, MW 3800) at about 0-3 wt%; glycerol at about 0-5 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and

minor ingredients (e.g., dispersants, suds suppressors, perfume, optical brightener) at about 0-5 wt%.

7) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising: fatty alcohol sulfate at about 5-10 wt%, ethoxylated fatty acid monoethanolamide at about 3-9 wt%; soap as fatty acid at about 0-3 wt%; sodium carbonate at about 5-10 wt%; soluble silicate (e.g., Na_2O $2SiO_2$) at about 1-4 wt%; zeolite (e.g., $NaAlSiO_4$) at about 20-40 wt%; sodium sulfate at about 2-8 wt%; sodium perborate at about 12-18 wt%; TAED at about 2-7 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; other polymers (e.g., maleic/acrylic acid copolymer, PEG) at about 1-5 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., optical brightener, suds suppressors, perfumes) at about 0-5 wt%.

8) A detergent composition formulated as a granulate comprising: linear alkylbenzenesulfonate (calculated as acid) at about 8-14 wt%; ethoxylated fatty acid monoethanolamide at about 5-11 wt%; soap as fatty acid at about 0-3 wt%; sodium carbonate at about 4-10 wt%; soluble silicate (e.g., Na_2O $2SiO_2$) at about 1-4 wt%; zeolite (e.g., $NaAlSiO_4$) at about 30-50 wt%; sodium sulfate at about 3-11 wt%; sodium citrate at about 5-12 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; other polymers (e.g., PVP, maleic/acrylic acid copolymer, PEG) at about 1-5 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., suds suppressors, perfumes) at about 0-5 wt%.

9) A detergent composition formulated as a granulate comprising: linear alkylbenzenesulfonate (calculated as acid) at about 6-12 wt%; nonionic surfactant at about 1-4 wt%; soap as fatty acid at about 2-6 wt%; sodium carbonate at about 14-22 wt%; zeolite (e.g., $NaAlSiO_4$) at about 18-32 wt%; sodium sulfate at about 5-20 wt%; sodium citrate at about 3-8 wt%; sodium perborate at about 4-9 wt%; bleach activator (e.g., NOBS or TAED) at about 1-5 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; other polymers (e.g., polycarboxylate or PEG) at about 1-5 wt%; optionally an enzyme(s) (calculated

as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., optical brightener, perfume) at about 0-5 wt%.

10) An aqueous liquid detergent composition comprising: linear alkylbenzenesulfonate (calculated as acid) at about 15-23 wt%; alcohol ethoxysulfate (e.g., C12-15 alcohol, 2-3 EO) at about 8-15 wt%; alcohol ethoxylate (e.g., C12-15 alcohol, 7 EO; or C12-15 alcohol, 5 EO) at about 3-9 wt%; soap as fatty acid (e.g., lauric acid) at about 0-3 wt%; aminoethanol at about 1-5 wt%; sodium citrate at about 5-10 wt%; hydrotrope (e.g., sodium toluenesulfonate) at about 2-6 wt%; borate at about 0-2 wt%; poly alpha-1,3-1,6-glucan ether up to about 1 wt%; ethanol at about 1-3 wt%; propylene glycol at about 2-5 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., dispersants, perfume, optical brighteners) at about 0-5 wt%.

11) An aqueous liquid detergent composition comprising: linear alkylbenzenesulfonate (calculated as acid) at about 20-32 wt%; alcohol ethoxylate (e.g., C12-15 alcohol, 7 EO; or C12-15 alcohol, 5 EO) at about 6-12 wt%; aminoethanol at about 2-6 wt%; citric acid at about 8-14 wt%; borate at about 1-3 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; ethanol at about 1-3 wt%; propylene glycol at about 2-5 wt%; other polymers (e.g., maleic/acrylic acid copolymer, anchoring polymer such as lauryl methacrylate/acrylic acid copolymer) at about 0-3 wt%; glycerol at about 3-8 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., hydrotropes, dispersants, perfume, optical brighteners) at about 0-5 wt%.

12) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising: anionic surfactant (e.g., linear alkylbenzenesulfonate, alkyl sulfate, alpha-olefinsulfonate, alpha-sulfo fatty acid methyl esters, alkanesulfonates, soap) at about 25-40 wt%; nonionic surfactant (e.g., alcohol ethoxylate) at about 1-10 wt%; sodium carbonate at about 8-25 wt%; soluble silicate (e.g., Na_2O $2SiO_2$) at about 5-15 wt%; sodium sulfate at about 0-5 wt%; zeolite ($NaAlSiO_4$) at about 15-28 wt%; sodium perborate at

about 0-20 wt%; bleach activator (e.g., TAED or NOBS) at about 0-5 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., perfume, optical brighteners) at about 0-3 wt%.

13) Detergent compositions as described in (1)-(12) above, but in which all or part of the linear alkylbenzenesulfonate is replaced by C12-C18 alkyl sulfate.

14) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising: C12-C18 alkyl sulfate at about 9-15 wt%; alcohol ethoxylate at about 3-6 wt%; polyhydroxy alkyl fatty acid amide at about 1-5 wt%; zeolite (e.g., $NaAlSiO_4$) at about 10-20 wt%; layered disilicate (e.g., SK56 from Hoechst) at about 10-20 wt%; sodium carbonate at about 3-12 wt%; soluble silicate (e.g., Na_2O $2SiO_2$) at 0-6 wt%; sodium citrate at about 4-8 wt%; sodium percarbonate at about 13-22 wt%; TAED at about 3-8 wt%; poly alpha-1,3-1,6-glucan ether up to about 2 wt%; other polymers (e.g., polycarboxylates and PVP) at about 0-5 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., optical brightener, photobleach, perfume, suds suppressors) at about 0-5 wt%.

15) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising: C12-C18 alkyl sulfate at about 4-8 wt%; alcohol ethoxylate at about 11-15 wt%; soap at about 1-4 wt%; zeolite MAP or zeolite A at about 35-45 wt%; sodium carbonate at about 2-8 wt%; soluble silicate (e.g., Na_2O $2SiO_2$) at 0-4 wt%; sodium percarbonate at about 13-22 wt%; TAED at about 1-8 wt%; poly alpha-1,3-1,6-glucan ether up to about 3 wt%; other polymers (e.g., polycarboxylates and PVP) at about 0-3 wt%; optionally an enzyme(s) (calculated as pure enzyme protein) at about 0.0001-0.1 wt%; and minor ingredients (e.g., optical brightener, phosphonate, perfume) at about 0-3 wt%.

16) Detergent formulations as described in (1)-(15) above, but that contain a stabilized or encapsulated peracid, either as an additional component or as a substitute for an already specified bleach system(s).

17) Detergent compositions as described in (1), (3), (7), (9) and (12) above, but in which perborate is replaced by percarbonate.

18) Detergent compositions as described in (1), (3), (7), (9), (12), (14) and (15) above, but that additionally contain a manganese catalyst. A manganese catalyst, for example, is one of the compounds described by Hage et al. (1994, *Nature* 369:637-639), which is incorporated herein by reference.

19) Detergent compositions formulated as a non-aqueous detergent liquid comprising a liquid non-ionic surfactant (e.g., a linear alkoxylated primary alcohol), a builder system (e.g., phosphate), poly alpha-1,3-1,6-glucan ether, optionally an enzyme(s), and alkali. The detergent may also comprise an anionic surfactant and/or bleach system.

It is believed that numerous commercially available detergent formulations can be adapted to include a poly alpha-1,3-1,6-glucan ether compound. Examples include PUREX® ULTRAPACKS (Henkel), FINISH® QUANTUM (Reckitt Benckiser), CLOROX™ 2 PACKS (Clorox), OXICLEAN MAX FORCE POWER PAKS (Church & Dwight), TIDE® STAIN RELEASE, CASCADE® ACTIONPACS, and TIDE® PODS™ (Procter & Gamble).

Compositions disclosed herein can be in the form of an oral care composition. Examples of oral care compositions include dentifrices, toothpaste, mouth wash, mouth rinse, chewing gum, and edible strips that provide some form of oral care (e.g., treatment or prevention of cavities [dental caries], gingivitis, plaque, tartar, and/or periodontal disease). An oral care composition can also be for treating an “oral surface”, which encompasses any soft or hard surface within the oral cavity including surfaces of the tongue, hard and soft palate, buccal mucosa, gums and dental surfaces. A “dental surface” herein is a surface of a natural tooth or a hard surface of artificial dentition including a crown, cap, filling, bridge, denture, or dental implant, for example.

One or more poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compounds comprised in an oral care composition typically are provided therein as a thickening agent and/or dispersion agent, which may be useful to impart a desired consistency and/or mouth feel to the composition. An oral care

composition herein can comprise about 0.01-15.0 wt% (e.g., ~0.1-10 wt% or ~0.1-5.0 wt%, ~0.1-2.0 wt%) of one or more poly alpha-1,3-1,6-glucan and/or poly alpha-1,3-1,6-glucan ether compounds disclosed herein (e.g., a carboxyalkyl poly alpha-1,3-1,6-glucan ether such as carboxymethyl poly alpha-1,3-1,6-glucan), for example. One or more other thickening or dispersion agents can also be provided in an oral care composition herein, such as a carboxyvinyl polymer, carrageenan (e.g., L-carrageenan), natural gum (e.g., karaya, xanthan, gum arabic, tragacanth), colloidal magnesium aluminum silicate, or colloidal silica, for example.

An oral care composition herein may be a toothpaste or other dentifrice, for example. Such compositions, as well as any other oral care composition herein, can additionally comprise, without limitation, one or more of an anticaries agent, antimicrobial or antibacterial agent, anticalculus or tartar control agent, surfactant, abrasive, pH-modifying agent, foam modulator, humectant, flavorant, sweetener, pigment/colorant, whitening agent, and/or other suitable components. Examples of oral care compositions to which one or more poly alpha-1,3-1,6-glucan ether compounds can be added are disclosed in U.S. Patent Appl. Publ. Nos. 2006/0134025, 2002/0022006 and 2008/0057007, which are incorporated herein by reference.

An anticaries agent herein can be an orally acceptable source of fluoride ions. Suitable sources of fluoride ions include fluoride, monofluorophosphate and fluorosilicate salts as well as amine fluorides, including olaflur (N'-octadecyltrimethylendiamine-N,N,N'- tris(2-ethanol)-dihydrofluoride), for example. An anticaries agent can be present in an amount providing a total of about 100-20000 ppm, about 200-5000 ppm, or about 500-2500 ppm, fluoride ions to the composition, for example. In oral care compositions in which sodium fluoride is the sole source of fluoride ions, an amount of about 0.01-5.0 wt%, about 0.05-1.0 wt%, or about 0.1-0.5 wt%, sodium fluoride can be present in the composition, for example.

An antimicrobial or antibacterial agent suitable for use in an oral care composition herein includes, for example, phenolic compounds (e.g., 4-

allylcatechol; p-hydroxybenzoic acid esters such as benzylparaben, butylparaben, ethylparaben, methylparaben and propylparaben; 2-benzylphenol; butylated hydroxyanisole; butylated hydroxytoluene; capsaicin; carvacrol; creosol; eugenol; guaiacol; halogenated bisphenolics such as hexachlorophene and bromochlorophene; 4-hexylresorcinol; 8-hydroxyquinoline and salts thereof; salicylic acid esters such as menthyl salicylate, methyl salicylate and phenyl salicylate; phenol; pyrocatechol; salicylanilide; thymol; halogenated diphenylether compounds such as triclosan and triclosan monophosphate), copper (II) compounds (e.g., copper (II) chloride, fluoride, sulfate and hydroxide), zinc ion sources (e.g., zinc acetate, citrate, gluconate, glycinate, oxide, and sulfate), phthalic acid and salts thereof (e.g., magnesium monopotassium phthalate), hexetidine, octenidine, sanguinarine, benzalkonium chloride, domiphen bromide, alkylpyridinium chlorides (e.g. cetylpyridinium chloride, tetradecylpyridinium chloride, N-tetradecyl-4-ethylpyridinium chloride), iodine, sulfonamides, bisbiguanides (e.g., alexidine, chlorhexidine, chlorhexidine digluconate), piperidino derivatives (e.g., delmopinol, octapinol), magnolia extract, grapeseed extract, rosemary extract, menthol, geraniol, citral, eucalyptol, antibiotics (e.g., augmentin, amoxicillin, tetracycline, doxycycline, minocycline, metronidazole, neomycin, kanamycin, clindamycin), and/or any antibacterial agents disclosed in U.S. Patent No. 5776435, which is incorporated herein by reference. One or more antimicrobial agents can optionally be present at about 0.01-10 wt% (e.g., 0.1-3 wt%), for example, in the disclosed oral care composition.

An anticalculus or tartar control agent suitable for use in an oral care composition herein includes, for example, phosphates and polyphosphates (e.g., pyrophosphates), polyaminopropanesulfonic acid (AMPS), zinc citrate trihydrate, polypeptides (e.g., polyaspartic and polyglutamic acids), polyolefin sulfonates, polyolefin phosphates, diphosphonates (e.g.,azacycloalkane-2,2-diphosphonates such as azacycloheptane-2,2-diphosphonic acid), N-methyl azacyclopentane-2,3-diphosphonic acid, ethane-1-hydroxy-1,1-diphosphonic acid (EHDP), ethane-1-amino-1,1-diphosphonate, and/or phosphonoalkane carboxylic acids and salts thereof (e.g., their alkali metal and ammonium salts). Useful inorganic phosphate

and polyphosphate salts include, for example, monobasic, dibasic and tribasic sodium phosphates, sodium tripolyphosphate, tetrapolyphosphate, mono-, di-, tri- and tetra-sodium pyrophosphates, disodium dihydrogen pyrophosphate, sodium trimetaphosphate, sodium hexametaphosphate, or any of these in which sodium is replaced by potassium or ammonium. Other useful anticalculus agents in certain embodiments include anionic polycarboxylate polymers (e.g., polymers or copolymers of acrylic acid, methacrylic, and maleic anhydride such as polyvinyl methyl ether/maleic anhydride copolymers). Still other useful anticalculus agents include sequestering agents such as hydroxycarboxylic acids (e.g., citric, fumaric, malic, glutaric and oxalic acids and salts thereof) and aminopolycarboxylic acids (e.g., EDTA). One or more anticalculus or tartar control agents can optionally be present at about 0.01-50 wt% (e.g., about 0.05-25 wt% or about 0.1-15 wt%), for example, in the disclosed oral care composition.

A surfactant suitable for use in an oral care composition herein may be anionic, non-ionic, or amphoteric, for example. Suitable anionic surfactants include, without limitation, water-soluble salts of C_{8-20} alkyl sulfates, sulfonated monoglycerides of C_{8-20} fatty acids, sarcosinates, and taurates. Examples of anionic surfactants include sodium lauryl sulfate, sodium coconut monoglyceride sulfonate, sodium lauryl sarcosinate, sodium lauryl isoethionate, sodium laureth carboxylate and sodium dodecyl benzenesulfonate. Suitable non-ionic surfactants include, without limitation, poloxamers, polyoxyethylene sorbitan esters, fatty alcohol ethoxylates, alkylphenol ethoxylates, tertiary amine oxides, tertiary phosphine oxides, and dialkyl sulfoxides. Suitable amphoteric surfactants include, without limitation, derivatives of C_{8-20} aliphatic secondary and tertiary amines having an anionic group such as a carboxylate, sulfate, sulfonate, phosphate or phosphonate. An example of a suitable amphoteric surfactant is cocoamidopropyl betaine. One or more surfactants are optionally present in a total amount of about 0.01-10 wt% (e.g., about 0.05-5.0 wt% or about 0.1-2.0 wt%), for example, in the disclosed oral care composition.

An abrasive suitable for use in an oral care composition herein may include, for example, silica (e.g., silica gel, hydrated silica, precipitated silica), alumina, insoluble phosphates, calcium carbonate, and resinous abrasives (e.g., a urea-formaldehyde condensation product). Examples of insoluble phosphates useful as abrasives herein are orthophosphates, polymetaphosphates and pyrophosphates, and include dicalcium orthophosphate dihydrate, calcium pyrophosphate, beta-calcium pyrophosphate, tricalcium phosphate, calcium polymetaphosphate and insoluble sodium polymetaphosphate. One or more abrasives are optionally present in a total amount of about 5-70 wt% (e.g., about 10-56 wt% or about 15-30 wt%), for example, in the disclosed oral care composition. The average particle size of an abrasive in certain embodiments is about 0.1-30 microns (e.g., about 1-20 microns or about 5-15 microns).

An oral care composition in certain embodiments may comprise at least one pH-modifying agent. Such agents may be selected to acidify, make more basic, or buffer the pH of a composition to a pH range of about 2-10 (e.g., pH ranging from about 2-8, 3-9, 4-8, 5-7, 6-10, or 7-9). Examples of pH-modifying agents useful herein include, without limitation, carboxylic, phosphoric and sulfonic acids; acid salts (e.g., monosodium citrate, disodium citrate, monosodium malate); alkali metal hydroxides (e.g. sodium hydroxide, carbonates such as sodium carbonate, bicarbonates, sesquicarbonates); borates; silicates; phosphates (e.g., monosodium phosphate, trisodium phosphate, pyrophosphate salts); and imidazole.

A foam modulator suitable for use in an oral care composition herein may be a polyethylene glycol (PEG), for example. High molecular weight PEGs are suitable, including those having an average molecular weight of about 200000-7000000 (e.g., about 500000-5000000 or about 1000000-2500000), for example. One or more PEGs are optionally present in a total amount of about 0.1-10 wt% (e.g. about 0.2-5.0 wt% or about 0.25-2.0 wt%), for example, in the disclosed oral care composition.

An oral care composition in certain embodiments may comprise at least one humectant. A humectant in certain embodiments may be a polyhydric

alcohol such as glycerin, sorbitol, xylitol, or a low molecular weight PEG. Most suitable humectants also may function as a sweetener herein. One or more humectants are optionally present in a total amount of about 1.0-70 wt% (e.g., about 1.0-50 wt%, about 2-25 wt%, or about 5-15 wt%), for example, in the disclosed oral care composition.

A natural or artificial sweetener may optionally be comprised in an oral care composition herein. Examples of suitable sweeteners include dextrose, sucrose, maltose, dextrin, invert sugar, mannose, xylose, ribose, fructose, levulose, galactose, corn syrup (e.g., high fructose corn syrup or corn syrup solids), partially hydrolyzed starch, hydrogenated starch hydrolysate, sorbitol, mannitol, xylitol, maltitol, isomalt, aspartame, neotame, saccharin and salts thereof, dipeptide-based intense sweeteners, and cyclamates. One or more sweeteners are optionally present in a total amount of about 0.005-5.0 wt%, for example, in the disclosed oral care composition.

A natural or artificial flavorant may optionally be comprised in an oral care composition herein. Examples of suitable flavorants include vanillin; sage; marjoram; parsley oil; spearmint oil; cinnamon oil; oil of wintergreen (methylsalicylate); peppermint oil; clove oil; bay oil; anise oil; eucalyptus oil; citrus oils; fruit oils; essences such as those derived from lemon, orange, lime, grapefruit, apricot, banana, grape, apple, strawberry, cherry, or pineapple; bean- and nut-derived flavors such as coffee, cocoa, cola, peanut, or almond; and adsorbed and encapsulated flavorants. Also encompassed within flavorants herein are ingredients that provide fragrance and/or other sensory effect in the mouth, including cooling or warming effects. Such ingredients include, without limitation, menthol, menthyl acetate, menthyl lactate, camphor, eucalyptus oil, eucalyptol, anethole, eugenol, cassia, oxanone, Irisone®, propenyl guaiethol, thymol, linalool, benzaldehyde, cinnamaldehyde, N-ethyl-p-menthan-3-carboxamine, N,2,3-trimethyl-2-isopropylbutanamide, 3-(1-menthoxy)-propane-1,2-diol, cinnamaldehyde glycerol acetal (CGA), and menthone glycerol acetal (MGA). One or more flavorants are optionally present in a total amount of about

0.01-5.0 wt% (e.g., about 0.1-2.5 wt%), for example, in the disclosed oral care composition.

An oral care composition in certain embodiments may comprise at least one bicarbonate salt. Any orally acceptable bicarbonate can be used, including alkali metal bicarbonates such as sodium or potassium bicarbonate, and ammonium bicarbonate, for example. One or more bicarbonate salts are optionally present in a total amount of about 0.1-50 wt% (e.g., about 1-20 wt%), for example, in the disclosed oral care composition.

An oral care composition in certain embodiments may comprise at least one whitening agent and/or colorant. A suitable whitening agent is a peroxide compound such as any of those disclosed in U.S. Patent No. 8540971, which is incorporated herein by reference. Suitable colorants herein include pigments, dyes, lakes and agents imparting a particular luster or reflectivity such as pearling agents, for example. Specific examples of colorants useful herein include talc; mica; magnesium carbonate; calcium carbonate; magnesium silicate; magnesium aluminum silicate; silica; titanium dioxide; zinc oxide; red, yellow, brown and black iron oxides; ferric ammonium ferrocyanide; manganese violet; ultramarine; titaniated mica; and bismuth oxychloride. One or more colorants are optionally present in a total amount of about 0.001-20 wt% (e.g., about 0.01-10 wt% or about 0.1-5.0 wt%), for example, in the disclosed oral care composition.

Additional components that can optionally be included in an oral composition herein include one or more enzymes (above), vitamins, and anti-adhesion agents, for example. Examples of vitamins useful herein include vitamin C, vitamin E, vitamin B5, and folic acid. Examples of suitable anti-adhesion agents include solbrol, ficin, and quorum-sensing inhibitors.

The disclosed invention also concerns a method for increasing the viscosity of an aqueous composition. This method comprises contacting one or more poly alpha-1,3-1,6-glucan ether compounds with the aqueous composition, wherein:

(i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,6 linkages,

(iii) the poly alpha-1,3-1,6-glucan ether compound has a weight average degree of polymerization (DP_w) of at least 1000;

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of the poly alpha-1,3-1,6-glucan ether compound do not consecutively alternate with each other; and

(v) the compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0..

The contacting step in this method results in increasing the viscosity of the aqueous composition. Any hydrocolloid and aqueous solution disclosed herein can be produced using this method.

An aqueous composition herein can be water (e.g., de-ionized water), an aqueous solution, or a hydrocolloid, for example. The viscosity of an aqueous composition before the contacting step, measured at about 20-25 °C, can be about 0-10000 cPs (or any integer between 0-10000 cPs), for example. Since the aqueous composition can be a hydrocolloid or the like in certain embodiments, it should be apparent that the method can be used to increase the viscosity of aqueous compositions that are already viscous.

Contacting a poly alpha-1,3-1,6-glucan ether compound disclosed herein with an aqueous composition increases the viscosity of the aqueous composition in certain embodiments. This increase in viscosity can be an increase of at least about 1%, 10%, 100%, 1000%, 100000%, or 1000000% (or any integer between 1% and 1000000%), for example, compared to the viscosity of the aqueous composition before the contacting step. It should be apparent that very large percent increases in viscosity can be obtained with the disclosed method when the aqueous composition has little to no viscosity before the contacting step.

Contacting a poly alpha-1,3-1,6-glucan ether compound disclosed herein with an aqueous composition increases the shear thinning behavior or the shear

thickening behavior of the aqueous composition in certain embodiments. Thus, a poly alpha-1,3-1,6-glucan ether compound rheologically modifies the aqueous composition in these embodiments. The increase in shear thinning or shear thickening behavior can be an increase of at least about 1%, 10%, 100%, 1000%, 100000%, or 1000000% (or any integer between 1% and 1000000%), for example, compared to the shear thinning or shear thickening behavior of the aqueous composition before the contacting step. It should be apparent that very large percent increases in rheologic modification can be obtained with the disclosed method when the aqueous composition has little to no rheologic behavior before the contacting step.

The contacting step in a method for increasing the viscosity of an aqueous composition can be performed by mixing or dissolving any poly alpha-1,3-1,6-glucan ether compound(s) disclosed herein in the aqueous composition by any means known in the art. For example, mixing or dissolving can be performed manually or with a machine (e.g., industrial mixer or blender, orbital shaker, stir plate, homogenizer, sonicator, bead mill). Mixing or dissolving can comprise a homogenization step in certain embodiments. Homogenization (as well as any other type of mixing) can be performed for about 5 to 60, 5 to 30, 10 to 60, 10 to 30, 5 to 15, or 10 to 15 seconds (or any integer between 5 and 60 seconds), or longer periods of time as necessary to mix a poly alpha-1,3-1,6-glucan ether compound with the aqueous composition. A homogenizer can be used at about 5000 to 30000 rpm, 10000 to 30000 rpm, 15000 to 30000 rpm, 15000 to 25000 rpm, or 20000 rpm (or any integer between 5000 and 30000 rpm), for example. Hydrocolloids and aqueous solutions disclosed herein prepared using a homogenization step can be termed as homogenized hydrocolloids and aqueous solutions.

After a poly alpha-1,3-1,6-glucan ether compound is mixed with or dissolved into an aqueous composition, the resulting aqueous composition may be filtered, or may not be filtered. For example, an aqueous composition prepared with a homogenization step may or may not be filtered.

Certain embodiments of the above method can be used to prepare an aqueous composition disclosed herein, such as a household product (e.g., laundry detergent, fabric softener, dishwasher detergent), personal care product (e.g., a water-containing dentifrice such as toothpaste), or industrial product.

The disclosed invention also concerns a method of treating a material. This method comprises contacting a material with an aqueous composition comprising at least one poly alpha-1,3-1,6-glucan ether compound disclosed herein. A poly alpha-1,3-1,6-glucan ether compound(s) used in this method has the following features: (i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,3 linkages, (ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan ether compound are alpha-1,6 linkages, (iii) the poly alpha-1,3-1,6-glucan ether compound has a weight average degree of polymerization (DP_w) of at least 1000; (iv) the alpha-1,3 linkages and alpha-1,6 linkages of the poly alpha-1,3-1,6-glucan ether compound do not consecutively alternate with each other, and (v) the poly alpha-1,3-1,6-glucan ether compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0.

A material contacted with an aqueous composition in a contacting method herein can comprise a fabric in certain embodiments. A fabric herein can comprise natural fibers, synthetic fibers, semi-synthetic fibers, or any combination thereof. A semi-synthetic fiber herein is produced using naturally occurring material that has been chemically derivatized, an example of which is rayon. Non-limiting examples of fabric types herein include fabrics made of (i) cellulosic fibers such as cotton (e.g., broadcloth, canvas, chambray, chenille, chintz, corduroy, cretonne, damask, denim, flannel, gingham, jacquard, knit, matelassé, oxford, percale, poplin, plissé, sateen, seersucker, sheers, terry cloth, twill, velvet), rayon (e.g., viscose, modal, lyocell), linen, and Tencel®; (ii) proteinaceous fibers such as silk, wool and related mammalian fibers; (iii) synthetic fibers such as polyester, acrylic, nylon, and the like; (iv) long vegetable fibers from jute, flax, ramie, coir, kapok, sisal, henequen, abaca, hemp and sunn; and (v) any combination of a fabric of (i)-(iv). Fabric comprising a combination of

fiber types (e.g., natural and synthetic) include those with both a cotton fiber and polyester, for example. Materials/articles containing one or more fabrics herein include, for example, clothing, curtains, drapes, upholstery, carpeting, bed linens, bath linens, tablecloths, sleeping bags, tents, car interiors, etc. Other materials comprising natural and/or synthetic fibers include, for example, non-woven fabrics, paddings, paper, and foams.

An aqueous composition that is contacted with a fabric can be, for example, a fabric care composition (e.g., laundry detergent, fabric softener). Thus, a treatment method in certain embodiments can be considered a fabric care method or laundry method if employing a fabric care composition therein. A fabric care composition herein can effect one or more of the following fabric care benefits (i.e., surface substantive effects): wrinkle removal, wrinkle reduction, wrinkle resistance, fabric wear reduction, fabric wear resistance, fabric pilling reduction, fabric color maintenance, fabric color fading reduction, fabric color restoration, fabric soiling reduction, fabric soil release, fabric shape retention, fabric smoothness enhancement, anti-redeposition of soil on fabric, anti-greying of laundry, improved fabric hand/handle, and/or fabric shrinkage reduction.

Examples of conditions (e.g., time, temperature, wash/rinse volumes) for conducting a fabric care method or laundry method herein are disclosed in WO1997/003161 and U.S. Patent Nos. 4794661, 4580421 and 5945394, which are incorporated herein by reference. In other examples, a material comprising fabric can be contacted with an aqueous composition herein: (i) for at least about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 120 minutes; (ii) at a temperature of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 °C (e.g., for laundry wash or rinse: a “cold” temperature of about 15-30 °C, a “warm” temperature of about 30-50 °C, a “hot” temperature of about 50-95 °C); (iii) at a pH of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 (e.g., pH range of about 2-12, or about 3-11); (iv) at a salt (e.g., NaCl) concentration of at least about 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, or 4.0 wt%; or any combination of (i)-(iv).

The contacting step in a fabric care method or laundry method can comprise any of washing, soaking, and/or rinsing steps, for example. Contacting a material or fabric in still further embodiments can be performed by any means known in the art, such as dissolving, mixing, shaking, spraying, treating, immersing, flushing, pouring on or in, combining, painting, coating, applying, affixing to, and/or communicating an effective amount of a poly alpha-1,3-1,6-glucan ether compound herein with the fabric or material. In still further embodiments, contacting may be used to treat a fabric to provide a surface substantive effect. As used herein, the term "fabric hand" or "handle" refers to a person's tactile sensory response towards fabric which may be physical, physiological, psychological, social or any combination thereof. In one embodiment, the fabric hand may be measured using a PhabrOmeter® System for measuring relative hand value (available from Nu Cybertek, Inc. Davis, CA) (American Association of Textile Chemists and Colorists (AATCC test method "202-2012, Relative Hand Value of Textiles: Instrumental Method")).

In certain embodiments of treating a material comprising fabric, a poly alpha-1,3-1,6-glucan ether compound component(s) of the aqueous composition adsorbs to the fabric. This feature is believed to render poly alpha-1,3-1,6-glucan ether compounds (e.g., anionic glucan ether compounds such as carboxymethyl poly-alpha-1,3-1,6-glucan) useful as anti-redeposition agents and/or anti-greying agents in fabric care compositions disclosed herein (in addition to their viscosity-modifying effect). An anti-redeposition agent or anti-greying agent herein helps keep soil from redepositing onto clothing in wash water after the soil has been removed. It is further contemplated that adsorption of one or more poly alpha-1,3-1,6-glucan ether compounds herein to a fabric enhances mechanical properties of the fabric.

Adsorption of a poly alpha-1,3-1,6-glucan ether compound to a fabric herein can be measured following the methodology disclosed in the below Examples, for example. Alternatively, adsorption can be measured using a colorimetric technique (e.g., Dubois et al., 1956, *Anal. Chem.* 28:350-356;

Zemljič et al., 2006, *Lenzinger Berichte* 85:68-76; both incorporated herein by reference) or any other method known in the art.

Other materials that can be contacted in the above treatment method include surfaces that can be treated with a dish detergent (e.g., automatic dishwashing detergent or hand dish detergent). Examples of such materials include surfaces of dishes, glasses, pots, pans, baking dishes, utensils and flatware made from ceramic material, china, metal, glass, plastic (e.g., polyethylene, polypropylene, polystyrene, etc.) and wood (collectively referred to herein as "tableware"). Thus, the treatment method in certain embodiments can be considered a dishwashing method or tableware washing method, for example. Examples of conditions (e.g., time, temperature, wash volume) for conducting a dishwashing or tableware washing method herein are disclosed in U.S. Patent No. 8575083, which is incorporated herein by reference. In other examples, a tableware article can be contacted with an aqueous composition herein under a suitable set of conditions such as any of those disclosed above with regard to contacting a fabric-comprising material.

Other materials that can be contacted in the above treatment method include oral surfaces such as any soft or hard surface within the oral cavity including surfaces of the tongue, hard and soft palate, buccal mucosa, gums and dental surfaces (e.g., natural tooth or a hard surface of artificial dentition such as a crown, cap, filling, bridge, denture, or dental implant). Thus, a treatment method in certain embodiments can be considered an oral care method or dental care method, for example. Conditions (e.g., time, temperature) for contacting an oral surface with an aqueous composition herein should be suitable for the intended purpose of making such contact. Other surfaces that can be contacted in a treatment method also include a surface of the integumentary system such as skin, hair or nails.

Thus, certain embodiments of the disclosed invention concern material (e.g., fabric) that comprises a poly alpha-1,3-1,6-glucan ether compound herein. Such material can be produced following a material treatment method as disclosed, for example. A material may comprise a glucan ether compound in

certain embodiments if the compound is adsorbed to, or otherwise in contact with, the surface of the material.

Certain embodiments of a method of treating a material herein further comprise a drying step, in which a material is dried after being contacted with the aqueous composition. A drying step can be performed directly after the contacting step, or following one or more additional steps that might follow the contacting step (e.g., drying of a fabric after being rinsed, in water for example, following a wash in an aqueous composition herein). Drying can be performed by any of several means known in the art, such as air drying (e.g., ~20-25 °C), or at a temperature of at least about 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 170, 175, 180, or 200 °C, for example. A material that has been dried herein typically has less than 3, 2, 1, 0.5, or 0.1 wt% water comprised therein. Fabric is a preferred material for conducting an optional drying step.

An aqueous composition used in a treatment method herein can be any aqueous composition disclosed herein, such as in the above embodiments or in the below Examples. Thus, the poly alpha-1,3-1,6-glucan ether component(s) of an aqueous composition can be any as disclosed herein. Examples of aqueous compositions include detergents (e.g., laundry detergent or dish detergent) and water-containing dentifrices such as toothpaste.

The disclosed invention also concerns a method for producing a poly alpha-1,3-1,6-glucan ether compound. This method comprises: contacting poly alpha-1,3-1,6-glucan in a reaction under alkaline conditions with at least one etherification agent comprising an organic group, wherein the organic group is etherified to the poly alpha-1,3-1,6-glucan thereby producing a poly alpha-1,3-1,6-glucan ether compound. Further regarding this method:

(i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,6 linkages,

(iii) the poly alpha-1,3-1,6-glucan has a weight average degree of polymerization (DP_w) of at least 1000,

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of the poly alpha-1,3-1,6-glucan do not consecutively alternate with each other, and

(v) the poly alpha-1,3-1,6-glucan ether compound has a degree of substitution (DoS) with the organic group of about 0.05 to about 3.0.

A poly alpha-1,3-1,6-glucan ether compound produced by this method can optionally be isolated. This method can be considered to comprise an etherification reaction.

The following steps can be taken to prepare the above etherification reaction. Poly alpha-1,3-1,6-glucan is contacted in a reaction under alkaline conditions with at least one etherification agent comprising an organic group. This step can be performed, for example, by first preparing alkaline conditions by contacting poly alpha-1,3-1,6-glucan with a solvent and one or more alkali hydroxides to provide a mixture (e.g., slurry) or solution. The alkaline conditions of the etherification reaction can thus comprise an alkali hydroxide solution. The pH of the alkaline conditions can be at least about 11.0, 11.2, 11.4, 11.6, 11.8, 12.0, 12.2, 12.4, 12.6, 12.8, or 13.0.

Various alkali hydroxides can be used, such as sodium hydroxide, potassium hydroxide, calcium hydroxide, lithium hydroxide, and/or tetraethylammonium hydroxide. The concentration of alkali hydroxide in a preparation with poly alpha-1,3-1,6-glucan and a solvent can be from about 1-70 wt%, 5-50 wt%, 5-10 wt%, 10-50 wt%, 10-40 wt%, or 10-30 wt% (or any integer between 1 and 70 wt%). Alternatively, the concentration of alkali hydroxide such as sodium hydroxide can be at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 wt%. An alkali hydroxide used to prepare alkaline conditions may be in a completely aqueous solution or an aqueous solution comprising one or more water-soluble organic solvents such as ethanol or isopropanol. Alternatively, an alkali hydroxide can be added as a solid to provide alkaline conditions.

Various organic solvents that can optionally be included or used as the main solvent when preparing the etherification reaction include alcohols, acetone, dioxane, isopropanol and toluene, for example; none of these solvents

dissolve poly alpha-1,3-1,6-glucan. Toluene or isopropanol can be used in certain embodiments. An organic solvent can be added before or after addition of alkali hydroxide. The concentration of an organic solvent (e.g., isopropanol or toluene) in a preparation comprising poly alpha-1,3-1,6-glucan and an alkali hydroxide can be at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, or 90 wt% (or any integer between 10 and 90 wt%).

Alternatively, solvents that can dissolve poly alpha-1,3-1,6-glucan can be used when preparing the etherification reaction. These solvents include, but are not limited to, lithium chloride(LiCl)/N,N-dimethyl-acetamide (DMAc), SO_2/diethylamine (DEA)/dimethyl sulfoxide (DMSO), LiCl/1,3-dimethy-2-imidazolidinone (DMI), N,N-dimethylformamide (DMF)/N_2O_4, DMSO/tetrabutyl-ammonium fluoride trihydrate (TBAF), N-methylmorpholine-N-oxide (NMMO), $Ni(tren)(OH)_2$ [tren¼tris(2-aminoethyl)amine] aqueous solutions and melts of $LiClO_4 \cdot 3H_2O$, NaOH/urea aqueous solutions, aqueous sodium hydroxide, aqueous potassium hydroxide, formic acid, and ionic liquids.

Poly alpha-1,3-1,6-glucan can be contacted with a solvent and one or more alkali hydroxides by mixing. Such mixing can be performed during or after adding these components with each other. Mixing can be performed by manual mixing, mixing using an overhead mixer, using a magnetic stir bar, or shaking, for example. In certain embodiments, poly alpha-1,3-1,6-glucan can first be mixed in water or an aqueous solution before it is mixed with a solvent and/or alkali hydroxide.

After contacting poly alpha-1,3-1,6-glucan, solvent, and one or more alkali hydroxides with each other, the resulting composition can optionally be maintained at ambient temperature for up to 14 days. The term “ambient temperature” as used herein refers to a temperature between about 15-30 °C or 20-25 °C (or any integer between 15 and 30 °C). Alternatively, the composition can be heated with or without reflux at a temperature from about 30 °C to about 150 °C (or any integer between 30 and 150 °C) for up to about 48 hours. The composition in certain embodiments can be heated at about 55 °C for about 30 minutes or about 60 minutes. Thus, a composition obtained from mixing a poly

alpha-1,3-1,6-glucan, solvent, and one or more alkali hydroxides with each other can be heated at about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 °C for about 30-90 minutes.

After contacting poly alpha-1,3-1,6-glucan, solvent, and one or more alkali hydroxides with each other, the resulting composition can optionally be filtered (with or without applying a temperature treatment step). Such filtration can be performed using a funnel, centrifuge, press filter, or any other method and/or equipment known in the art that allows removal of liquids from solids. Though filtration would remove much of the alkali hydroxide, the filtered poly alpha-1,3-1,6-glucan would remain alkaline (i.e., mercerized poly alpha-1,3-1,6-glucan), thereby providing alkaline conditions.

An etherification agent comprising an organic group can be contacted with poly alpha-1,3-1,6-glucan in a reaction under alkaline conditions in a method herein of producing poly alpha-1,3-1,6-glucan ether compounds. For example, an etherification agent can be added to a composition prepared by contacting poly alpha-1,3-1,6-glucan, solvent, and one or more alkali hydroxides with each other as described above. Alternatively, an etherification agent can be included when preparing the alkaline conditions (e.g., an etherification agent can be mixed with poly alpha-1,3-1,6-glucan and solvent before mixing with alkali hydroxide).

An etherification agent herein can refer to an agent that can be used to etherify one or more hydroxyl groups of glucose monomeric units of poly alpha-1,3-1,6-glucan with an organic group as disclosed herein. Examples of organic groups include alkyl groups, hydroxy alkyl groups, and carboxy alkyl groups. One or more etherification agents may be used in the reaction.

Etherification agents suitable for preparing an alkyl poly alpha-1,3-1,6-glucan ether compound include, for example, dialkyl sulfates, dialkyl carbonates, alkyl halides (e.g., alkyl chloride), iodoalkanes, alkyl triflates (alkyl trifluoromethanesulfonates) and alkyl fluorosulfonates. Thus, examples of etherification agents for producing methyl poly alpha-1,3-1,6-glucan ethers include dimethyl sulfate, dimethyl carbonate, methyl chloride, iodomethane,

methyl triflate and methyl fluorosulfonate. Examples of etherification agents for producing ethyl poly alpha-1,3-1,6-glucan ethers include diethyl sulfate, diethyl carbonate, ethyl chloride, iodoethane, ethyl triflate and ethyl fluorosulfonate. Examples of etherification agents for producing propyl poly alpha-1,3-1,6-glucan ethers include dipropyl sulfate, dipropyl carbonate, propyl chloride, iodopropane, propyl triflate and propyl fluorosulfonate. Examples of etherification agents for producing butyl poly alpha-1,3-1,6-glucan ethers include dibutyl sulfate, dibutyl carbonate, butyl chloride, iodobutane and butyl triflate.

Etherification agents suitable for preparing a hydroxyalkyl poly alpha-1,3-1,6-glucan ether compound include, for example, alkylene oxides such as ethylene oxide, propylene oxide (e.g., 1,2-propylene oxide), butylene oxide (e.g., 1,2-butylene oxide; 2,3-butylene oxide; 1,4-butylene oxide), or combinations thereof. As examples, propylene oxide can be used as an etherification agent for preparing hydroxypropyl poly alpha-1,3-1,6-glucan, and ethylene oxide can be used as an etherification agent for preparing hydroxyethyl poly alpha-1,3-1,6-glucan. Alternatively, hydroxyalkyl halides (e.g., hydroxyalkyl chloride) can be used as etherification agents for preparing hydroxyalkyl poly alpha-1,3-1,6-glucan. Examples of hydroxyalkyl halides include hydroxyethyl halide, hydroxypropyl halide (e.g., 2-hydroxypropyl chloride, 3-hydroxypropyl chloride) and hydroxybutyl halide. Alternatively, alkylene chlorohydrins can be used as etherification agents for preparing hydroxyalkyl poly alpha-1,3-1,6-glucan. Alkylene chlorohydrins that can be used include, but are not limited to, ethylene chlorohydrin, propylene chlorohydrin, butylene chlorohydrin, or combinations of these.

Etherification agents suitable for preparing a dihydroxyalkyl poly alpha-1,3-1,6-glucan ether compound include dihydroxyalkyl halides (e.g., dihydroxyalkyl chloride) such as dihydroxyethyl halide, dihydroxypropyl halide (e.g., 2,3-dihydroxypropyl chloride [i.e., 3-chloro-1,2-propanediol]), or dihydroxybutyl halide, for example. 2,3-dihydroxypropyl chloride can be used to prepare dihydroxypropyl poly alpha-1,3-1,6-glucan, for example.

Etherification agents suitable for preparing a carboxyalkyl poly alpha-1,3-1,6-glucan ether compound may include haloalkylates (e.g., chloroalkylate). Examples of haloalkylates include haloacetate (e.g., chloroacetate), 3-halopropionate (e.g., 3-chloropropionate) and 4-halobutyrate (e.g., 4-chlorobutyrate). For example, chloroacetate (monochloroacetate) (e.g., sodium chloroacetate or chloroacetic acid) can be used as an etherification agent to prepare carboxymethyl poly alpha-1,3-1,6-glucan.

An etherification agent herein can alternatively comprise a positively charged organic group.

An etherification agent in certain embodiments can etherify poly alpha-1,3-1,6-glucan with a positively charged organic group, where the carbon chain of the positively charged organic group only has a substitution with a positively charged group (e.g., substituted ammonium group such as trimethylammonium). Examples of such etherification agents include dialkyl sulfates, dialkyl carbonates, alkyl halides (e.g., alkyl chloride), iodoalkanes, alkyl triflates (alkyl trifluoromethanesulfonates) and alkyl fluorosulfonates, where the alkyl group(s) of each of these agents has one or more substitutions with a positively charged group (e.g., substituted ammonium group such as trimethylammonium). Other examples of such etherification agents include dimethyl sulfate, dimethyl carbonate, methyl chloride, iodomethane, methyl triflate and methyl fluorosulfonate, where the methyl group(s) of each of these agents has a substitution with a positively charged group (e.g., substituted ammonium group such as trimethylammonium). Other examples of such etherification agents include diethyl sulfate, diethyl carbonate, ethyl chloride, iodoethane, ethyl triflate and ethyl fluorosulfonate, where the ethyl group(s) of each of these agents has a substitution with a positively charged group (e.g., substituted ammonium group such as trimethylammonium). Other examples of such etherification agents include dipropyl sulfate, dipropyl carbonate, propyl chloride, iodopropane, propyl triflate and propyl fluorosulfonate, where the propyl group(s) of each of these agents has one or more substitutions with a positively charged group (e.g., substituted ammonium group such as trimethylammonium). Other examples of

such etherification agents include dibutyl sulfate, dibutyl carbonate, butyl chloride, iodobutane and butyl triflate, where the butyl group(s) of each of these agents has one or more substitutions with a positively charged group (e.g., substituted ammonium group such as trimethylammonium).

An etherification agent alternatively may be one that can etherify poly alpha-1,3-1,6-glucan with a positively charged organic group, where the carbon chain of the positively charged organic group has a substitution (e.g., hydroxyl group) in addition to a substitution with a positively charged group (e.g., substituted ammonium group such as trimethylammonium). Examples of such etherification agents include hydroxyalkyl halides (e.g., hydroxyalkyl chloride) such as hydroxypropyl halide and hydroxybutyl halide, where a terminal carbon of each of these agents has a substitution with a positively charged group (e.g., substituted ammonium group such as trimethylammonium); an example is 3-chloro-2-hydroxypropyl-trimethylammonium. Other examples of such etherification agents include alkylene oxides such as propylene oxide (e.g., 1,2-propylene oxide) and butylene oxide (e.g., 1,2-butylene oxide; 2,3-butylene oxide), where a terminal carbon of each of these agents has a substitution with a positively charged group (e.g., substituted ammonium group such as trimethylammonium).

A substituted ammonium group comprised in any of the foregoing etherification agent examples can be a primary, secondary, tertiary, or quaternary ammonium group. Examples of secondary, tertiary and quaternary ammonium groups are represented in structure I, where R_2, R_3 and R_4 each independently represent a hydrogen atom or an alkyl group such as a methyl, ethyl, propyl, or butyl group.

Etherification agents herein typically can be provided as a fluoride, chloride, bromide, or iodide salt (where each of the foregoing halides serve as an anion).

When producing a poly alpha-1,3-1,6-glucan ether compound with two or more different organic groups, two or more different etherification agents would be used, accordingly. For example, both an alkylene oxide and an alkyl chloride

could be used as etherification agents to produce an alkyl hydroxyalkyl poly alpha-1,3-1,6-glucan ether. Any of the etherification agents disclosed herein may therefore be combined to produce poly alpha-1,3-1,6-glucan ether compounds with two or more different organic groups. Such two or more etherification agents may be used in the reaction at the same time, or may be used sequentially in the reaction. When used sequentially, any of the temperature-treatment (e.g., heating) steps disclosed below may optionally be used between each addition. One may choose sequential introduction of etherification agents in order to control the desired DoS of each organic group. In general, a particular etherification agent would be used first if the organic group it forms in the ether product is desired at a higher DoS compared to the DoS of another organic group to be added.

The amount of etherification agent to be contacted with poly alpha-1,3-1,6-glucan in a reaction under alkaline conditions can be determined based on the DoS required in the poly alpha-1,3-1,6-glucan ether compound being produced. The amount of ether substitution groups on each glucose monomeric unit in poly alpha-1,3-1,6-glucan ether compounds produced herein can be determined using nuclear magnetic resonance (NMR) spectroscopy. The molar substitution (MS) value for poly alpha-1,3-1,6-glucan has no upper limit. In general, an etherification agent can be used in a quantity of at least about 0.05 mole per mole of poly alpha-1,3-1,6-glucan. There is no upper limit to the quantity of etherification agent that can be used.

Reactions for producing poly alpha-1,3-1,6-glucan ether compounds herein can optionally be carried out in a pressure vessel such as a Parr reactor, an autoclave, a shaker tube or any other pressure vessel well known in the art.

A reaction herein can optionally be heated following the step of contacting poly alpha-1,3-1,6-glucan with an etherification agent under alkaline conditions. The reaction temperatures and time of applying such temperatures can be varied within wide limits. For example, a reaction can optionally be maintained at ambient temperature for up to 14 days. Alternatively, a reaction can be heated, with or without reflux, between about 25 °C to about 200 °C (or any integer

between 25 and 200 °C). Reaction time can be varied correspondingly: more time at a low temperature and less time at a high temperature.

In certain embodiments of producing carboxymethyl poly alpha-1,3-1,6-glucan, a reaction can be heated to about 55 °C for about 3 hours. Thus, a reaction for preparing a carboxyalkyl poly alpha-1,3-1,6-glucan herein can be heated to about 50 °C to about 60 °C (or any integer between 50 and 60 °C) for about 2 hours to about 5 hours, for example. Etherification agents such as a haloacetate (e.g., monochloroacetate) may be used in these embodiments, for example.

Optionally, an etherification reaction herein can be maintained under an inert gas, with or without heating. As used herein, the term "inert gas" refers to a gas which does not undergo chemical reactions under a set of given conditions, such as those disclosed for preparing a reaction herein.

All of the components of the reactions disclosed herein can be mixed together at the same time and brought to the desired reaction temperature, whereupon the temperature is maintained with or without stirring until the desired poly alpha-1,3-1,6-glucan ether compound is formed. Alternatively, the mixed components can be left at ambient temperature as described above.

Following etherification, the pH of a reaction can be neutralized. Neutralization of a reaction can be performed using one or more acids. The term "neutral pH" as used herein, refers to a pH that is neither substantially acidic or basic (e.g., a pH of about 6-8, or about 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, or 8.0). Various acids that can be used for this purpose include, but are not limited to, sulfuric, acetic (e.g., glacial acetic), hydrochloric, nitric, any mineral (inorganic) acid, any organic acid, or any combination of these acids.

A poly alpha-1,3-1,6-glucan ether compound produced in a reaction herein can optionally be washed one or more times with a liquid that does not readily dissolve the compound. For example, poly alpha-1,3-1,6-glucan ether can typically be washed with alcohol, acetone, aromatics, or any combination of these, depending on the solubility of the ether compound therein (where lack of solubility is desirable for washing). In general, a solvent comprising an organic

solvent such as alcohol is preferred for washing a poly alpha-1,3-1,6-glucan ether. A poly alpha-1,3-1,6-glucan ether product can be washed one or more times with an aqueous solution containing methanol or ethanol, for example. For example, 70-95 wt% ethanol can be used to wash the product. A poly alpha-1,3-1,6-glucan ether product can be washed with a methanol:acetone (e.g., 60:40) solution in another embodiment.

A poly alpha-1,3-1,6-glucan ether produced in the disclosed reaction can be isolated. This step can be performed before or after neutralization and/or washing steps using a funnel, centrifuge, press filter, or any other method or equipment known in the art that allows removal of liquids from solids. An isolated poly alpha-1,3-1,6-glucan ether product can be dried using any method known in the art, such as vacuum drying, air drying, or freeze drying.

Any of the above etherification reactions can be repeated using a poly alpha-1,3-1,6-glucan ether product as the starting material for further modification. This approach may be suitable for increasing the DoS of an organic group, and/or adding one or more different organic groups to the ether product.

The structure, molecular weight and DoS of a poly alpha-1,3-1,6-glucan ether product can be confirmed using various physiochemical analyses known in the art such as NMR spectroscopy and size exclusion chromatography (SEC).

Any of the embodiments of poly alpha-1,3-1,6-glucan described above can be used in an etherification reaction herein. For example, the poly alpha-1,3-1,6-glucan used in an etherification reaction herein can be a product of a glucosyltransferase enzyme comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10. Alternatively, the glucosyltransferase enzyme can comprise an amino acid sequence that is at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, or 100% identical to, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

Poly alpha-1,3-1,6-glucan used for preparing poly alpha-1,3-1,6-glucan ether compounds herein can be enzymatically produced from sucrose using one

or more glucosyltransferase (gtf) enzymes. The poly alpha-1,3-1,6-glucan product of this enzymatic reaction can be purified before using it to prepare an ether. Alternatively, a poly alpha-1,3-1,6-glucan product of a gtf reaction can be used with little or no processing for preparing poly alpha-1,3-1,6-glucan ether compounds.

A poly alpha-1,3-1,6-glucan slurry can be used directly in any of the above processes for producing a poly alpha-1,3-1,6-glucan ether compound disclosed herein. As used herein, a "poly alpha-1,3-1,6-glucan slurry" refers to a mixture comprising the components of a gtf enzymatic reaction. A gtf enzymatic reaction can include, in addition to poly alpha-1,3-1,6-glucan itself, various components such as sucrose, one or more gtf enzymes, glucose, fructose, leucrose, buffer, FermaSure®, soluble oligosaccharides, oligosaccharide primers, bacterial enzyme extract components, borates, sodium hydroxide, hydrochloric acid, cell lysate, proteins and/or nucleic acids. Minimally, the components of a gtf enzymatic reaction can include, in addition to poly alpha-1,3-1,6-glucan itself, sucrose, one or more gtf enzymes, glucose and fructose, for example. In another example, the components of a gtf enzymatic reaction can include, in addition to poly alpha-1,3-1,6-glucan itself, sucrose, one or more gtf enzymes, glucose, fructose, leucrose and soluble oligosaccharides (and optionally bacterial enzyme extract components). It should be apparent that poly alpha-1,3-1,6-glucan, when in a slurry as disclosed herein, has not been purified or washed. It should also be apparent that a slurry represents a gtf enzymatic reaction that is complete or for which an observable amount of poly alpha-1,3-1,6-glucan has been produced, which forms a solid since it is insoluble in the aqueous reaction milieu (pH of 5-7, for example). A poly alpha-1,3-1,6-glucan slurry can be prepared by setting up a gtf reaction as disclosed herein.

Alternatively, a wet cake of poly alpha-1,3-1,6-glucan can be used directly in any of the above processes for producing a poly alpha-1,3-1,6-glucan ether compound disclosed herein. A "wet cake of poly alpha-1,3-1,6-glucan" as used herein refers to poly alpha-1,3-1,6-glucan that has been separated (e.g., filtered) from a slurry and washed with water or an aqueous solution. A wet cake can be

washed at least 1, 2, 3, 4, 5, or more times, for example. The poly alpha-1,3-1,6-glucan is not dried when preparing a wet cake. A wet cake is termed as "wet" given the retention of water by the washed poly alpha-1,3-1,6-glucan.

A wet cake of poly alpha-1,3-1,6-glucan can be prepared using any device known in the art for separating solids from liquids, such as a filter or centrifuge. For example, poly alpha-1,3-1,6-glucan solids in a slurry can be collected on a funnel using a mesh screen over filter paper. Filtered wet cake can be resuspended in water (e.g., deionized water) and filtered one or more times to remove soluble components of the slurry such as sucrose, fructose and leucrose. As another example for preparing a wet cake, poly alpha-1,3-1,6-glucan solids from a slurry can be collected as a pellet via centrifugation, resuspended in water (e.g., deionized water), and re-pelleted and resuspended one or more additional times.

Non-limiting examples of compositions and methods disclosed herein include:

1. A composition comprising poly alpha-1,3-1,6-glucan, wherein
   (i) at least 30% of the glycosidic linkages of the glucan are alpha-1,3 linkages,
   (ii) at least 30% of the glycosidic linkages of the glucan are alpha-1,6 linkages,
   (iii) the glucan has a weight average degree of polymerization (DP_w) of at least 1000; and
   (iv) the alpha-1,3 linkages and alpha-1,6 linkages of the glucan do not consecutively alternate with each other.
2. The composition of embodiment 1, wherein at least 60% of the glycosidic linkages of the glucan are alpha-1,6 linkages.
3. The composition of embodiment 1 or 2, wherein the DP_w of the glucan is at least 10000.
4. The composition of embodiment 1, 2, or 3, wherein the glucan is a product of a glucosyltransferase enzyme comprising an amino acid sequence that

is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

5. A composition comprising a poly alpha-1,3-1,6-glucan ether compound, wherein:

(i) at least 30% of the glycosidic linkages of the compound are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of the compound are alpha-1,6 linkages,

(iii) the compound has a weight average degree of polymerization (DP_w) of at least 1000,

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of the compound do not consecutively alternate with each other, and

(v) the compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0.

6. The composition of embodiment 5, wherein at least 60% of the glycosidic linkages of the compound are alpha-1,6 linkages.

7. The composition of embodiment 5 or 6, wherein at least one organic group is selected from the group consisting of carboxy alkyl group, hydroxy alkyl group, and alkyl group.

8. The composition of embodiment 7, wherein at least one organic group is selected from the group consisting of carboxymethyl, hydroxypropyl, dihydroxypropyl, hydroxyethyl, methyl, and ethyl group.

9. The composition of embodiment 5 or 6, wherein at least one organic group is a positively charged organic group.

10. The composition of embodiment 5, 6, 7, 8, or 9, wherein the poly alpha-1,3-1,6-glucan from which the compound is derived is a product of a glucosyltransferase enzyme comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

11. The composition of embodiment 5, 6, 7, 8, or 9, wherein the composition is a hydrocolloid or aqueous solution having a viscosity of at least about 10 cPs.

12. The composition of embodiment 11, wherein the hydrocolloid or aqueous solution is in the form of a personal care product, pharmaceutical product, food product, household product, or industrial product.

13. A method of producing a poly alpha-1,3-1,6-glucan ether compound, the method comprising:

(a) contacting poly alpha-1,3-1,6-glucan in a reaction under alkaline conditions with at least one etherification agent comprising an organic group, wherein at least one organic group is etherified to the poly alpha-1,3-1,6-glucan thereby producing a poly alpha-1,3-1,6-glucan ether compound,

wherein

(i) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of the poly alpha-1,3-1,6-glucan are alpha-1,6 linkages,

(iii) the poly alpha-1,3-1,6-glucan has a weight average degree of polymerization (DP_w) of at least 1000,

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of the poly alpha-1,3-1,6-glucan do not consecutively alternate with each other, and

(v) the compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0; and

(b) optionally, isolating the poly alpha-1,3-1,6-glucan ether compound produced in step (a).

14. The method of embodiment 13, wherein the alkaline conditions comprise an alkali hydroxide solution.

15. A method for increasing the viscosity of an aqueous composition, the method comprising:

contacting a poly alpha-1,3-1,6-glucan ether compound with the aqueous composition, wherein the viscosity of the aqueous composition is increased by the compound,

wherein

(i) at least 30% of the glycosidic linkages of the compound are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of the compound are alpha-1,6 linkages,

(iii) the compound has a weight average degree of polymerization (DP_w) of at least 1000,

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of the compound do not consecutively alternate with each other, and

(v) the compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0.

16. A method of treating a material, the method comprising:

contacting a material with an aqueous composition comprising a poly alpha-1,3-1,6-glucan ether compound, wherein:

(i) at least 30% of the glycosidic linkages of the compound are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of the compound are alpha-1,6 linkages,

(iii) the compound has a weight average degree of polymerization (DP_w) of at least 1000,

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of the compound do not consecutively alternate with each other, and

(v) the compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0.

EXAMPLES

The disclosed invention is further defined in Examples 1-8 provided below. It should be understood that these Examples, while indicating certain preferred aspects of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various uses and conditions.

Abbreviations

The meanings of some of the abbreviations used herein are as follows: "g" means gram(s), "h" means hour(s), "mL" means milliliter(s), "psi" means pound(s) per square inch, "wt%" means weight percentage, "μm" means micrometer(s), "°C" means degrees Celsius, "mg" means milligram(s), "mm" means millimeter(s), "μL" means microliter(s), "mmol" means millimole(s), "min" means minute(s), "mol%" means mole percent, "M" means molar, "mM" means millimolar, "N" means normal, "rpm" means revolutions per minute, "w/v" means weight for volume, "MPa" means megaPascal(s), "LB means Luria broth, "nm means nanometer(s), "OD" means optical density, "IPTG" means isopropyl-beta-D-thio-galactoside, "x*g*" means gravitational force, "SDS-PAGE" means sodium dodecyl sulfate polyacrylamide electrophoresis, "DTT" means dithiothreitol, "BCA" means bicinchoninic acid, "DMAc" means N,N'-dimethyl acetamide, "DMSO" means dimethylsulfoxide, "NMR" means nuclear magnetic resonance, "SEC" means size exclusion chromatography, "DI water" means deionized water.

Materials

T10 dextran (D9260), IPTG, (cat#I6758), triphenyltetrazolium chloride, and BCA protein assay kit/reagents were obtained from the Sigma Co. (St. Louis, MO). BELLCO spin flasks were from the Bellco Co. (Vineland, NJ). LB medium was from Becton, Dickinson and Company (Franklin Lakes, NJ). Suppressor 7153 antifoam was obtained from Cognis Corporation (Cincinnati, OH). All other chemicals were obtained from commonly used suppliers of such chemicals.

Seed Medium

The seed medium used to grow starter cultures for the fermenters contained: yeast extract (AMBEREX 695, 5.0 grams per liter, g/L), K_2HPO_4 (10.0 g/L), KH_2PO_4 (7.0 g/L), sodium citrate dihydrate (1.0 g/L), $(NH_4)_2SO_4$ (4.0 g/L), $MgSO_4$ heptahydrate (1.0 g/L) and ferric ammonium citrate (0.10 g/L). The pH of the medium was adjusted to 6.8 using either 5N NaOH or H_2SO_4 and the medium was sterilized in the flask. Post-sterilization additions included glucose (20 mL/L of a 50% w/w solution) and ampicillin (4 mL/L of a 25 mg/mL stock solution).

Fermenter Medium

The growth medium used in the fermenter contained: KH_2PO_4 (3.50 g/L), $FeSO_4$ heptahydrate (0.05 g/L), $MgSO_4$ heptahydrate (2.0 g/L), sodium citrate dihydrate (1.90 g/L), yeast extract (AMBEREX 695, 5.0 g/L), Suppressor 7153 antifoam (0.25 mL/L), NaCl (1.0 g/L), $CaCl_2$ dihydrate (10 g/L), and NIT trace elements solution (10 mL/L). The NIT trace elements solution contained citric acid monohydrate (10 g/L), $MnSO_4$ hydrate (2 g/L), NaCl (2 g/L), $FeSO_4$ heptahydrate (0.5 g/L), $ZnSO_4$ heptahydrate (0.2 g/L), $CuSO_4$ pentahydrate (0.02 g/L) and $NaMoO_4$ dihydrate (0.02 g/L). Post-sterilization additions included glucose (12.5 g/L of a 50% w/w solution) and ampicillin (4 mL/L of a 25 mg/mL stock solution).

GENERAL METHODS

Production of Recombinant Glucosyltransferase (gtf) Enzymes in Fermentation

Production of a recombinant gtf enzyme in a fermenter was initiated by preparing a pre-seed culture of an *E. coli* strain expressing the gtf enzyme. A 10-mL aliquot of seed medium was added into a 125-mL disposable baffled flask and inoculated with a 1.0-mL aliquot of the *E. coli* strain in 20% glycerol. The culture was allowed to grow at 37 °C while shaking at 300 rpm for 3 hours.

A seed culture, which was used for starting growth for gtf fermentation, was prepared by charging a 2-L shake flask with 0.5 L of seed medium. 1.0 mL of the pre-seed culture was aseptically transferred into 0.5-L seed medium in the flask and cultivated at 37 °C and 300 rpm for 5 hours. The seed culture was

transferred at an optical density 550 nm (OD_{550}) > 2 to a 14-L fermenter (Braun, Perth Amboy, NJ) containing 8 L of fermenter medium at 37 °C.

The *E. coli* strain was allowed to grow in the fermenter medium. Glucose (50% w/w glucose solution containing 1% w/w $MgSO_4 \cdot 7H_2O$) was fed to this culture when its glucose concentration decreased to 0.5 g/L. The glucose feed was started at 0.36 grams feed per minute (g feed/min) and increased progressively each hour to 0.42, 0.49, 0.57, 0.66, 0.77, 0.90, 1.04, 1.21, 1.41 1.63, 1.92, and 2.2 g feed/min, respectively. The feed rate remained constant afterwards. Glucose concentration in the medium was monitored using an YSI glucose analyzer (YSI, Yellow Springs, OH). When glucose concentration exceeded 0.1 g/L, the feed rate was decreased or stopped temporarily. Induction of gtf enzyme expression, which was performed when cells reached an OD_{550} of 70, was initiated by adding 9 mL of 0.5 M IPTG. The dissolved oxygen (DO) concentration was controlled at 25% of air saturation. The DO was controlled first by impeller agitation rate (400 to 1200 rpm) and later by aeration rate (2 to 10 standard liters per minute, slpm). Culture pH was controlled at 6.8 using NH_4OH (14.5% w/v) and H_2SO_4 (20% w/v). Back pressure was maintained at 0.5 bars. At various intervals (20, 25 and 30 hours), 5 mL of Suppressor 7153 antifoam was added to the fermenter to suppress foaming. Cells were harvested by centrifugation 8 hours post IPTG addition and were stored at -80 °C as a cell paste.

The cell paste obtained from fermentation for each gtf enzyme was suspended at 150 g/L in 50 mM potassium phosphate buffer, pH 7.2, to prepare a slurry. The slurry was homogenized at 12,000 psi (Rannie-type machine, APV-1000 or APV 16.56) and the homogenate chilled to 4 °C. With moderately vigorous stirring, 50 g of a floc solution (Sigma Aldrich no. 409138, 5% in 50 mM sodium phosphate buffer, pH 7.0) was added per liter of cell homogenate. Agitation was reduced to light stirring for 15 minutes. The cell homogenate was then clarified by centrifugation at 4500 rpm for 3 hours at 5-10 °C. Supernatant, containing gtf enzyme, was concentrated (approximately 5X) with a 30 kiloDalton (kDa) cut-off membrane to render a gtf extract.

Determination of Gtf Enzymatic Activity

Gtf enzyme activity was confirmed by measuring the production of reducing sugars (fructose and glucose) in a gtf reaction solution. A reaction solution was prepared by adding a gtf extract (prepared as above) to a mixture containing sucrose (50 g/L), potassium phosphate buffer (pH 6.5, 50 mM), and dextran T10 (1 mg/mL); the gtf extract was added to 5% by volume. The reaction solution was then incubated at 22-25 °C for 24-30 hours, after which it was centrifuged. Supernatant (0.01 mL) was added to a mixture containing 1 N NaOH and 0.1% triphenyltetrazolium chloride (Sigma-Aldrich). The mixture was incubated for five minutes after which its OD_{480nm} was determined using an ULTROSPEC spectrophotometer (Pharmacia LKB, New York, NY) to gauge the presence of the reducing sugars fructose and glucose.

Determination of Glycosidic Linkages

Glycosidic linkages in glucan products synthesized by a gtf enzyme were determined by ^{13}C NMR (nuclear magnetic resonance) or ^{1}H NMR.

For ^{13}C NMR, dry glucan polymer (25-30 mg) was dissolved in 1 mL of deuterated DMSO containing 3% by weight of LiCl with stirring at 50 °C. Using a glass pipet, 0.8 mL of the solution was transferred into a 5-mm NMR tube. A quantitative ^{13}C NMR spectrum was acquired using a Bruker Avance 500-MHz NMR spectrometer (Billerica, MA) equipped with a CPDUL cryoprobe at a spectral frequency of 125.76 MHz, using a spectral window of 26041.7 Hz. An inverse-gated decoupling pulse sequence using waltz decoupling was used with an acquisition time of 0.629 second, an inter-pulse delay of 5 seconds, and 6000 pulses. The time domain data was transformed using an exponential multiplication of 2.0 Hz.

For ^{1}H NMR, approximately 20 mg of a glucan polymer sample was weighed into a vial on an analytical balance. The vial was removed from the balance and 0.8 mL of deuterated DMSO (DMSO-d6), containing 3% by weight of LiCl, was added to the vial. The mixture was stirred with a magnetic stir bar and warmed to 90 °C until the glucan sample dissolved. The solution was allowed to cool to room temperature. While stirring at room temperature, 0.2 mL

of a 20% by volume solution of trifluoroacetic acid (TFA) in DMSO-d6 was added to the polymer solution. The TFA was added in order to move all hydroxyl proton signals out of the region of the spectrum where carbohydrate ring proton signals occur. A portion, 0.8 mL, of the final solution was transferred, using a glass pipet, into a 5-mm NMR tube. A quantitative ^{1}H NMR spectrum was acquired using an NMR spectrometer with a proton frequency of 500 MHz or greater. The spectrum was acquired using a spectral window of 11.0 ppm and a transmitter offset of 5.5 ppm. A 90° pulse was applied for 32 pulses with an inter-pulse delay of 10 seconds and an acquisition time of 1.5 seconds. The time domain data were transformed using an exponential multiplication of 0.15 Hz.

Determination of Weight Average Degree of Polymerization (DP_w)

The DP_w of a glucan product synthesized by a gtf enzyme was determined by SEC. Dry glucan polymer was dissolved in DMAc and 5% LiCl (0.5 mg/mL) with shaking overnight at 100 °C. The SEC system used was an Alliance™ 2695 separation module from Waters Corporation (Milford, MA) coupled with three on-line detectors: a differential refractometer 2410 from Waters, a multiangle light scattering photometer Heleos™ 8+ from Wyatt Technologies (Santa Barbara, CA), and a differential capillary viscometer ViscoStar™ from Wyatt. The columns used for SEC were four styrene-divinyl benzene columns from Shodex (Japan) and two linear KD-806M, KD-802 and KD-801 columns to improve resolution at the low molecular weight region of a polymer distribution. The mobile phase was DMAc with 0.11% LiCl. The chromatographic conditions used were 50 °C in the column and detector compartments, 40 °C in the sample and injector compartment, a flow rate of 0.5 mL/min, and an injection volume of 100 μL. The software packages used for data reduction were Empower™ version 3 from Waters (calibration with broad glucan polymer standard) and Astra® version 6 from Wyatt (triple detection method with column calibration).

EXAMPLE 1

Production of Gtf Enzyme 4297 (SEQ ID NO:2)

This Example describes preparing an N-terminally truncated version of a *Streptococcus oralis* gtf enzyme identified in GENBANK under GI number 7684297 (SEQ ID NO:2, encoded by SEQ ID NO:1; herein referred to as "4297").

A nucleotide sequence encoding gtf 4297 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc., Menlo Park, CA). The nucleic acid product (SEQ ID NO:1), encoding gtf 4297 (SEQ ID NO:2), was subcloned into pJexpress404® (DNA2.0, Inc.) to generate the plasmid construct identified as pMP70. This plasmid construct was used to transform *E. coli* MG1655 (ATCC™ 47076) cells to generate the strain identified as MG1655/pMP70.

Production of gtf 4297 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The linkage profile and DP_w of glucan produced by 4297 are shown in Table 2 (see Example 6 below).

EXAMPLE 2

Production of Gtf Enzyme 3298 (SEQ ID NO:4)

This Example describes preparing an N-terminally truncated version of a *Streptococcus* sp. C150 gtf enzyme identified in GENBANK under GI number 322373298 (SEQ ID NO:4, encoded by SEQ ID NO:3; herein referred to as "3298").

A nucleotide sequence encoding gtf 3298 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:3), encoding gtf 3298 (SEQ ID NO:4), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP98. This plasmid construct was used to transform *E. coli* MG1655 (ATCC™ 47076) cells to generate the strain identified as MG1655/pMP98.

Production of gtf 3298 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the

General Methods section. The linkage profile and DP_w of glucan produced by 3298 are shown in Table 2 (see Example 6 below).

EXAMPLE 3

Production of Gtf Enzyme 0544 (SEQ ID NO:6)

This Example describes preparing an N-terminally truncated version of a *Streptococcus mutans* gtf enzyme identified in GENBANK under GI number 290580544 (SEQ ID NO:6, encoded by SEQ ID NO:5; herein referred to as "0544").

A nucleotide sequence encoding gtf 0544 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:5), encoding gtf 0544 (SEQ ID NO:6), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP67. This plasmid construct was used to transform *E. coli* MG1655 (ATCC™ 47076) cells to generate the strain identified as MG1655/pMP67.

Production of gtf 0544 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The linkage profile and DP_w of glucan produced by 0544 are shown in Table 2 (see Example 6 below).

EXAMPLE 4

Production of Gtf Enzyme 5618 (SEQ ID NO:8)

This Example describes preparing an N-terminally truncated version of a *Streptococcus sanguinis* gtf enzyme identified in GENBANK under GI number 328945618 (SEQ ID NO:8, encoded by SEQ ID NO:7; herein referred to as "5618").

A nucleotide sequence encoding gtf 5618 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:7), encoding gtf 5618 (SEQ ID NO:8), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP72. This plasmid construct was used to transform *E. coli* MG1655 (ATCC™ 47076) cells to generate the strain identified as MG1655/pMP72.

Production of gtf 5618 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The linkage profile and DP_w of glucan produced by 5618 are shown in Table 2 (see Example 6 below).

EXAMPLE 5

Production of Gtf Enzyme 2379 (SEQ ID NO:10)

This Example describes preparing an N-terminally truncated version of a *Streptococcus salivarius* gtf enzyme identified in GENBANK under GI number 662379 (SEQ ID NO:10, encoded by SEQ ID NO:9; herein referred to as "2379").

A nucleotide sequence encoding gtf 2379 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:9), encoding gtf 2379 (SEQ ID NO:10), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP65. This plasmid construct was used to transform *E. coli* MG1655 (ATCC™ 47076) cells to generate the strain identified as MG1655/pMP65.

Production of gtf 2379 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The linkage profile and DP_w of glucan produced by 2379 are shown in Table 2 (see Example 6 below).

EXAMPLE 6

Production of Insoluble Glucan Polymer with Gtf Enzymes

This Example describes using the gtf enzymes prepared in the above Examples to synthesize glucan polymer.

Reactions were performed with each of the above gtf enzymes following the procedures disclosed in the General Methods section. Briefly, gtf reaction solutions were prepared comprising sucrose (50 g/L), potassium phosphate buffer (pH 6.5, 50 mM) and a gtf enzyme (2.5% extract by volume). After 24-30 hours at 22-25 °C, insoluble glucan polymer product was harvested by centrifugation, washed three times with water, washed once with ethanol, and dried at 50 °C for 24-30 hours.

Following the procedures disclosed in the General Methods section, the glycosidic linkages in the insoluble glucan polymer product from each reaction were determined by ^{13}C NMR, and the DP_w for each product was determined by SEC. The results of these analyses are shown in Table 2.

Table 2

Linkages and DP_w of Glucan Produced by Various Gtf Enzymes

Gtf	SEQ ID NO.	Glucan Alpha Linkages		DP_w
		% 1,3	% 1,6	
4297	2	31	67	10540
3298	4	50	50	1235
0544	6	62	36	3815
5618	8	34	66	3810
2379	10	37	63	1640

Thus, gtf enzymes capable of producing insoluble glucan polymer having a heterogeneous glycosidic linkage profile (alpha-1,3 and 1,6 linkages) and a DP_w of at least 1000 were identified. These enzymes can be used to produce insoluble poly alpha-1,3-1,6-glucan suitable for derivatization to downstream products such as glucan ether, as demonstrated below in Example 7.

EXAMPLE 7

Preparation of Carboxymethyl Poly Alpha-1,3-1,6-Glucan

This Example describes producing the glucan ether derivative, carboxymethyl poly alpha-1,3-1,6-glucan.

Poly alpha-1,3-1,6-glucan was first prepared as in Example 6, but with a few modifications. Specifically, a glucan polymerization reaction solution was prepared comprising sucrose (300 g), potassium phosphate buffer (pH 5.5; 8.17 g), gtf enzyme 4297 extract (90 mL) in 3 L distilled water. After 24-30 hours at 22-25 °C, insoluble glucan polymer was harvested by centrifugation, filtered, washed three times with water, washed twice with ethanol, and dried at 50 °C for 24-30 hours. About 12 g of poly alpha-1,3-1,6-glucan was obtained.

The DP_w and glycosidic linkages of the insoluble glucan polymer was determined as described in the General Methods. The polymer had a DPw of 10,540 and a linkage profile of 31% alpha-1,3 and 67% alpha-1,6. It had a

weight-average molecular weight (M_w) of 1100000. This solid glucan was used to prepare carboxymethyl poly alpha-1,3-1,6-glucan as follows.

1 g of the poly alpha-1,3-1,6-glucan was added to 20 mL of isopropanol in a 50-mL capacity round bottom flask fitted with a thermocouple for temperature monitoring and a condenser connected to a recirculating bath, and a magnetic stir bar. Sodium hydroxide (40 mL of a 15% solution) was added dropwise to the preparation, which was then heated to 25 °C on a hotplate. The preparation was stirred for 1 hour before the temperature was increased to 55 °C. Sodium monochloroacetate (0.3 g) was then added to provide a reaction, which was held at 55 °C for 3 hours before being neutralized with glacial acetic acid. The solid material was then collected by vacuum filtration and washed with ethanol (70%) four times, dried under vacuum at 20-25 °C, and analyzed by NMR to determine degree of substitution (DoS) of the solid. The solid was identified as sodium carboxymethyl poly alpha-1,3-1,6-glucan with a DoS of 0.464 (sample 1D in Table 3).

Table 3 provides a list of DoS measurements for additional samples of carboxymethyl poly alpha-1,3-1,6-glucan prepared using processes similar to the above process, but with certain modifications as indicated in the table. Each reaction listed in Table 3 used poly alpha-1,3-1,6-glucan with an M_w of 1100000 as substrate. The results in Table 3 indicate that by altering the reagent amounts and time of the etherification reaction, product DoS can be altered.

Table 3

Samples of Sodium Carboxymethyl Poly Alpha-1,3-1,6-Glucan Prepared from Poly Alpha-1,3-1,6-Glucan

Product Sample Designation	Reagent[a]:Glucan Molar Ratio[b]	NaOH:Glucan Molar Ratio[b]	Reaction Time (hours)	DoS
1A	1.66	1.68	3	0.827
1B	0.83	1.92	1.5	0.648
1C	0.83	1.08	3	0.627

1D	0.41	1.08	3	0.464

[a] Reagent refers to sodium monochloroacetate.
[b] Molar ratios calculated as moles of reagent per moles of poly alpha-1,3-1,6-glucan (second column), or moles of NaOH per moles of poly alpha-1,3-1,6-glucan (third column).

Thus, the glucan ether derivative, carboxymethyl poly alpha-1,3-1,6-glucan, was prepared and isolated.

EXAMPLE 8

Viscosity Modification Using Carboxymethyl Poly Alpha-1,3-1,6-Glucan

This Example describes the effect of carboxymethyl poly alpha-1,3-1,6-glucan on the viscosity of an aqueous composition.

Various sodium carboxymethyl poly alpha-1,3-1,6 glucan samples (1A-1D) were prepared as described in Example 72. To prepare 0.6 wt% solutions of each of these samples, 0.102 g of sodium carboxymethyl poly alpha-1,3-1,6-glucan was added to DI water (17 g). Each preparation was then mixed using a bench top vortexer at 1000 rpm until the solid was completely dissolved.

To determine the viscosity of carboxymethyl poly alpha-1,3-1,6-glucan, each solution of the dissolved glucan ether samples was subjected to various shear rates using a Brookfield III+ viscometer equipped with a recirculating bath to control temperature (20 °C). The shear rate was increased using a gradient program which increased from 0.1-232.5 rpm and the shear rate was increased by 4.55 (1/s) every 20 seconds. Results of this experiment at 14.72 (1/s) are listed in Table 4.

Table 4

Viscosity of Carboxymethyl Poly Alpha-1,3-1,6-Glucan Solutions at Various Shear Rates

Sample	Sample Loading (wt%)	Viscosity (cPs)
1A	0.6	106.35[a]
1B	0.6	48.92[a]
1C	0.6	633.83[a]
1D	0.6	2008.45[b]

[a] Viscosity at 14.72 rpm.
[b] Viscosity at 17.04 rpm

The results summarized in Table 4 indicate that a low concentration (0.6 wt%) of carboxymethyl poly alpha-1,3-1,6-glucan can increase the viscosity of DI water when dissolved therein. Also, the results in Table 4 indicate that a relatively low DoS (e.g., as low as 0.464, refer to sample 1D in Tables 3 and 4) is sufficient for carboxymethyl poly alpha-1,3-1,6-glucan to be an effective viscosity modifier of an aqueous composition.

It is noteworthy that the viscosity levels obtained with carboxymethyl poly alpha-1,3-1,6-glucan are substantially higher than the viscosity levels observed using carboxymethyl dextran (refer to comparative Example 10) and carboxymethyl poly alpha-1,3-glucan (refer to comparative Example 12) (compare Table 4 with Tables 6 and 8). This was despite these other agents having DoS levels similar with those of the above carboxymethyl poly alpha-1,3-1,6-glucan samples (compare Table 3 with Tables 5 and 7) and using these other agents at the same concentration (0.6 wt%).

EXAMPLE 9 (Comparative)

Preparation of Carboxymethyl Dextran from Solid Dextran

This Example describes producing carboxymethyl dextran for use in Example 10.

0.5 g of solid dextran (M_w = 750000) was added to 10 mL of isopropanol in a 50-mL capacity round bottom flask fitted with a thermocouple for temperature monitoring and a condenser connected to a recirculating bath, and a magnetic stir bar. Sodium hydroxide (0.9 mL of a 15% solution) was added dropwise to the preparation, which was then heated to 25 °C on a hotplate. The preparation was stirred for 1 hour before the temperature was increased to 55 °C. Sodium monochloroacetate (0.15 g) was then added to provide a reaction, which was held at 55 °C for 3 hours before being neutralized with glacial acetic acid. The solid material was then collected by vacuum filtration and washed with ethanol (70%) four times, dried under vacuum at 20-25 °C, and analyzed by NMR to determine degree of substitution (DoS) of the solid. The solid was identified as sodium carboxymethyl dextran.

Additional sodium carboxymethyl dextran was prepared using dextran of different M_w. The DoS values of carboxymethyl dextran samples prepared in this example are provided in Table 5.

Table 5

Samples of Sodium Carboxymethyl Dextran Prepared from Solid Dextran

Product Sample Designation	Dextran M_w	Reagent[a]:Dextran Molar Ratio[b]	NaOH:Dextran Molar Ratio[b]	Reaction Time (hours)	DoS
2A	750000	0.41	1.08	3	0.64
2B	1750000	0.41	0.41	3	0.49

[a] Reagent refers to sodium monochloroacetate.
[b] Molar ratios calculated as moles of reagent per moles of dextran (third column), or moles of NaOH per moles of dextran (fourth column).

These carboxymethyl dextran samples were tested for their viscosity modification effects in Example 10.

EXAMPLE 10 (Comparative)

Effect of Shear Rate on Viscosity of Carboxymethyl Dextran

This Example describes the viscosity, and the effect of shear rate on viscosity, of solutions containing the carboxymethyl dextran samples prepared in Example 9.

Various sodium carboxymethyl dextran samples (2A and 2B) were prepared as described in Example 9. To prepare 0.6 wt% solutions of each of these samples, 0.102 g of sodium carboxymethyl dextran was added to DI water (17 g). Each preparation was then mixed using a bench top vortexer at 1000 rpm until the solid was completely dissolved.

To determine the viscosity of carboxymethyl dextran at various shear rates, each solution of the dissolved dextran ether samples was subjected to various shear rates using a Brookfield III+ viscometer equipped with a recirculating bath to control temperature (20 °C). The shear rate was increased using a gradient program which increased from 0.1-232.5 rpm and the shear rate was increased by 4.55 (1/s) every 20 seconds. The results of this experiment at 14.72 (1/s) are listed in Table 6.

Table 6

Viscosity of Carboxymethyl Dextran Solutions at Various Shear Rates

Sample	Sample Loading (wt%)	Viscosity (cPs) @ 66.18 rpm	Viscosity (cPs) @ 110.3 rpm	Viscosity (cPs) @ 183.8 rpm	Viscosity (cPs) @ 250 rpm
2A	0.6	4.97	2.55	4.43	3.88
2B	0.6	6.86	5.68	5.28	5.26

The results summarized in Table 6 indicate that 0.6 wt% solutions of carboxymethyl dextran have viscosities of about 2.5-7 cPs. These viscosity levels are substantially lower than the viscosity levels observed using carboxymethyl poly alpha-1,3-1,6-glucan samples at the same low concentration (0.6 wt%) in water. Specifically, Table 4 indicates that carboxymethyl poly alpha-1,3-1,6-glucan solutions have viscosities of about 48-2010 cPs. This difference in viscosity modification is further noteworthy with respect to carboxymethyl dextran sample 2B, which likely has a higher molecular weight than the molecular weights of the carboxymethyl poly alpha-1,3-1,6-glucan samples. Despite having a higher molecular weight, carboxymethyl dextran sample 2B exhibited a substantially lower viscosity-modifying effect than carboxymethyl poly alpha-1,3-1,6-glucan.

Thus, it is believed that carboxymethyl poly alpha-1,3-1,6-glucan has a greater viscosity-modifying effect than carboxymethyl dextran.

EXAMPLE 11 (Comparative)

Preparation of Carboxymethyl Poly Alpha-1,3-Glucan

This Example describes producing carboxymethyl poly alpha-1,3-glucan for use in Example 12.

Poly alpha-1,3-glucan was prepared using a gtfJ enzyme preparation as described in U.S. Patent Appl. Publ. No. 2013/0244288, which is incorporated herein by reference in its entirety.

150 g of poly alpha-1,3-glucan (M_w = 192000) was added to 3000 mL of isopropanol in a 500-mL capacity round bottom flask fitted with a thermocouple for temperature monitoring and a condenser connected to a recirculating bath, and a magnetic stir bar. Sodium hydroxide (600 mL of a 15% solution) was

added dropwise to the preparation, which was then heated to 25 °C on a hotplate. The preparation was stirred for 1 hour before the temperature was increased to 55 °C. Sodium monochloroacetate was then added to provide a reaction, which was held at 55 °C for 3 hours before being neutralized with 90% acetic acid. The solid material was then collected by vacuum filtration and washed with ethanol (70%) four times, dried under vacuum at 20-25 °C, and analyzed by NMR to determine degree of substitution (DoS) of the solid. The solid was identified as sodium carboxymethyl poly alpha-1,3-glucan.

Additional sodium carboxymethyl poly alpha-1,3-glucan was prepared using processes similar to the above process, but with certain modifications as indicated in the Table 7. Each reaction listed in Table 7 used poly alpha-1,3-glucan with an M_w of 192000 as substrate.

Table 7

Samples of Carboxymethyl Poly Alpha-1,3-Glucan

Product Sample Designation	Reagent[a]:Glucan Molar Ratio[b]	NaOH:Glucan Molar Ratio[b]	Reaction Time (hours)	DoS
C1A	3.297	2.4	3	0.977
C1B	1.65	2.4	3	0.514

[a] Reagent refers to sodium monochloroacetate.

[b] Molar ratios calculated as moles of reagent per moles of poly alpha-1,3-glucan (second column), or moles of NaOH per moles of poly alpha-1,3-glucan (third column).

These carboxymethyl poly alpha-1,3-glucan samples were tested for their viscosity modification effects in Example 12.

EXAMPLE 12 (Comparative)

Viscosity Modification Using Carboxymethyl Poly Alpha-1,3-Glucan

This Example describes the effect of carboxymethyl poly alpha-1,3-glucan on the viscosity of an aqueous composition.

Various sodium carboxymethyl poly alpha-1,3-glucan samples (C1A and C1B) were prepared as described in Example 11. To prepare 0.6 wt% solutions of each of these samples, 0.102 g of sodium carboxymethyl poly alpha-1,3-

glucan was added to DI water (17 g). Each preparation was then mixed using a bench top vortexer at 1000 rpm until the solid was completely dissolved.

To determine the viscosity of carboxymethyl poly alpha-1,3-glucan at various shear rates, each solution of the dissolved glucan ether samples was subjected to various shear rates using a Brookfield III+ viscometer equipped with a recirculating bath to control temperature (20 °C). The shear rate was increased using a gradient program which increased from 0.1-232.5 rpm and the shear rate was increased by 4.55 (1/s) every 20 seconds. Results of this experiment at 14.72 (1/s) are listed in Table 8.

Table 8

Viscosity of Carboxymethyl Poly Alpha-1,3-Glucan Solutions

Sample	Sample Loading (wt%)	Viscosity (cPs) @ 14.9 rpm
C1A	0.6	6.38
C1B	0.6	21.27

The results summarized in Table 8 indicate that 0.6 wt% solutions of carboxymethyl poly alpha-1,3-glucan have viscosities of about 6-22 cPs. These viscosity levels are lower than the viscosity levels observed using carboxymethyl poly alpha-1,3-1,6-glucan samples at the same low concentration (0.6 wt%) in water. Specifically, Table 4 indicates that carboxymethyl poly alpha-1,3-1,6-glucan solutions have viscosities of about 48-2010 cPs.

Thus, it is believed that carboxymethyl poly alpha-1,3-1,6-glucan may have a greater viscosity-modifying effect than carboxymethyl poly alpha-1,3-glucan.

EXAMPLE 13 (Comparative)

Viscosity Modification Using Carboxymethyl Cellulose

This Example describes the effect of carboxymethyl cellulose (CMC) on the viscosity of an aqueous composition.

CMC samples (C3A and C3B, Table 9) obtained from DuPont Nutrition & Health (Danisco) were dissolved in DI water to prepare 0.6 wt% solutions of each sample.

To determine the viscosity of CMC at various shear rates, each solution of the dissolved CMC samples was subjected to various shear rates using a Brookfield III+ viscometer equipped with a recirculating bath to control temperature (20 °C). The shear rate was increased using a gradient program which increased from 0.1-232.5 rpm and the shear rate was increased by 4.55 (1/s) every 20 seconds. Results of this experiment at 14.72 (1/s) are listed in Table 9.

Table 9

Viscosity of CMC Solutions

Sample	Molecular Weight (Mw)	DoS	Sample Loading (wt%)	Viscosity (cPs) @ 14.9 rpm
C3A (BAK 130)	~130000	0.66	0.6	235.03
C3B (BAK 550)	~550000	0.734	0.6	804.31

CMC (0.6 wt%) therefore can increase the viscosity of an aqueous solution. However, it is believed that this ability to increase viscosity is lower than the ability of carboxymethyl poly alpha-1,3-1,6-glucan to increase viscosity.

Thus, it is believed that carboxymethyl poly alpha-1,3-1,6-glucan may have a greater viscosity-modifying effect than CMC.

EXAMPLE 14

Creating Calibration Curves for Direct Red 80 and Toluidine Blue O Dyes Using UV Absorption

This example discloses creating calibration curves that could be useful for determining the relative level of adsorption of poly alpha-1,3-1,6-glucan ether derivatives onto fabric surfaces.

Solutions of known concentration (ppm) were made using Direct Red 80 and Toluidine Blue O dyes. The absorbance of these solutions were measured using a LAMOTTE SMART2 Colorimeter at either 520 or 620 nm. The absorption information was plotted in order that it can be used to determine dye concentration of solutions exposed to fabric samples. The concentration and absorbance of each calibration curve are provided in Tables 10 and 11.

Table 10

Direct Red 80 Dye Calibration Curve Data

Dye Concentration (ppm)	Average Absorbance @520 nm
25	0.823333333
22.5	0.796666667
20	0.666666667
15	0.51
10	0.37
5	0.2

Table 11

Toluidine Blue O Dye Calibration Curve Data

Dye Concentration (ppm)	Average Absorbance @620 nm
12.5	1.41
10	1.226666667
7	0.88
5	0.676666667
3	0.44
1	0.166666667

Thus, calibration curves were prepared that may be useful for determining the relative level of adsorption of poly alpha-1,3-1,6-glucan ether derivatives onto fabric surfaces. These calibration curves would be utilized in Examples 15 and 16.

EXAMPLE 15

Adsorption of Quaternary Ammonium Poly Alpha-1,3-1,6-Glucan Ether on Various Fabrics

This example discloses how one could test the degree of adsorption of a quaternary ammonium poly alpha-1,3-1,6-glucan (trimethylammonium hydroxypropyl poly alpha-1,3-1,6-glucan) on different types of fabrics.

A 0.07 wt% solution of trimethylammonium hydroxypropyl poly alpha-1,3-1,6-glucan is made by dissolving 0.105 g of the polymer in 149.89 g of deionized water. This solution is divided into several aliquots with different concentrations

of polymer (Table 12). Other components are added such as acid (dilute hydrochloric acid) or base (sodium hydroxide) to modify pH, or NaCl salt.

Table 12

Quaternary Ammonium Poly Alpha-1,3-1,6-Glucan Solutions Useful in Fabric Adsorption Studies

Amount of NaCl (g)	Amount of Solution (g)	Polymer Concentration (wt%)	Final pH
0	15	0.07	~7
0.15	14.85	0.0693	~7
0.3	14.7	0.0686	~7
0.45	14.55	0.0679	~7
0	9.7713	0.0683	~3
0	9.7724	0.0684	~5
0	10.0311	0.0702	~9
0	9.9057	0.0693	~11

Four different fabric types (cretonne, polyester, 65:35 polyester/cretonne, bleached cotton) are cut into 0.17 g pieces. Each piece is placed in a 2-mL well in a 48-well cell culture plate. Each fabric sample is exposed to 1 mL of each of the above solutions (Table 12) for a total of 36 samples (a control solution with no polymer is included for each fabric test). The fabric samples are allowed to sit for at least 30 minutes in the polymer solutions. The fabric samples are removed from the polymer solutions and rinsed in DI water for at least one minute to remove any unbound polymer. The fabric samples are then dried at 60 °C for at least 30 minutes until constant dryness is achieved. The fabric samples are weighed after drying and individually placed in 2-mL wells in a clean 48-well cell culture plate. The fabric samples are then exposed to 1 mL of a 250 ppm Direct Red 80 dye solution. The samples are left in the dye solution for at least 15 minutes. Each fabric sample is removed from the dye solution, afterwhich the dye solution is diluted 10x.

The absorbance of the diluted solutions is measured compared to a control sample. A relative measure of glucan polymer adsorbed to the fabric is calculated based on the calibration curve created in Example 14 for Direct Red 80 dye. Specifically, the difference in UV absorbance for the fabric samples

exposed to polymer compared to the controls (fabric not exposed to polymer) represents a relative measure of polymer adsorbed to the fabric. This difference in UV absorbance could also be expressed as the amount of dye bound to the fabric (over the amount of dye bound to control), which is calculated using the calibration curve (i.e., UV absorbance is converted to ppm dye). A positive value represents the dye amount that is in excess to the dye amount bound to the control fabric, whereas a negative value represents the dye amount that is less than the dye amount bound to the control fabric. A positive value would reflect that the glucan ether compound adsorbed to the fabric surface.

It is believed that this assay would demonstrate that quaternary ammonium poly alpha-1,3-1,6-glucan can adsorb to various types of fabric under different salt and pH conditions. This adsorption would suggest that cationic poly alpha-1,3-1,6-glucan ether derivatives are useful in detergents for fabric care (e.g., as anti-redeposition agents).

EXAMPLE 16

Adsorption of Carboxymethyl Poly Alpha-1,3-1,6-Glucan (CMG) on Various Fabrics

This example discloses how one could test the degree of adsorption of a poly alpha-1,3-1,6-glucan ether compound (CMG) on different types of fabrics.

A 0.25 wt% solution of CMG is made by dissolving 0.375 g of the polymer in 149.625 g of deionized water. This solution is divided into several aliquots with different concentrations of polymer (Table 13). Other components are added such as acid (dilute hydrochloric acid) or base (sodium hydroxide) to modify pH, or NaCl salt.

Table 13

CMG Solutions Useful in Fabric Adsorption Studies

Amount of NaCl (g)	Amount of Solution (g)	Polymer Concentration (wt%)	Final pH
0	15	0.25	~7
0.15	14.85	0.2475	~7
0.3	14.7	0.245	~7
0.45	14.55	0.2425	~7

0	9.8412	0.2459	~3
0	9.4965	0.2362	~5
0	9.518	0.2319	~9
0	9.8811	0.247	~11

Four different fabric types (cretonne, polyester, 65:35 polyester/cretonne, bleached cotton) are cut into 0.17 g pieces. Each piece is placed in a 2-mL well in a 48-well cell culture plate. Each fabric sample is exposed to 1 mL of each of the above solutions (Table 13) for a total of 36 samples (a control solution with no polymer is included for each fabric test). The fabric samples are allowed to sit for at least 30 minutes in the polymer solutions. The fabric samples are removed from the polymer solutions and rinsed in DI water for at least one minute to remove any unbound polymer. The fabric samples are then dried at 60 °C for at least 30 minutes until constant dryness is achieved. The fabric samples are weighed after drying and individually placed in 2-mL wells in a clean 48-well cell culture plate. The fabric samples are then exposed to 1 mL of a 250 ppm Toluidine Blue dye solution. The samples are left in the dye solution for at least 15 minutes. Each fabric sample is removed from the dye solution, afterwhich the dye solution is diluted 10x.

The absorbance of the diluted solutions is measured compared to a control sample. A relative measure of glucan polymer adsorbed to the fabric is calculated based on the calibration curve created in Example 14 for Toluidine Blue dye. Specifically, the difference in UV absorbance for the fabric samples exposed to polymer compared to the controls (fabric not exposed to polymer) represents a relative measure of polymer adsorbed to the fabric. This difference in UV absorbance could also be expressed as the amount of dye bound to the fabric (over the amount of dye bound to control), which is calculated using the calibration curve (i.e., UV absorbance is converted to ppm dye). A positive value represents the dye amount that is in excess to the dye amount bound to the control fabric, whereas a negative value represents the dye amount that is less than the dye amount bound to the control fabric. A positive value would reflect that the glucan ether compound adsorbed to the fabric surface.

It is believed that this assay would demonstrate that CMG polymer can adsorb to various types of fabric under different salt and pH conditions. This adsorption would suggest that poly alpha-1,3-1,6-glucan ether derivatives are useful in detergents for fabric care (e.g., as anti-redeposition agents).

EXAMPLE 17

Effect of Cellulase on Carboxymethyl Poly Alpha-1,3-1,6-Glucan (CMG)

This example discloses how one could test the stability of a poly alpha-1,3-1,6-glucan ether, CMG, in the presence of cellulase compared to the stability of carboxymethyl cellulose (CMC). Stability to cellulase would indicate applicability of CMG to use in cellulase-containing compositions/processes such as in fabric care.

Solutions (1 wt%) of CMC (M_w = 90000, DoS = 0.7) or CMG (Sample 1A, 1B, 1C, or 1D; Table 3) are treated with cellulase or amylase as follows. CMG or CMC polymer (100 mg) is added to a clean 20-mL glass scintillation vial equipped with a PTFE stir bar. Water (10.0 mL) that has been previously adjusted to pH 7.0 using 5 vol% sodium hydroxide or 5 vol% sulfuric acid is then added to the scintillation vial, and the mixture is agitated until a solution (1 wt%) forms. A cellulase or amylase enzyme is added to the solution, which is then agitated for 24 hours at room temperature (~25 °C). Each enzyme-treated sample is analyzed by SEC (above) to determine the molecular weight of the treated polymer. Negative controls are conducted as above, but without the addition of a cellulase or amylase. Various enzymatic treatments of CMG and CMC that could be performed are listed in Table 14, for example.

Table 14

Measuring Stability of CMG and CMC Against Degradation by Cellulase or Amylase

Polymer	Enzyme	Enzyme Type	Enzyme Loading
CMC	none	N/A	-
CMC	PURADAX HA 1200E	Cellulase	1 mg/mL
CMC	PREFERENZ S 100	Amylase	3 μL/mL

CMG	none	N/A	-
CMG	PURADAX HA 1200E	Cellulase	1 mg/mL
CMG	PREFERENZ S 100	Amylase	3 μL/mL
CMG	PURASTAR ST L	Amylase	3 μL/mL
CMG	PURADAX EG L	Cellulase	3 μL/mL

It is believed that the enzymatic studies in Table 14 would indicate that CMC is highly susceptible to degradation by cellulase, whereas CMG is resistant to this degradation. It is also believed that these studies would indicate that both CMC and CMG are largely stable to amylase.

Since high polymer molecular weight is a key characteristic of a soluble polysaccharide ether for providing viscosity to aqueous compositions, use of CMC for providing viscosity to an aqueous composition (e.g., laundry or dishwashing detergent) containing cellulase would be unacceptable. CMG on the other hand, given its stability to cellulase, would be useful for providing viscosity to cellulase-containing aqueous compositions such as detergents.

EXAMPLE 18

Preparation of Quaternary Ammonium Poly Alpha-1,3-1,6-Glucan

This Example describes how one could produce a quaternary ammonium poly alpha-1,3-1,6-glucan ether derivative. Specifically, trimethylammonium hydroxypropyl poly alpha-1,3-1,6-glucan can be produced.

10 g of poly alpha-1,3-1,6-glucan (prepared as in Examples 6 or 7) is added to 100 mL of isopropanol in a 500-mL capacity round bottom flask fitted with a thermocouple for temperature monitoring and a condenser connected to a recirculating bath, and a magnetic stir bar. 30 mL of sodium hydroxide (17.5% solution) is added dropwise to this preparation, which is then heated to 25 °C on a hotplate. The preparation is stirred for 1 hour before the temperature is increased to 55 °C. 3-chloro-2-hydroxypropyl-trimethylammonium chloride (31.25 g) is then added to provide a reaction, which is held at 55 °C for 1.5 hours before being neutralized with 90% acetic acid. The solid that forms

(trimethylammonium hydroxypropyl poly alpha-1,3-1,6-glucan) is collected by vacuum filtration and washed with ethanol (95%) four times, dried under vacuum at 20-25 °C, and analyzed by NMR and SEC to determine molecular weight and DoS.

Thus, the quaternary ammonium glucan ether derivative, trimethylammonium hydroxypropyl poly alpha-1,3-1,6-glucan, can be prepared and isolated.

EXAMPLE 19

Effect of Shear Rate on Viscosity of Quaternary Ammonium Poly Alpha-1,3-1,6-Glucan

This Example describes how one could test the effect of shear rate on the viscosity of trimethylammonium hydroxypropyl poly alpha-1,3-1,6-glucan. It is contemplated that this glucan ether derivative exhibits shear thinning or shear thickening behavior.

Samples of trimethylammonium hydroxypropyl poly alpha-1,3-1,6-glucan are prepared as described in Example 18. To prepare a 2 wt% solution of each sample, 1 g of sample is added to 49 g of DI water. Each preparation is then homogenized for 12-15 seconds at 20,000 rpm to dissolve the trimethylammonium hydroxypropyl poly alpha-1,3-1,6-glucan sample in the water.

To determine the viscosity of each 2 wt% quaternary ammonium glucan solution at various shear rates, each solution is subjected to various shear rates using a Brookfield DV III+ Rheometer equipped with a recirculating bath to control temperature (20 °C) and a ULA (ultra low adapter) spindle and adapter set. The shear rate is increased using a gradient program which increases from 10-250 rpm and the shear rate is increased by 4.9 1/s every 20 seconds for the ULA spindle and adapter.

It is contemplated that the viscosity of each of the quaternary ammonium poly alpha-1,3-1,6-glucan solutions would change (reduced or increased) as the shear rate is increased, thereby indicating that the solutions demonstrate shear thinning or shear thickening behavior. Such would indicate that quaternary

ammonium poly alpha-1,3-1,6-glucan could be added to an aqueous liquid to modify its rheological profile.

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CLAIMS

What is claimed is:

1. A composition comprising poly alpha-1,3-1,6-glucan, wherein
   (i) at least 30% of the glycosidic linkages of said glucan are alpha-1,3 linkages,
   (ii) at least 30% of the glycosidic linkages of said glucan are alpha-1,6 linkages,
   (iii) said glucan has a weight average degree of polymerization (DP_w) of at least 1000; and
   (iv) the alpha-1,3 linkages and alpha-1,6 linkages of said glucan do not consecutively alternate with each other.

2. The composition of claim 1, wherein at least 60% of the glycosidic linkages of said glucan are alpha-1,6 linkages.

3. The composition of claim 1, wherein the DP_w of said glucan is at least 10000.

4. The composition of claim 1, wherein said glucan is a product of a glucosyltransferase enzyme comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

5. A composition comprising a poly alpha-1,3-1,6-glucan ether compound, wherein:
   (i) at least 30% of the glycosidic linkages of said compound are alpha-1,3 linkages,
   (ii) at least 30% of the glycosidic linkages of said compound are alpha-1,6 linkages,
   (iii) said compound has a weight average degree of polymerization (DP_w) of at least 1000,

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of said compound do not consecutively alternate with each other, and

(v) the compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0.

6. The composition of claim 5, wherein at least 60% of the glycosidic linkages of said compound are alpha-1,6 linkages.

7. The composition of claim 5, wherein at least one organic group is selected from the group consisting of carboxy alkyl group, hydroxy alkyl group, and alkyl group.

8. The composition of claim 7, wherein at least one organic group is selected from the group consisting of carboxymethyl, hydroxypropyl, dihydroxypropyl, hydroxyethyl, methyl, and ethyl group.

9. The composition of claim 5, wherein at least one organic group is a positively charged organic group.

10. The composition of claim 5, wherein the poly alpha-1,3-1,6-glucan from which said compound is derived is a product of a glucosyltransferase enzyme comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

11. The composition of claim 5, wherein the composition is a hydrocolloid or aqueous solution having a viscosity of at least about 10 cPs.

12. The composition of claim 11, wherein the hydrocolloid or aqueous solution is in the form of a personal care product, pharmaceutical product, food product, household product, or industrial product.

13. A method of producing a poly alpha-1,3-1,6-glucan ether compound, the method comprising:

(a) contacting poly alpha-1,3-1,6-glucan in a reaction under alkaline conditions with at least one etherification agent comprising an organic group, wherein at least one organic group is etherified to the poly alpha-1,3-1,6-glucan thereby producing a poly alpha-1,3-1,6-glucan ether compound,

wherein

(i) at least 30% of the glycosidic linkages of said poly alpha-1,3-1,6-glucan are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of said poly alpha-1,3-1,6-glucan are alpha-1,6 linkages,

(iii) said poly alpha-1,3-1,6-glucan has a weight average degree of polymerization (DP_w) of at least 1000,

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of said poly alpha-1,3-1,6-glucan do not consecutively alternate with each other, and

(v) the compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0; and

(b) optionally, isolating the poly alpha-1,3-1,6-glucan ether compound produced in step (a).

14. The method of claim 13, wherein said alkaline conditions comprise an alkali hydroxide solution.

15. A method for increasing the viscosity of an aqueous composition, the method comprising:

contacting a poly alpha-1,3-1,6-glucan ether compound with the aqueous composition, wherein the viscosity of the aqueous composition is increased by said compound,

wherein

(i) at least 30% of the glycosidic linkages of said compound are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of said compound are alpha-1,6 linkages,

(iii) said compound has a weight average degree of polymerization (DP_w) of at least 1000,

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of said compound do not consecutively alternate with each other, and

(v) the compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0.

16. A method of treating a material, said method comprising:

contacting a material with an aqueous composition comprising a poly alpha-1,3-1,6-glucan ether compound, wherein:

(i) at least 30% of the glycosidic linkages of said compound are alpha-1,3 linkages,

(ii) at least 30% of the glycosidic linkages of said compound are alpha-1,6 linkages,

(iii) said compound has a weight average degree of polymerization (DP_w) of at least 1000,

(iv) the alpha-1,3 linkages and alpha-1,6 linkages of said compound do not consecutively alternate with each other, and

(v) the compound has a degree of substitution (DoS) with at least one organic group of about 0.05 to about 3.0.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/015452

A. CLASSIFICATION OF SUBJECT MATTER
INV. C08B37/00 C09D105/00 C08L5/00 C11D3/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C08B C09D C08L C11D

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/008618 A2 (TNO [NL]; VAN GEEL-SCHUTTEN GERRITDINA H [NL]) 30 January 2003 (2003-01-30) claims 1, 3-6, 16-20 examples 4, 8, 9 paragraphs [0007], [0009], [0014] - [0025]	1-16
X	TSUMORI H ET AL: "PURIFICATION AND PROPERTIES OF EXTRACELLULAR GLUCOSYLTRANSFERASE SYNTHESIZING 1,6-, 1,3-X-D-GLUCAN FROM STREPTOCOCCUS MUTANS SEROTYPE A", JOURNAL OF GENERAL MICROBIOLOGY, SOCIETY FOR MICROBIOLOGY, READING, GB, vol. 131, 1 January 1985 (1985-01-01), pages 3347-3353, XP000937830, ISSN: 0022-1287 abstract; table 2	1-12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 May 2015	12/06/2015

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/015452

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