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Benjamin et al.

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[54] ENZYME-CONTAINING DETERGENT
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195/63, 68; 424/94

[56]

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3,451,935 6/1969 Roald 252/99 UX

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[57]

ABSTRACT

Soil- and stain-removing detergent compositions consisting essentially of a water-soluble synthetic organic detergent and from 0.01 to 2 percent of a lipoxidase having enzymatic activity over the ranges of 5° C. to 70° C. and pH 5 to 11 are disclosed. The lipoxidase-containing detergent compositions can optionally contain a fatty acid substrate having cis-, cis-double bonds or an alkyl ester thereof for coupled oxidation of stains. Inorganic peroxy compounds can also be employed. The compositions of the invention are particularly adapted to the treatment of textile materials having soils or stains which have a content of polyunsaturated components.

10 Claims, No Drawings

ENZYME-CONTAINING DETERGENT COMPOSITIONS

BACKGROUND OF THE INVENTION

This invention relates to enzyme-containing detergent compositions. More particularly, it relates to enzyme-containing detergent compositions useful as laundering or presoaking compositions and containing an organic synthetic detergent and a lipoxidase.

The employment of enzymes in admixture with detergent compositions is known as described, for example, in U.S. Pat. No. 1,882,279 issued Oct. 11, 1932. Similarly, British Pat. No. 814,772 issued June 10, 1959, East German Pat. NO. 14,296 published Jan. 6, 1958, and Jaag, Seifen, Ole Fette, Wachse 88, No. 24, pp. 789-793 (Nov. 1962) disclose detergent compositions containing enzymes. While the employment of proteolytic and amylolytic enzymes in detergent compositions to degrade or otherwise alter proteinaceous and starchy soils and stains has been known, the formulation of detergent compositions which permit the removal of additional soils and stains is a desirable objective. It will be appreciated that a normal household laundering situation presents a complex and difficult cleaning problem inasmuch as the soils and stains normally encountered in a household laundering situation are comprised of a broad spectrum of substance of varying composition. Notwithstanding the employment of soaking or laundering compositions containing proteases and/or amylases, certain difficulty removable soils and stains either do not respond to such treatment or are not substantially degraded or altered and thereby rendered more easily removable by the deterative action of a detergent compound. In some instances these difficulty removable soils and stains serve as binding agents for nondigestible and relatively unalterable soils and stains. Examples of difficulty removable stains are the oily and greasy stains having constituents of polyunsaturated character. These stains include French salad dressing, grease, mayonnaise and the like.

The formulation of detergent compositions having enzymes which facilitate the removal of the soils and stains which frequently remain notwithstanding the use of conventional enzyme-containing detergent compositions is desirable from the standpoint of making possible the removal of a greater range or spectrum of soils and stains.

It is an object of the present invention to provide detergent compositions having superior cleaning and laundering properties.

It is another object of the present invention to provide cleaning and laundering compositions which facilitate the removal of difficulty removable soils and stains.

Still another object is the provision of detergent compositions which facilitate the removal of soils and stains having a content of polyunsaturated fats or derivatives thereof.

Another object of the invention is the provision of laundering compositions adapted to the removal of a wide spectrum of soils and stains.

Other objects of the invention will become apparent from a consideration of the invention described in detail hereinafter.

SUMMARY OF THE INVENTION

This invention is based in part on the discovery that detergent compositions comprising a water-soluble organic detergent compound and a lipoxidase, alternatively termed a lipoxigenase, facilitate the removal by soaking and laundering methods of soils and stains having polyunsaturated components from textile materials and thereby permit the removal of soils and stains which are difficulty removable by the action of conventional enzyme-containing detergent compositions. The invention thus involves the provision of laundering and soaking detergent compositions consisting essentially of:

- A. from 1 percent to 50 percent of a water-soluble synthetic organic detergent; and
- B. from 0.01 percent to 2 percent of a lipoxidase having lipoxidase enzymatic activity at a temperature of from 5° C. to 70° C. in the pH range of from 5 to 11.

DETAILED DESCRIPTION OF THE INVENTION

The lipoxidase-containing detergent compositions of the invention contain as an essential component a water-soluble synthetic detergent. The organic detergent compounds which can be used include soap and anionic, nonionic, ampholytic and zwitterionic synthetic detergents and mixtures thereof exemplified as follows:

a. Water-soluble soap: Examples of suitable soaps for use in this invention are the sodium, potassium, ammonium and alkanol ammonium (e.g., triethanolammonium) salts of higher fatty acids containing from about 10 to about 22 carbon atoms. Particularly useful are the sodium and potassium salts of the mixture of fatty acids derived from coconut oil and tallow, i.e., sodium and potassium tallow and coconut soap.

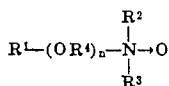
b. Anionic synthetic nonsoap detergents, a preferred class, can be broadly described as the water-soluble salts, particularly the alkali metal salts, of organic sulfuric reaction products having in their molecular structure an alkyl radical containing from about eight to about 22 carbon atoms and a radical selected from the group consisting of sulfonic acid and sulfuric acid ester radicals. (Included in the term alkyl is the alkyl portion of higher acyl radicals.) Important examples of the synthetic detergents which form a part of the preferred compositions of the present invention are the sodium or potassium alkyl sulfates, especially those obtained by sulfating the higher alcohols (C_8 - C_{18} carbon atoms) produced by reducing the glycerides of tallow or coconut oil; sodium or potassium alkyl benzene sulfonates, in which the alkyl group contains from about nine to about 15 carbon atoms, including those of the types described in U.S. Pat. Nos. 2,220,099 and 2,477,383 (the alkyl radical can be a straight or branched aliphatic chain); sodium alkyl glyceryl ether sulfonates, especially those ethers of the higher alcohols derived from tallow and coconut oil; sodium coconut oil fatty acid monoglyceride sulfates and sulfonates; sodium or potassium salts or sulfuric acid esters of the reaction product of one mole of a higher fatty alcohol (e.g., tallow or coconut oil alcohols) and about 1 to 6 moles of ethylene oxide; sodium or potassium salts of alkyl phenol ethylene oxide ether sulfate with about 1 to about 10 units of ethylene oxide per molecule and in which the alkyl radicals contain from eight to about 12 carbon atoms; the reaction product of fatty acids esterified with isethionic acid and neutralized with sodium hydroxide where, for example, the fatty acids are derived from coconut oil; sodium or potassium salts of fatty acid amide of a methyl tauride in which the fatty acids, for example, are derived from coconut oil; and others known in the art, a number specifically set forth in U.S. Pat. Nos. 2,486,921, 2,486,922 and 2,396,278. Other important anionic detergents, sulfonated olefins, are described in the U.S. Pat. No. 3,332,880 to Phillip E. Pflaumer and Adrian Kessler issued July 25, 1967.

c. Nonionic synthetic detergents: One class can be broadly defined as compounds produced by the condensation of alkylene oxide groups (hydrophilic in nature) with an organic hydrophobic compound, which may be aliphatic or alkyl aromatic in nature. The length of the hydrophilic or polyoxyalkylene radical which is condensed with any particular hydrophobic group can be readily adjusted to yield a water-soluble compound having the desired degree of balance between hydrophilic and hydrophobic elements. Another class has semipolar characteristics. Preferred classes of nonionic synthetic detergents are as follows:

1. A class of nonionic synthetic detergents under the trade name of "Pluronic." These compounds are formed by condensing ethylene oxide with a hydrophobic base formed by the condensation of propylene oxide with propylene glycol. The hydrophobic portion of the molecule which, of course, exhibits water insolubility, has a molecular weight of from about 1,500 to 1,800. The addition of polyoxyethylene radicals to this hydrophobic portion tends to increase the water solubility of the molecule as a whole and the liquid character of the product is retained up to the point where the polyox-

ethylene content is about 50 percent of the total weight of the condensation product.

2. The polyethylene oxide condensates of alkyl phenols, e.g., the condensation products of alkyl phenols having an alkyl group containing from about six to 12 carbon atoms in either a straight-chain or branched-chain configuration with ethylene oxide, the said ethylene oxide being present in amounts equal to 5 to 25 moles of ethylene oxide per mole of alkyl phenol. The alkyl substituent in such compounds may be derived from polymerized propylene, diisobutylene, octene, or nonene, for example.
3. Those nonionic synthetic detergents derived from the condensation of ethylene oxide with the product resulting from the reaction of propylene oxide and ethylene diamine. For example, compounds containing from about 40 percent to about 80 percent polyoxyethylene by weight and having a molecular weight of from about 5,000 to about 11,000 resulting from the reaction of ethylene oxide groups with a hydrophobic base constituted of the reaction product of ethylene diamine and excess propylene oxide, said base having a molecular weight of the order of 2,500 to 3,000 are satisfactory.
4. The condensation product of aliphatic alcohols having from eight to 22 carbon atoms, in either straight-chain or branched-chain configuration, with ethylene oxide, e.g., a coconut alcohol ethylene oxide condensate having from 5 to 40 moles of ethylene oxide per mole of coconut alcohol, the coconut alcohol fraction having from 10 to 14 carbon atoms.
5. The ammonia, monoethanol and diethanol amides of fatty acids having an acyl moiety of from about eight to about 18 carbon atoms. These acyl moieties are normally derived from naturally occurring glycerides, e.g., coconut oil, palm oil, soybean oil and tallow, but can be derived synthetically, e.g., by the oxidation of petroleum, or by hydrogenation of carbon monoxide by the Fischer-Tropsch process.
6. Long chain tertiary amine oxides corresponding to the following general formula



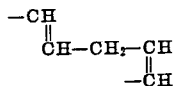
wherein R^1 is an alkyl radical of from about eight to about 24 carbon atoms, R^2 and R^3 are each methyl, ethyl or hydroxyethyl radicals, R^4 is ethylene, and n equals from 0 to about 10. The arrow in the formula is a conventional representation of a semipolar bond. Specific examples of amine oxide detergents include: dimethyldodecylamine oxide; cetyl-dimethylamine oxide; bis-(2-hydroxyethyl) dodecylamine oxide; bis-(hydroxyethyl)-3-dodecoxy-1-hydroxypropyl amine oxide.

7. Long chain tertiary phosphine oxides corresponding to the following general formula $\text{RR}'\text{R}''\text{P}=\text{O}$ wherein R is an alkyl, alkenyl or monohydroxyalkyl radical ranging from 10 to 24 carbon atoms in chain length and R' and R'' are each alkyl or monohydroxyalkyl groups containing from one to three carbon atoms. The arrow in the formula is a conventional representation of a semipolar bond. Examples of suitable phosphine oxides are found in U.S. Pat. No. 3,304,263 of Feb. 14, 1967, and include: dimethyldodecylphosphine oxide; diethyldodecylphosphine oxide; dimethyl-(2-hydroxydodecyl) phosphine oxide.
8. Long chain sulfoxides having the formula



- 5 wherein R^5 is an alkyl radical containing from about 10 to about 28 carbon atoms, from zero to about five ether linkages and from zero to about two hydroxyl substituents, at least one moiety of R^5 being an alkyl radical containing zero ether linkages and containing from about 10 to about 18 carbon atoms, and wherein R^6 is an alkyl radical containing from one to three carbon atoms and from one to two hydroxyl groups. Specific examples of these sulfoxides are: dodecyl methyl sulfoxide; 3-hydroxy tridecyl methyl sulfoxide; 3-methoxy tridecyl methyl sulfoxide; 3-hydroxy-4-dodecoxybutyl methyl sulfoxide.
- 10 d. Ampholytic synthetic detergents can be broadly described as derivatives of aliphatic secondary and tertiary amines, in which the aliphatic radical may be straight-chain or branched and wherein one of the aliphatic substituents contains from about eight to 18 carbon atoms and one contains an anionic water-solubilizing group, e.g., carboxy, sulfo, sulfato, phosphato, or phosphono. Examples of compounds falling within this definition are sodium-3-dodecylaminopropionate and sodium-3-dodecylaminopropane sulfonate.
- 15 e. Zwitterionic synthetic detergents can be broadly described as derivatives of aliphatic quaternary ammonium, phosphonium, and sulfonium compounds, in which the aliphatic radical may be straight-chain or branched, and wherein one of the aliphatic substituents contains from about eight to 18 carbon atoms and one contains an anionic water-solubilizing group, e.g., carboxy, sulfo, sulfato, phosphato, or phosphono. Examples of compounds falling within this definition are 3-(N,N-dimethyl-N-hexadecylammonio)propane-1-sulfonate and 3-(N,N-dimethyl-N-hexadecylammonio)-2-hydroxy propane-1-sulfonate which are especially preferred for their excellent cool water detergency characteristics. See, for example, Snoddy, et al., Canadian Pat. No. 708,148 issued Apr. 20, 1965.
- 20 Preferred detergents for use in the compositions of the invention include the condensation products of 1 mole of aliphatic alcohol having eight to 22 carbon atoms with from 5 to 40 moles of ethylene oxide, e.g., tallow alcohol ethoxylated with 11 or 30 moles of ethylene oxide and coconut alcohol ethoxylated with 6 moles of ethylene oxide. Also preferred are
- 25 the 3-(N,N-dimethyl-N-alkylammonio)-2-hydroxypropane-1-sulfonates wherein the alkyl has from eight to 22 carbon atoms, e.g., 3-(N,N-dimethyl-N-coconutalkylammonio)-2-hydroxypropane-1-sulfonate and the 3-(N,N-dimethyl-N-alkylammonio) propane-1-sulfonates wherein the alkyl has from
- 30 eight to 22 carbon atoms, e.g., 3-(N,N-dimethyl-N-hexadecylammonio) propane-1-sulfonate. These detergents are especially suitable herein by reason of their excellent cleaning properties, compatibility with lipoxidase and ready availability.
- 35 The organic detergent employed in the compositions of the invention can vary in amount from 1 percent to 50 percent by weight of the total composition. Compositions consisting essentially of a major proportion of a suitable detergent compound and a minor amount of a lipoxidase can be suitably employed. Variations in the amount of detergent will depend on the intended use of the detergent composition. Thus, compositions intended for use as washing formulations will normally contain an amount of from 1 percent to 25 percent. Similarly, compositions intended for use as soaking formulations preparatory to washing with a conventional or enzyme-containing detergent composition will normally contain a lesser proportion of detergent active corresponding to an amount of from 1 percent to 20 percent and, preferably, 3 percent to 10 percent.
- 40 The lipoxidases of the invention are those which exhibit lipoxidase activity under the conditions of temperature and pH normally encountered in laundry situations. The lipoxidases suitable herein are those which are characterized by sufficient enzymatic activity at a temperature of from 5° C. to
- 45 70° C. in a pH range of from 5 to 11 to alter or otherwise

render more easily removable the soils and stains which are normally encountered in a laundering situation. While the precise mechanism by which the lipoxidases of the invention function to remove soils and stains in laundry soaking and washing operations is not completely understood, it is believed that the lipoxidase is involved in the oxidation of unsaturated fatty acids and esters containing the cis, cis-1,4-pentadiene system. The enzymatic attack appears to involve the methylene-interrupted multiply-unsaturated system in which the double bonds are cis, the simplest case of which is as follows:



While applicants do not wish to be bound by any precise theory or mechanism, it is believed that the formation of hydroperoxide compounds from unsaturated fatty acids or esters by the action of lipoxidase and dissolved oxygen is involved. The conversion of such unsaturated compounds via an oxidative mechanism to species which are either less colored or are more readily removed from textile materials is believed to be responsible at least in part for the desirable soil- and stain-removing properties of the compositions of the invention. The oxidative mechanism involving lipoxidase dissolved oxygen and a cis, cis-1,4-pentadiene system and described by A. L. Tappel in "Enzymes," Boyer, Lardy and Myrback (editors) Academic Press, p. 275 (1963) is believed to be in marked contrast to the degradative, fragmentative and digestive mechanisms thought to be involved in the removal of stains from textile materials which are laundered with hydrolytic enzymes such as the proteases and amylases.

Suitable lipoxidases of the invention include those of plant or microbiological origin. Suitable plant lipoxidases include those derived by known methods from legumes, cereals, grains and oil seeds. Examples of such sources include soybeans, urd beans, lentils, green peas, mung beans, peanuts, navy beans, red beans, lima beans, alfalfa, wheat, barley and sunflower seeds.

A preferred lipoxidase of the invention is that derived from soybeans. Soybean lipoxidase is well known, commercially available and provides desirable removal of fatty and other stains in presoaking and washing methods. The lipoxidases of the invention, e.g., soybean lipoxidase, can be employed in either pure or impure form, the latter being preferred from the standpoints of availability and ease of handling. Pure crystalline lipoxidase can be isolated from legumes such as soybeans or from green peas, pea seeds, wheat, green beans, green bean seeds and the like by known methods. A suitable extraction method for soybean lipoxidase involves aqueous extraction at pH 4.5 from soybean meal, followed by ammonium sulfate precipitation. Suitable extraction methods are described by R. T. Holman, et al., "The Enzymes," 1st ed., Vol. II, Part I, pp. 564-565 (1951) and by S. A. Hale, et al., "Lipids," Vol. 4, No. 3, pp. 209-215 (May 1969).

Preferred herein are lipoxidase preparations obtained commercially in combination with inert carrier or vehicle materials such as carbohydrates, agglutinating proteins, inorganic salts such as calcium sulfate, trypsin inhibitor, proteases, and the like. In such preparations, the lipoxidase constitutes a minor component and comprises from about 1 percent to 50 percent. The remaining 50 percent to 90 percent is comprised of the hereinbefore described carrier materials. The commercially available lipoxidase-containing preparations are preferred herein inasmuch as they are more readily available than pure crystalline lipoxidase and provide desirable levels of lipoxidase activity. Suitable examples of such commercially available lipoxidase-containing preparations include the soybean lipoxidase preparations available from the following suppliers: P-L Biochemicals, Inc., Milwaukee, Wis.; Sigma

Chemical Co., St. Louis, Mo.; Nutritional Biochemical Corp., Cleveland, Ohio; Worthington Biochemical Corp., Freehold, N.J.; Gallard-Schlesinger Chem. Mfg. Corp., Carle Place, Long Island, N.Y.; Mann Research Laboratories, New York, N.Y.

Lipoxidases of microbial origin and suitable for use herein include bacterial and fungal lipoxidases derived from fermentation broths. Suitable examples of such lipoxidases are those obtained from *Aspergillus sojae*, *Aspergillus flavus*, *Aspergillus glaucus*, *Aspergillus niger*, *Aspergillus elegans*, *Rhizopus usami*, *Rhizopus G. 34 Yamasake*, *Rhizopus G. 36 Yamasake*, *Rhizopus tritici*, *Penicillium rugulosum* and *Penicillium* 15 described by H. Fukuba, Nippon Nanyo Kaishi 26, 167 (1952). Also suitable are the lipoxidases derived from *Aspergillus parasiticus* (ATCC 11906) and *Aspergillus flavus* (ATCC 1003) which can be obtained from the permanent collection of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md.

The amount of lipoxidase employed in the compositions of the invention is an amount which provides sufficient lipoxidase enzymatic activity to alter or otherwise facilitate removal of the stains and fatty soils normally encountered under laundry situations. It will be appreciated that the amount of lipoxidase employed will depend upon enzyme activity and purity or concentration of the enzyme, conditions of pH and temperature, the nature of the soils or stains to be removed, substrate concentration and the like. As used herein, enzyme activity refers to the property of an enzyme to attack or otherwise alter a substrate molecule. Pure or substantially pure lipoxidases such as those obtained by extraction from vegetable sources or from microbiological sources are characterized by high enzymatic activity and are employed in smaller amounts than the less active lipoxidase-containing preparations having present additional carrier or vehicle materials such as those described hereinbefore. Pure lipoxidases are employed in an amount of from 0.01 percent to 2 percent and, preferably, from 0.1 percent to 1 percent by weight of the compositions of the invention.

Lipoxidase-containing preparations wherein the lipoxidase is combined with carriers or inert materials and comprises from 1 percent to 50 percent by weight of such preparation are normally employed in a lesser amount of from 0.1 percent to 20 percent by weight of the detergent compositions of the invention.

The lipoxidase component of the compositions of the invention, whether incorporated as a pure or substantially pure component or as a commercially available preparation, is employed in an amount sufficient to incorporate from 0.01 percent to 2 percent lipoxidase on a pure enzyme basis. This amount provides sufficient enzymatic activity to facilitate the removal of soils and stains from laundered goods.

Lipoxidase activity can be determined by known methods. For example, oxygen uptake and measurement of peroxide formation by thiocyanate method can be employed. Assay methods involving destruction of a substrate such as carotene under prescribed conditions can also be used. A suitable and preferred method is a spectrophotometric assay method whereby a homogeneous substrate and the products of the primary reaction are measured by ultraviolet light absorption. Peroxide formation is proportional to time and to enzyme concentration of wide ranges. Lipoxidase activity can be determined by spectrophotometric assay according to the following procedure.

To the main compartment of a side arm test tube is added 1.0 ml. of substrate solution containing 2 mg. linoleic acid in borate buffer at pH 9.0. The enzyme in 0.2 ml. borate buffer is pipetted into the side arm, the tube is flushed with O_2 and stoppered. After the temperature has been adjusted to 20° C. by placing in a water bath, the tube is rocked to mix the contents, and at the end of 2 minutes 2.0 ml. absolute ethanol are added to stop the reaction. The mixture is diluted 10 times with 60 percent alcohol and the absorption of light at 2340 Å. is measured with a Beckman spectrophotometer. Correction is

made for the light absorption of the enzyme preparation and the substrate. Activity of an enzyme or enzyme-containing composition is expressed in units/mg. and is determined according to the following relationship

$$\text{activity} = \Delta A / \text{min.} \times 1,000 / \text{mg. enzyme}$$

wherein ΔA is the change in absorption. Activity values employed in the specification and claims herein refer to those determined by the hereinbefore described method. This method is described by R. T. Holman, et al., "The Enzymes," 1st ed. Vol. II, Part I, pp. 563-4 (1951).

The activity of suitable lipoxidases of the invention, determined by the hereinbefore described spectrophotometric assay method, will vary depending upon factors hereinbefore mentioned. For example, pure lipoxidases may have an activity of 100,000 units/mg. or more. The more readily available lipoxidase-containing preparations such as the commercially available preparations derived from soybeans range in activity from about 10,000 to 50,000 units/mg. These compositions employed in the detergent compositions of the invention in an amount by weight of from 0.1 percent to 20 percent as hereinbefore described provide lipid soil and stain removal in soaking and washing solutions.

The detergent compositions described herein are not restricted or limited to any special physical form. They can, for example, be solids such as granular compositions made by spray-drying or coagglomeration processes or liquid or paste compositions. They can be employed in the form of liquid compositions for application directly as by spraying onto stained fabrics or added to conventional detergent compositions. Granular compositions can be employed to advantage as presoaking or washing compositions.

The lipoxidase-containing detergent compositions of the invention can contain additional or minor amounts of materials which make the compositions more effective or attractive. The usual detergent adjuvants, diluents and additives can be employed, the following being mentioned by way of example. Soluble sodium carboxymethyl cellulose can be added in minor amounts to inhibit soil redeposition. A tarnish inhibitor such as benzotriazole or ethylenethiourea can also be added in amounts up to about 2 percent. Fluorescers, perfumes, dyes, suds builders, suds depressors, bacteriostats and the like can be employed herein without detracting from the advantageous properties of the composition of the invention.

The detergent compositions of this invention can contain water-soluble alkaline detergency builder salts, either of the organic or inorganic types. The ratio of builder salts to organic detergent is preferably from about 1:4 to about 20:1, more preferably from about 0.7:1 to about 9:1. Examples of suitable water-soluble inorganic alkaline detergency builder salts are alkali metal carbonates, borates, phosphates, polyphosphates, bicarbonates and silicates. Specific examples of such salts are sodium and potassium tetraborates, bicarbonates, carbonates, tripolyphosphates, pyrophosphates, orthophosphates, and hexametaphosphates. Examples of suitable organic alkaline detergency builder salts are: (1) Water-soluble aminopolycarboxylates [e.g., sodium and potassium ethylenediaminetetraacetates, nitrilo triacetates, and N-(2-hydroxyethyl)-nitrilo diacetates]; (2) Water-soluble salts of phytic acid (e.g., sodium and potassium phytates—see U.S. Pat. No. 2,739,942); (3) Water-soluble salts of ethane-1,1-dihydroxy-1,1-diphosphonate (e.g., the trisodium and tripotassium salts—see U.S. Pat. No. 3,159,581); (4) Water-soluble salts of methylene diphosphonic acid (e.g., trisodium and tripotassium methylene diphosphonate and the other salts described in the copending application of Francis L. Diehl, Ser. No. 266,025, filed Mar. 18, 1963, now U.S. Pat. No. 3,213,030); (5) Water-soluble salts of substituted methylene diphosphonic acids (e.g., trisodium and tripotassium ethylidene, isopropylidene, benzylmethylidene, and halomethylidene diphosphonates and the other substituted methylene diphosphonates disclosed in U.S. Pat. No. 3,422,021 to

Clarence H. Roy, issued Jan. 14, 1969; (6) Water-soluble salts of polycarboxylate polymers and copolymers as described in the copending application of Francis L. Diehl, Ser. No. 269,359, filed Apr. 1, 1963, now U.S. Pat. No. 3,260,153.

(Specifically, a polyelectrolyte builder material comprising a water-soluble salt of a polymeric aliphatic polycarboxylic acid having the following structural relationships as to the position of the carboxylate groups and possessing the following prescribed physical characteristics: (a) a minimum molecular weight of about 350 calculated as to the acid form; (b) an equivalent weight of about 50 to about 80 calculated as to acid form; (c) at least 45 mole percent of the monomeric species having at least two carboxyl radicals separated from each other by not more than two carbon atoms; (d) the site of attachment to the polymer chain of any carboxyl-containing radical being separated by not more than three carbon atoms along the polymer chain from the site of attachment of the next carboxyl-containing radical. Specific examples are polymers of itaconic acid, aconitic acid, maleic acid, mesaconic acid, fumaric acid, methylene malonic acid, and citraconic acid and copolymers with themselves and other compatible monomers such as ethylene), and (7) mixtures thereof.

Mixtures of organic and/or inorganic builders can be used and are generally desirable. Especially preferred are the mixtures of builders disclosed in U.S. Pat. No. 3,392,121 to Burton H. Gedge, issued July 9, 1968, e.g., ternary mixtures of sodium tripolyphosphate, sodium nitrilotriacetate, and trisodium ethane-1,1-dihydroxy-1,1-diphosphonate.

It is preferred that the compositions of the invention contain in addition certain proteolytic and amylolytic enzymes. The enzymes include the alkaline, neutral and acid proteases and amylases which aid materially the removal of proteinaceous and starchy soils and stains from laundered textiles. The employment of proteolytic and amylolytic enzymes in combination with the lipoxidases of the present invention is preferred from the standpoint of facilitating the removal of a broad spectrum of varied soils and stains. The preferred proteolytic enzymes are the subtilisins obtained from the bacterial organism, *Bacillus subtilis* and from Streptomyces organisms. Preferred amylases are those derived from *Bacillus subtilis* by fermentation methods known in the art. Examples of proteases and amylases are described for example in U.S. Pat. No. 3,451,935 to Roald, et al., issued June 24, 1969. The proteases and amylases are employed in an amount each of from 0.001 percent to 10 percent by weight, on a pure enzyme basis, of the enzyme-containing detergent compositions of the invention.

The compositions of the invention preferably contain a water-soluble inorganic electrolyte salt. It has been found that the presence of such a salt preserves enzymatic activity in soaking and washing solutions and in some instances enhances the detergency properties of the composition. The presence of such salts inhibits loss of lipoxidase activity thereby maximizing the oxidative and/or altering effects of lipoxidase. Suitable electrolyte salts, employed in an amount of from 1 percent to 50 percent by weight of the compositions of the invention, include the water-soluble alkali metal, alkaline earth metal and ammonium chlorides, sulfates, borates, nitrates, acetates and the like. Examples include sodium chloride, calcium chloride, potassium chloride, magnesium chloride, sodium sulfate, sodium phosphate, sodium borate, magnesium sulfate, magnesium nitrate, sodium acetate, borax and the like.

According to a preferred embodiment of the invention, the compositions of the invention contain a fatty acid component, or lower alkyl ester thereof, characterized by having from 12 to 20 carbon atoms, and preferably 18 to 20 carbon atoms, in the fatty acid moiety and having methylene-interrupted cis-, cis-double bonds. Compounds having less than 12 carbons in the fatty acid chain tend to be less detergents while those having more than 20 tend to be difficultly soluble in water. While applicants do not wish to be bound by theory, the added fatty acid or derivative is believed to act as a substrate which is con-

verted by the action of a lipoxidase and dissolved oxygen to a hydroperoxide specie. This specie in turn is believed to aid the laundering process by bleaching, decolorizing and/or removing soils and stains from laundered goods or by preventing the redeposition of soils and stains by modification of their color characteristics or affinity for textile fabrics. The employment of added substrate to facilitate stain removal by an oxidative mechanism, termed herein coupled oxidation, makes possible the oxidation of components such as carotene, chlorophyll, hemoglobin and lycopene which occur in carrot, grass, blood and tomato stains and the like.

Suitable unsaturated fatty acids for purposes of coupled oxidation are the fatty acids having from 12 to 20 carbon atoms and include the readily available linoleic, linolenic and arachidonic acids. Lower alkyl esters such as the C₁- to C₅-alkyl, e.g., methyl-, ethyl-, *n*-propyl- and isopropyl-, esters can also be employed to advantage.

The compositions of the invention can additionally contain a component which provides an available source of oxygen for the lipoxidase of the invention. While the amount of oxygen or air present in an agitated washing solution is sufficient to permit stain removal by a lipoxidase, an added source of oxygen can be incorporated into the compositions of the invention. Suitable oxygen-yielding compounds are the inorganic peroxy compounds including the peracids and persalts. Suitable examples are the alkali metal (e.g. sodium and potassium) and ammonium perborates, percarbonates, persulfates and perpyrophosphates. These compounds are employed in an amount of from 1 percent to 30 percent or more of the total composition. In addition to providing available oxygen to facilitate lipoxidase catalysis, the presence of a peroxy compound in some instances tends to reduce the amount of redeposition of soils and stains onto a soaked or laundered fabric. The presence of such peroxy compound for its improved antiredeposition properties, thus, constitutes a preferred embodiment.

Inorganic or enzymatic catalysts which facilitate evolution of oxygen or degradation of the peroxy compound to thereby provide available oxygen can also be employed. Suitable catalysts, employed in an amount up to 1 percent, include catalase, manganese dioxide, finely divided metals such as powdered copper or iron, water-soluble halites and hypohalites as, for example, sodium chlorite and sodium hypochlorite, molybdates and hemin-type compounds. Preferred herein is catalase.

The compositions of the invention are illustrated by the examples which follow. The examples are not to be regarded as limiting the invention. All amounts, percentages and ratios in the specification and claims are by weight unless otherwise indicated.

EXAMPLE I

The soil-removal properties of lipoxidase-containing detergent compositions of the invention were evaluated by a detergent test termed herein the Facial Swatch Test. This test involves a procedure of soiling a cloth swatch with natural soil by attaching a swatch (about 5 inches by 5 inches) to the plunger cup of an electric vibrator massager. Two swatches are soiled from an individual subject by massaging the right and left halves of the face respectively of 1 minute each. The soiled swatches are then randomized into groups to statistically provide equal numbers of left and right samples. Groups of six swatches each are then subjected to soaking and washing treatments in the compositions to be evaluated. Each group of swatches is subjected to four cycles, each cycle including soiling, soaking and washing. Whiteness variations are then measured.

The detergent compositions tested were soaking solutions having the components and soaking conditions described in table I. Amounts expressed in table I are weight percent.

TABLE I

Components

Soaking composition

	A	B	C
3-(N,N-dimethyl-N-hexadecyl ammonio)-propane sulfonate	0.05	0.05	0.05
Boric Acid, H ₃ BO ₃	0.31	0.31	0.31
Lipoxidase preparation (containing about 10 % soybean lipoxidase and having an activity of about 10,000 units/mg.)	0.01	0.01	0.01
Water	Balance to 100%		
Soaking conditions			
pH	9	7.5	7.5
Temperature (°F.)	80	80	80
U.S. hardness (grains/gallon)	0	1.5	5
Soaking time (hours)	4	4	4

Following each soaking, the swatches were rinsed twice in 85° F. water (0 grain/gallon hardness). Each washing treatment was conducted by washing the swatches in a mechanical washer equipped with an agitator and which simulates a home washing machine. The washing composition employed was a conventional built anionic-containing detergent formulation employed in an amount of 1.75 grams/1.5 gallon water (equivalent to 1 cup/17 gallons water). Each washing step (10 minutes) was conducted at 140° F., pH 10 in 0 grain/gallon water. The detergent composition employed in the washing step was as follows:

Ingredient	Parts by weight
A mixture of 55% sodium tallow alkyl sulfate and 45% sodium linear alkyl benzene sulfonate wherein the alkyl chain distribution is 16% C ₁₁ , 27% C ₁₂ , 35% C ₁₃ , and 22% C ₁₄ .	17.5
Sodium tripolyphosphate	50.0
Sodium silicate having an SiO ₂ :Na ₂ O ratio of 1.8:1	6.0
Coconut fatty acid ammonio amide	2.5
Sodium sulfate	14.0
Water	10.0

Following the washing of the swatches, they were rinsed and dried and then whiteness measurements were made with a commercially available photoelectric reflectometer, i.e., a Hunter Color and Color Difference meter (Model D25) manufactured by Hunter Associates Laboratory, Fairfax, Va. This instrument is designed to distinguish color differences and operates on the tristimulus colorimeter principle. According to this principle, a 45° diffuse reflectance of an incident light beam on a test specimen is measured through a combination of green, blue and amber filters. The electrical circuitry of the instrument is so designed that lightness and chromaticity values for the test specimen are read directly. The departure from white (MgO being taken as a standard white) of the test specimen is calculated by introducing the lightness and chromaticity values so obtained into a complex formula supplied by the manufacturer. An evaluation of relative whiteness performance compared to a standard treatment is thus obtained for the test formulations.

The measurements obtained by the foregoing procedure are given below in table II. The greater the Hunter value, the greater the whiteness level. A statistically significant difference is 0.24.

The measurements obtained by the foregoing procedure and presented in table II are compared with those obtained from swatches which were subjected to two control treatments. In the case of Control-1, swatches were treated as described above except that no soaking step was employed, i.e., the soiled swatches were washed in the conventional anionic-containing detergent formulation. In the case of Control-2, the swatches were soaked and laundered as described, the soaking being conducted in the same composition utilized in the washing step. The soaking step was conducted in a solution of the detergent composition of 1.75 grams/1.5 gallon water; 0 gram hardness; 4 hours; 80° F.; and pH 9.6.

TABLE II

Treatment	Hunter whiteness
Composition A	0.24
Composition B	0.21
Composition C	0.05
Control-1	-0.09
Control-2	0.00

It can be seen from the foregoing table that soaking with Compositions A, B and C of the invention effects significant soil removal compared to Controls 1 and 2. Extraction (with chloroform and methanol) of the fabrics after the last washing step confirmed the greater soil removal obtained by soaking in Compositions A, B and C. A lesser amount of soil was extracted from swatches treated with Compositions A, B or C than was extracted where the Control-1 and -2 treatments were employed.

EXAMPLE II

Muslin swatches were stained by passing strips of muslin through a padding bath containing the staining solution, passing the muslin through wringers and a drying oven. The stained strip was cut into 5x5¼ inch swatches. These swatches were laundered in an automatic miniature washer at 125° F. in water of 7 grain hardness for 10 minutes. The composition being tested was used to wash a soiled load consisting of three swatches each of (1) gravy (a substrate sensitive to proteolytic activity and having a content of cis-, cis- polyunsaturated component), (2) spinach (a substrate primarily sensitive to proteolytic activity), and (3) milk substitute (a substrate primarily sensitive to proteolytic activity), and (4) licorice (a substrate primarily sensitive to amylolytic activity) stained muslin in the presence of two untreated terrycloth swatches added to provide bulk to the washload.

The control composition used for a comparison was a conventional built anionic-containing detergent formulation and was employed in an amount of 6.75 grams/1½ gal. water (equivalent to 1 cup/17 gal. water). The lipoxidase to be evaluated (0.01 percent by weight of the washing solution) was added in the form of a water solution to provide the desired level of enzyme. The swatches were washed, dried, and ironed and their whiteness levels were measured employing a Hunter Color-Difference Meter described hereinbefore. The control detergent for comparison purposes was the anionic-containing composition described in example I. The lipoxidase preparation was a commercially available lipoxidase preparation con-

	Gravy Stain	Hunter whiteness Spinach Stain	Milk Stain	Licorice Stain
5 Control composition	75.56	37.03	105.43	94.93
Control composition + lipoxidase	80.26	36.63	103.30	94.19
	+4.70	-0.40	2.13	0.74

- 10 The effectiveness of the lipoxidase in removing gravy stains is readily apparent. As respects the gravy stain, a difference of 1.91 Hunter Whiteness units is a visually observable difference; with spinach stain, an observable difference is 2.47 units; with milk stain, 5.91 units; and with licorice stains, 2.76 units.

EXAMPLE III

- The stain-removing properties of a lipoxidase-containing composition of the invention were further evaluated by employing a test involving 10 stains and four fabrics. Duplicate swatches (16.5 cm.x22 cm.) of cotton, Dacron, nylon and a Dacron/polyester blend were stained with ten staining materials, each of the fabric swatches containing one stain from each of such materials. The following stains were placed onto the fabric swatches: felt-tipped pen ink (green); ballpoint pen ink (blue); liquid shoe polish (brown); dirty motor oil; mustard; chocolate; French salad dressing; lipstick; bacon grease; and grass. Four stained swatches (four fabrics) were soaked in a composition identical to Composition A of example I except that the lipoxidase preparation was a commercially available preparation containing about 35 percent soybean lipoxidase and having an activity of about 35,000 units/mg. Four duplicate swatches were soaked under identical conditions in a control composition identical to Composition A but containing no lipoxidase. In each instance soaking was conducted for 4 hours at 30° C. in water of 0 grain/gallon U.S. hardness and at a pH of 9.0. Following the soaking period, the swatches were wrung out by hand and washed in an automatic miniature washer at 60° C. for 10 minutes in water of 0 grain hardness at a pH of 10. The washing solution was a 0.15 percent solution of the anionic-containing detergent formulation described in examples I and II. The laundered swatches were rinsed, dried and ironed and their stain removal properties visually measured by a panel of three judges. Each stain on each of the stained and laundered fabrics was compared by each of the three judges with standard stained swatches having on each type of fabric a gradation of staining degrees ranging from zero (no stain) to 10 (corresponding to the degree of staining of the test swatches prior to treatment). The values reported in table IV represent an average of the three visual grades accorded each stain. A difference of one unit is considered to represent a visually detectable difference.

TABLE IV

Fabric	Felt-tipped pen ink	Ball-point pen ink	Liquid shoe polish	Dirty motor oil	Mustard	Chocolate	French Dressing	Lipstick	Bacon Grease	Grass
Control:										
Cotton.....	9	3	8	8	6	2	6	0.8	1	2
Dacron.....	10	0.5	8	10	1	0	8	1	0.7	2
Nylon.....	8	8	6	6	7	0	0.7	1	0	1
Dacron/polyester.....	9	6	7	9	6	0	2	2	0	2
Composition A:										
Cotton.....	8	4	8	8	4	3	0	0.7	0.7	0.7
Dacron.....	9	0.5	9	9	0	0	0	1	0.7	2
Nylon.....	4	9	7	5	7	0	0	0.8	0	0.5
Dacron/polyester.....	9	7	6	9	6	0	0	2	0	1

taining about 20 percent soybean lipoxidase and having an activity of about 18,000 units/mg.

Results of these stain tests are tabulated in table III as follows:

TABLE III

- As can be readily seen from the data of table IV, stains having a content of polyunsaturates, e.g., French salad dressing, are removed from textile materials by lipoxidase-containing detergent compositions. Stain removal was also observed in the case of the treatment of certain fabrics with stains which normally contain only minor amounts, if any, of polyunsaturated components. Thus, appreciable mustard stain removal from cotton and Dacron swatches is observed from the data of

table IV. Similarly, stain removal is observed in the case of cotton, Dacron and nylon swatches stained with felt-tipped pen ink.

Granular detergent washing compositions having excellent soil and stain-removing properties in the laundering of textile goods have the following compositions:

	1	2	3
Sodium C ₁₁ -alkyl benzenesulfonate (derived from tetrapropylene)	31.8	32.4	35.0
Sodium tripolyphosphate	30.7	22.7	24.5
Sodium silicate	6.1	6.2	6.7
Sodium sulfate	8.8	9.0	9.7
Tetrasodium pyrophosphate (Na ₂ P ₂ O ₇)	6.8	6.8	6.8
Alcalase (pyroteolytic enzyme)	0.72	0.72	0.72
Monsanto DA-10 (mixture of proteolytic and amylolytic enzymes)	0.72	0.72	0.72
Soybean lipoxidase preparation (having an activity of 50,000 units/mg.)	1		5
Water (moisture)	8.7	8.8	9.5
Miscellaneous detergent additives	Balance to 100%		

When in example IV any of the following detergents are substituted for the sodium alkyl benzene sulfonate detergent substantially similar results are obtained: sodium coconut soap; sodium linear alkyl benzene sulfonate having a chain length distribution of 10 percent C₁₀, 30 percent C₁₁, 35 percent C₁₂, 16.5 C₁₃, 8 percent C₁₄ and 0.5 C₁₅; sodium tallow alkyl sulfate; the condensation product of one mole of coconut alcohol with 5 moles of ethylene oxide; the condensation product of one mole of octyl phenol with 20 moles of ethylene oxide; the condensation product of one mole of coconut alcohol with 20 moles of ethylene oxide; dimethylhydroxydodecylamine oxide; cetyldimethylphosphine oxide; sodium-3-dodecylaminopropionate; and 3-(N,N-dimethyl-N-decylammonio)-2-hydroxypropane-1-sulfonate.

EXAMPLE V

An excellent presoaking composition effective in the removal of proteinaceous and starchy stains and stains having a content of cis-, cis-polyunsaturates has the following composition in parts by weight:

Ethoxylated tallow fatty alcohol 30 moles ethylene oxide per mole of alcohol	4.0
Sodium tripolyphosphate	69.3
Sodium perborate	25.0
Alcalase (proteolytic enzyme)	0.55
Maxatase (proteolytic enzyme)	0.55
Monsanto DA-10 (mixture of proteolytic and amylolytic enzymes)	0.10
Soybean lipoxidase preparation (having an activity of 39,000 units/mg.)	3
Miscellaneous (brightener, perfume, dye, etc.)	0.52

EXAMPLE VI

A granular presoak detergent composition having the following components in parts by weight provides soil- and stain-removal properties and improved anti-redeposition properties:

3-(N,N-dimethyl-N-hexadecylammonio)-propanesulfonate	12.6
Sodium sulfate	12.6
Sodium tripolyphosphate	37.5
Soybean lipoxidase preparation (having an activity of 50,000 units/mg.)	6.3
Linoleic acid	17.6
Sodium perborate, NaBO ₃ · 2 O	3.1
Catalase	0.02
Moisture	10.0

What is claimed is:

1. An enzyme-containing detergent composition having soil- and stain-removal properties consisting essentially of:

A. from 1 percent to 50 percent of a water-soluble synthetic organic detergent; and

B. from 0.01 percent to 2 percent by weight of the enzyme-containing detergent composition of a lipoxidase having lipoxidase enzymatic activity in the temperature range of from 5° C. to 70° C. and in the pH range of from 5 to 11.

2. The enzyme-containing detergent composition of claim 1 wherein the lipoxidase is a preparation consisting essentially of a mixture of from 1 percent to 50 percent lipoxidase and from 50 percent to 99 percent of inert carrier or vehicle materials.

3. The composition of claim 2 wherein the lipoxidase preparation has an activity of from 10,000 to 50,000 activity units/mg.

4. The detergent composition of claim 3 wherein the lipoxidase preparation is present in an amount by weight of from 0.1 percent to 20 percent.

5. The detergent composition of claim 4 wherein the lipoxidase is soybean lipoxidase.

6. The detergent composition of claim 5 wherein a water-soluble alkaline detergency builder salt is present in a ratio of said water-soluble alkaline detergency builder salt to water-soluble synthetic organic detergent of from 1:4 to 20:1.

7. The detergent composition of claim 6 wherein a fatty acid having from 12 to 20 carbon atoms and methylene-interrupted cis-, cis-double bonds or a C₁ to C₃ alkyl ester thereof is present.

8. The detergent composition of claim 7 wherein an inorganic peroxy compound is present in an amount of from 1 percent to 30 percent.

9. The detergent composition of claim 8 wherein the fatty acid is linoleic acid.

10. The detergent composition of claim 9 wherein the inorganic peroxy compound is sodium perborate.

* * * * *

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

16.5
252-99

Patent No. 3,635,828 Dated January 18, 1972

Inventor(s) Lawrence Benjamin and John F. Sullivan

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

- Col. 1, line 12, "NO" should be -- No. --.
- Col. 1, line 32, "difficulty removable" should be
-- difficultly-removable --.
- Col. 1, line 34, "difficulty removable" should be
-- difficultly-removable --.
- Col. 1, line 49, "difficulty removable" should be
-- difficultly-removable --.
- Col. 1, line 67, "difficulty" should be -- difficultly --.
- Col. 3, line 63, "RR'R"P O" should read -- RR'R"P → O --.
- Col. 4, line 1, the formula reads
- $$\text{"R}^5 - \overset{\text{O}}{\underset{|}{\text{S}}} - \text{R}^6 \text{"};$$
- it should read:
- $$\text{-- R}^5 - \overset{\text{O}}{\underset{\uparrow}{\text{S}}} - \text{R}^6 \text{--}.$$
- Col. 4, line 5, "R₅" should be -- R⁵ --.
- Col. 6, line 3, "Copr." should read -- Corp. --.
- Col. 8, line 64, "odium" should be -- sodium --.
- Col. 8, line 73, "difficulty" should read -- difficultly --.
- Col. 9, line 23, "can e" should be -- can be --.

Col. 13, between lines 3 and 4 insert

-- EXAMPLE IV --;

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,635,828 Dated January 18, 1972

Inventor(s) Lawrence Benjamin and John F. Sullivan

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Col. 13, line 18, after "(having" insert -- an activity of 50,000 units/mg.) --.

Col. 13, line 18, under heading "2" and after "1" insert -- 2 --.

Col. 14, line 13, " $\text{NaBO}_3 \cdot 20$ " should read -- $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ --.

Signed and sealed this 8th day of August 1972.

(SEAL)

Attest:

EDWARD M. FLETCHER, JR.
Attesting Officer

ROBERT GOTTSCHALK
Commissioner of Patents