STABILIZED AQUEOUS ENZYME CONTAINING COMPOSITIONS

11 Claims, No Drawings

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ABSTRACT: Stabilized aqueous enzyme compositions containing a protease and/or an α-amylase and a stabilizing agent selected from the group consisting of dialkyl glycol ethers; heterocyclic oxethers; and dialkyl ketones are described. These compositions, which can also contain a nonionic or zwitterionic detergent, are useful cleaning compositions particularly in the removal of soils and stains from textile materials.
STABILIZED AQUEOUS ENZYME CONTAINING COMPOSITIONS

FIELD OF THE INVENTION

This invention relates to stabilized proteolytic and/or amylolytic enzyme-containing compositions useful in the removal of stains and soils from textile materials. More particularly, it relates to aqueous enzyme-containing compositions containing a minor amount of a stabilizing agent which preserves the enzymatic activity of the proteolytic and/or amylolytic enzyme.

The use of proteolytic and amylolytic enzymes in laundry products is known. See for example, U.S. Pat. No. 1,882,270 to Frelinghusen Okt. 11, 1932) and Iaag, "Effect of Enzymatic Detergents," Seifen Ole, Fette, Wachse, 88, No. 24, pp. 789-793 (Nov. 1962). These enzymes aid in the laundry process by attacking soil and stains found on soiled fabrics and decomposing and/or altering them so as to render them more removable during laundering. Enzymatic materials suitable for laundering are expensive and powerful materials which must be judiciously formulated and used. These enzymes when employed in aqueous compositions are unstable and suffer appreciable destruction, particularly during long periods of storage, resulting in substantial loss of soil- and stain-removing efficacy. The loss in enzymatic activity is particularly severe under conditions of high temperature. Moreover, such aqueous solutions often contain additional components which are desirable from the standpoint of detergenting and cleaning but which exert a harmful effect on the enzymatic material. The result is further loss of enzymatic activity.

Numerous attempts have been made in the art to provide aqueous enzyme-containing compositions wherein the enzymatic activity is preserved by the incorporation of stabilizing agents. U.S. Pat. Nos. 3,050,445 (Aug. 21, 1962), 3,095,358 (June 25, 1963) and 3,352,364 (June 13, 1964) illustrate attempts to stabilize aqueous solutions of enzymes. None of the prior art methods of stabilizing aqueous enzyme-containing compositions against loss in enzymatic activity have been entirely satisfactory. Generally, these methods provide aqueous compositions which either lose appreciable enzymatic activity upon long periods of storage or require the employment of large amounts of stabilizing agents to effect suitable levels of enzyme stabilization.

It is therefore an object of this invention to provide stabilized aqueous solutions which retain substantially their enzyme activity upon storage.

It is another object of this invention to provide aqueous proteolytic and/or amylolytic enzyme-containing compositions stabilized substantially against loss of activity by the presence of minor amounts of an enzyme-stabilizing compound.

Other objects of this invention will be obvious from consideration of the invention which is more fully described hereinafter.

SUMMARY OF THE INVENTION

These and other objects of the present invention are achieved by the provision of aqueous proteolytic and/or amylolytic enzyme-containing compositions characterized by extended periods of stabilization and containing minor amounts of certain organic stabilizing agents. The aqueous compositions of the present invention can additionally contain nonionic or zwitterionic detergent components to enhance the stability of the enzymes in the aqueous compositions of this invention and to provide the stabilized compositions with excellent detergent properties. The present invention is based in part on the discovery that dialkyl glycol ethers, heterocyclic ethers, and dialkyl ketones, provide enzyme stabilization for extended periods of time.

The stabilized aqueous enzyme-containing compositions of this invention comprise:

1. from about 65 to about 97 percent water;

2. from about 0.001 to about 1.0 percent enzyme selected from the group consisting of proteases, a-amylases, and mixtures thereof;

3. from about 2 to about 27 percent of a stabilizing agent selected from the group consisting of dialkyl glycol ethers having the general formula

$$R_1O(CH_2CH_2O)xR_2$$

wherein $R_1$ and $R_2$ are each alkyl of one to about four carbon atoms and $x$ is one to about 10; heterocyclic oxoethers; and dialkyl ketones of the general formula

wherein $R_1$ and $R_2$ are each alkyl of one to about four carbon atoms; and

4. from zero to about 15 percent of a detergent selected from the group consisting of nonionic detergents and zwitterionic detergents.

The proteolytic enzymes which can be stabilized in aqueous solution by the action of the hereinbefore described organic stabilizing agents include alkaline proteases, neutral proteases, and acid proteases. Proteases in these classifications are generally derived from fungal and bacterial sources. Enzymes derived from plant and animal sources can also be utilized herein but are not as readily classified as alkaline, neutral, and acid subclasses. These enzymes are active in the pH range of from about 3 to about 11 and at temperature ranging from about 40 to about 170°F. Optimum activity of these proteases is generally exhibited in the pH range of from 5.0 to 10 and preferably from 6.0 to 9.5.

The proteases are particularly effective in degrading protein soil. These proteases catalyze the hydrolysis of the peptide linkage of proteins, polypeptides, and related compounds. Free amino and carboxy groups are thus obtained and the long chain protein structures are reduced to several shorter chains. These shorter chains can easily be removed from their environment with water of aqueous detergent compositions.

The alkaline proteases are particularly preferred enzymes for use herein. Alkaline proteases which are suitable for use in this invention include subtilisin, BPN', elastase, keratinase, carboxypeptidase, amino peptidase, aspergillopeptidase A and aspergillopeptidase B. Subtilisin and BPN' are especially preferred for use herein. The alkaline proteases are particularly preferred for use in this invention as they show optimum activity in the pH range of normal detergent components, i.e., 7.5 to 10.5, and the alkaline proteases show surprising stability in the compositions of this invention.

The neutral proteases which can also be utilized in the compositions of this invention include collagenase, chymotrypsin, and trypsin and those proteolytic enzymes isolated from Streptomyces species. Both chymotrypsin and trypsin show optimum activity in the neutral to alkaline range.

Examples of acid proteases suitable for use herein include pepsin, papain, and bromelin. Both papain and bromelin show optimum activity in the acidic range.

The a-amylases are also stabilized in the compositions of this invention. All of the a-amylases show optimum activity in the acid range. The a-amylases are particularly well suited for breaking down starch molecules as they attack the 1,4-glycosidic linkages in starch. The remaining shorter chains are easily removed from their environment with water or aqueous solutions of detergents. The a-amylases may be obtained from animal sources, cereal grains, bacterial or fungal sources.

Commercial enzyme compositions containing the above-described enzymes are suitable for use herein. These commercial enzyme compositions are generally sold in a dry powdered form and are comprised of from about 2 to about 80 percent active enzymes in combination with an inert powdered vehicle such as sodium or calcium sulfate or sodium chloride as the remaining 20 to 98 percent. The active enzyme content of commercial enzyme compositions is the result of manufacturing methods employed and is not critical herein so long as the
finished compositions of this invention have the specified enzyme content. The insoluble inert materials are generally removed from the compositions of this invention to provide compositions having suitable clarity and which are free of precipitates.

Specific examples of commercial enzyme compositions suitable for use herein and the manufacturer thereof include: Alcalase, Novo Industri, Copenhagen, Denmark; Maxatase, Koninklijke Nederlandse Spiritusfabriek De Delft, Netherlands; Protease B-400 and Protease AP, Schweizerische Ferment A.G., Basel, Switzerland; CRD-Protease, Monsanto Company, St. Louis, Mo.; Viokase, VioBin Corporation, Monticello, Ill.; Pronase-P, Pronase-E, Pronase-AS and Pronase-AF all of which are manufactured by Kaken Chemical Company, Japan; Bioprase, Nagase & Co., Ltd., Osaka, Japan; Rapidase P-2000, Rapidase, Seclin, France; Takamine, Bromelain 1:10, HI proteolytic enzyme 200, Enzyme L-W and a-amylase, Miles Chemical Company, Elkhart, Ind.; Rhomyl P-11 concentrate, Pectinol, Rhomyl PF, Rhomyl J-25, Rohm & Haas, Philadelphia, Pa. (Rhomyl PF and J-25 have salt and cornstarch vehicles and are proteases having diastase activity); Amaprozyme 200, Jacques Wolff, a subsidiary of Nopco Chemical Company, Newark, N.J. and Wallerstein 627P, Wallerstein Company, Staten Island, N.Y.

CRD-Protease (also known as Monsanto DA-10) is a useful powdered enzyme composition. CRD-Protease is reported to be obtained by mutation of a Bacillus subtilis organism. It is composed of neutral and alkaline proteases and a-amylase. The neutral protease has a molecular weight of about 44,000 and contains from one to two atoms of zinc per molecule. The CRD-Protease can be used in aqueous systems such as the present invention. The active enzyme content of CRD-Protease on a weight percent basis generally ranges from about 20 to about 75 percent.

Pronase-P, Pronase-E, Pronase-AS and Pronase-AF are powdered enzyme compositions which can also be used to advantage in this invention. These enzymes are produced from the culture broth of Streptomyces griseus used for streptomycin manufacture. They are isolated by a successive resin column treatment. The major component of Pronase is a neutral protease, Streptomyces griseus protease. This enzyme composition contains a calcium stabilizer salt and is fairly stable over a pH range, e.g., 4.0 to 10.0; and is fairly stable over a temperature range of 50° to 55°F.

Another enzyme composition preferred for use in the compositions of this invention is Alcalase which is manufactured and sold by Novo Industri A/S, Copenhagen, Denmark. Alcalase is described in a trade bulleting which was published by Novo Industry A/S, as a proteolytic enzyme prepared and manufactured by submerged fermentation of a special strain of Bacillus subtilis. The primary enzyme component of Alcalase is subtilisin. In addition to Proteases, Alcalase contains small amounts of a-amylase. Alcalase is a fine grayish free-flowing powder having a crystalline active enzyme content of about 6 percent. The remainder of the powder has a crystalline active enzyme content of about 6 percent. The remainder of the powder is comprised primarily of sodium sulfate, calcium sulfate and various inert organic and inorganic vehicle materials. Alcalase has unusually stable properties in the aqueous compositions of this invention.

Biophase is a powdered enzyme composition which contains alkaline proteases (BPN’) and a-amylases. This enzyme composition can be obtained with or without the presence of diuretics such as sodium and calcium sulfate.

Large variations in the amount of enzymes in the compositions of this invention are contemplated. The compositions can contain from about 0.001 to about 1.0 percent enzyme by weight of the composition. For best results, the compositions preferably contain from 0.01 to about 0.5 percent enzymes by weight. When one of the preferred enzyme compositions is utilized herein, the compositions of this invention preferably contain from about 0.1 to about 4.0 percent of the enzyme composition as it is sold in commercial form, e.g., from about 2 to about 80 percent active enzyme. The active enzyme content of the aqueous enzyme composition of this invention should, in any event, range between 0.001 and 1 percent as above delineated.

The stabilizing agents which stabilize the enzymes described above are water-soluble organic compounds selected from the group consisting of dialkyl glycol ethers, heterocyclic oxyethers and dialkyl ketones. Examples of dialkyl glycol ethers of the formula

$$R_1O(CH_2CH_2O)_nR_2$$

wherein $R_1$ and $R_2$ and $x$ have the same definitions hereinbefore described include ethylene glycol dimethyl ether, ethylene glycol diethyl ether, ethylene glycol di-n-propyl ether, diethylene glycol dimethyl ether, triethylene glycol dimethyl ether, the methyl ether of tetraoxyethylene methanol, the ethyl ether of pentaethoxyethylene ethanol, the methyl ether of hexaethoxyethylene butanol, the butyl ether of decaethoxyethylene butanol and the like. Preferred herein are the alkyl ethers of ethylene glycol, diethylene glycol and triethylene glycol, i.e., those wherein $x$ in the hereinbefore described formula is from 1 to 3. These compounds are preferred from the standpoint of enzyme stabilization and ready availability.

Suitable cyclic ethers include the 5 and 6-membered oxetethers. These include the water-soluble solvent ethers such as furan, tetrahydrofuran, dioxiane, pyran, and the like. Substituted derivatives, e.g., C,$_6$H$_5$ alkyl or alkoy-substituted or fused-ring cyclic ethers, can also be employed so long as the compounds are water soluble and do not denature or otherwise injure the enzyme. A preferred ether herein is tetrahydrofuran.

Examples of suitable ketones are acetone, methyl ethyl ketone, diethyl ketone, di-n-propyl ketone, diisopropyl ketone and the like. It will be appreciated that the hereinbefore described stabilizing agents can be employed singly or in combination.

The enzyme stabilizing compounds described above can be employed in the compositions of this invention in effective amounts ranging from about 2 to 27 percent by weight of the composition. The precise amount employed will depend in part on the solubility of the organic stabilizing compound, the nature of the enzyme employed, the desired level of stabilization and economic considerations. Preferably the stabilized compositions of the invention are prepared to contain from about 5 to about 20 percent of the stabilizing agent, the latter range being preferred from the standpoint of optimum stabilizing effects for most enzymes, particularly over long storage periods at high temperature.

It will be appreciated that variation in the levels of stabilization herein will depend as well on the nature of the proteolytic or amylolytic enzyme employed and the particular stabilizing agent employed. When an alkaline protease of bacterial origin is utilized in the aqueous compositions of the invention, best results are obtained when the organic stabilizing agent is ethylene glycol dimethyl ether (1,2-dimethoxyethane), diethylene glycol dimethyl ether, acetone or tetrahydrofuran, these compounds being preferred herein. These stabilizing agents employed in an amount of about 10 percent of the stabilized compositions of the invention are effective in the preservation of a substantial percentage of initial enzymatic activity. Table I, for example, illustrates the preservation of at least about half of initial activity after eight weeks of storage at 100°F.

Preferred compositions herein are those containing mixtures of ketooy and a-amylases, these being preferred by reason of their application in the removal of a wide variety of stains of proteaceous and starchy origin. These compositions preferably contain a mixture of organic stabilizers adapted to the stabilization of the particular enzymes employed. The stabilized compositions of the invention can additionally contain other enzyme stabilizing compounds, such as, alkylammonium alkylx substituted alkylam, polyhydric alcohols,
3,627,688

5 e.g., ethylene glycol, sorbitol or mannitol, gelatins and inorganic calcium or magnesium salts to provide additional enzymes stabilizing effect. These detergents enhance the storage stability of the enzymes employed herein and significantly improve the detergent characteristics of the compositions. Because of these useful characteristics, it is preferred to include nonionic and zwitterionic detergents in the aqueous enzyme compositions of this invention. The nonionics and zwitterionics can be utilized herein in amounts ranging from zero to about 15 percent, preferably from 4 to 10 percent, by weight of the enzyme compositions.

Examples of suitable nonionics for use herein includes:

1. 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 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, diisobutylenes, octene or noneone, for example.

2. 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 to about 80 percent polyoxyethylenes by weight and having a molecular weight of from about 5,000 to 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.

3. The condensation product of 1 mole of aliphatic alcohols having from eight to 22 carbon atoms, in higher straight chain or branched-chain configuration, with from 5 to 40 moles of 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.

4. The unsubstituted amides and the monoethanol and diethanol amides of fatty acid having acyl moieties of from about eight to about 22 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.

5. Long chain tertiary amine oxides corresponding to the following general formula

\[ R^1
\]
\[ +\![\text{O} \text{RO}]_n\text{N}\text{N}=\text{O}
\]
\[ R^2
\]

wherein \(R^1\) is an alkyl radical of from about eight to about 22 carbon atoms, \(R^2\) and \(R^n\) are each methyl, ethyl or hydroxyethyl radicals, \(R^3\) is ethylene, and \(n\) equals from zero 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 and bis-(2-hydroxyethyl)-dodecylamine oxide.

6. Long chain tertiary phosphate oxides corresponding to the following general formula \(\text{RR}^-\text{PO}_3^-\text{O}\text{W herein \(R\) is an alkyl, alkenyl or monohydroxyalkyl radical ranging from 10 to 22 carbon atoms in chain length and \(R^1\) and \(R^n\) 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 phosphate oxides are found in U.S. Pat. No. 3,304,263 which issued Feb. 14, 1967, and include: dimethyldodecylphosphine oxide and bis-(2-hydroxyethyl)dodecylphosphine oxide.

7. Long chain sulfonoxides having the formula

\[ \text{R}^1\text{O}^{-}\text{S}^{-}\text{O}^{-}\text{R}^2
\]

wherein \(R^2\) is an alkyl radical containing from about 10 to about 22 carbon atoms, from zero to about five ether linkages and from zero to about two hydroxyl substituents, at least one moiety of \(R^2\) being uninterrupted by ether linkages and containing from about 10 to about 18 carbon atoms, and wherein \(R^1\) is an alkyl radical containing from one to three carbon atoms and from zero to about 2 hydroxyl groups. Specific examples of these sulfonoxides are dodecyl methyl sulfoxide and 3-hydroxy tridecyl methyl sulfoxide.

The zwitterionic synthetic detergents suitable for use herein 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 22 carbon atoms and one contains an anionic water solubilizing group, e.g., carboxy, sulfon, sulfate, phosphate or phosphono. Examples of compounds falling within this definition are 3-(N,N-dimethyl-N-hexadecylammonio) propane-1-sulfonate and 3-(N,N-diethyl-N-hexadecy1ammonio)-2-hydroxy propane-1-sulfonate. For more examples of zwitterionic synthetic detergents, see Diehl and Smith, "Laundering Fabrics in Cold Water Containing a Synthetic Detergent Composition," Canadian Pat. No. 708,147, issued Apr. 20, 1965 at pp. 6, 1-2.

Mixtures of various nonionic detergents or mixtures of nonionic detergents and zwitterionic detergents can be utilized to advantage herein. Preferred herein are 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 the 3-(N,N-dimethyl-N-alkylammonio)-2-hydroxy propane-1-sulfonates wherein the alkyl has from eight to 22 carbon atoms, e.g., 3-(N,N-dimethyl-N-coconulalkylammonio)-2-hydroxy propane-1-sulfonate. These compounds provide enhancement of protease stability particularly at temperatures at which the unmodified enzyme is inactive and provide excellent detergency and cleaning properties to the compositions of the invention. In addition, they provide desirable levels of a-amylase stabilization.

The stabilized compositions of the present invention are prepared to contain from about 65 to about 97 percent by weight of water. Preferably from about 72 to about 95 percent is employed. Demineralized water is preferred, although not mandatory for use herein.

The various components of the enzyme compositions of this invention can be mixed together in any order. However, it is preferred that a stabilizer-water mixture be prepared first and the enzymes added thereto to prevent any degradation or deactivation in solutions predominately consisting of either water or organic stabilizing compound. The optional detergent components can be added at any time.

The pH of the stabilized aqueous enzyme compositions of this invention generally ranges from about 5.0 to 10.0 and preferably ranges from about 6.5 to about 8.5. Maximum stabilizing effects are obtained in the preferred pH range. The pH can be raised with a base, e.g., sodium or potassium hydroxide, or lowered with an acid, e.g., hydrochloric acid.

It is also preferred, although not mandatory, that a preservative be added to the compositions to prevent bacterial and fungal growth. Phenyl mercuric acetate which is generally utilized herein in amounts ranging from about 10 to about 40
parts per million of the compositions is an effective preservative. Any preservative compatible with the components of the compositions can be utilized herein.

The compositions of this invention can be employed as spot removers, detergent additives or as a detergent cleaning composition per se. These compositions can be packaged in spray-type bottles and conveniently used to remove relatively small spots from fabrics or can be employed in larger quantities as additives to other detergent compositions. These compositions can be substituted for hypochlorite bleaches as they remove many of the stains which these bleaches remove, do not weaken textile fibers, and do not attack or degrade fluorocarbons and whiteners. With the addition of optional nonionic and/or zwitterionic detergents, these compositions can be utilized as such or as excellent cleaning compositions under a variety of washing conditions.

The following stabilized aqueous enzyme compositions (examples 5 to 8) containing 1 percent of Monsanto CRD Protease (a commercially available mixture of protease and amylases derived from Bacillus subtilis) were prepared. Each sample contained sodium chloride, calcium acetate monohydrate, organic stabilizer and water in the amounts employed in examples 1 to 4. Control samples stored under identical conditions containing no organic stabilizer (Control-1) and no stabilizer, sodium chloride or calcium acetate (Control-2) were also evaluated for enzyme stability. The Azocoll method hereinbefore described was employed to measure the amount of alkaline protease activity remaining after storage. One ml. of 0.002 M disodium ethylene-diaminetetraacetate was employed in each Azocoll analysis to inhibit the activity of neutral protease. The results are tabulated in table II as follows:

<table>
<thead>
<tr>
<th>Example</th>
<th>Organic stabilizing agent</th>
<th>Percent remaining activity after storage at 100° F. for—</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 weeks 6 weeks 8 weeks</td>
</tr>
<tr>
<td>None (Control 1)</td>
<td>none (control 2)</td>
<td>9</td>
</tr>
<tr>
<td>Acetone</td>
<td>Acetone</td>
<td>28</td>
</tr>
<tr>
<td>Ethylene glycol dimethyl ether</td>
<td>Ethylene glycol dimethyl ether</td>
<td>37</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>Tetrahydrofuran</td>
<td>7</td>
</tr>
<tr>
<td>Diethylene glycol dimethyl ether</td>
<td>Diethylene glycol dimethyl ether</td>
<td>88</td>
</tr>
</tbody>
</table>

The following stabilized aqueous enzyme compositions (examples 9 to 20) described in table III were prepared. In each example, the water (containing calcium acetate monohydrate and sodium chloride) and stabilizing agent were thoroughly mixed, the nonionic or zwitterionic was added and the enzyme added last. The enzymes were stabilized in each example during high temperature (100° F.) storage. All perform well as spot removers, detergent additives and as detergents per se. The nonionic and the zwitterionic additives enhance storage stability, particularly at temperature of up to about 80° F., and provide excellent detergent properties which make the compositions especially suited as spot removers, detergent additives and as cleaning compositions per se.

<table>
<thead>
<tr>
<th>Example</th>
<th>Organic stabilizing agent</th>
<th>Nonionic or zwitterionic detergent</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethylene glycol dimethyl ether</td>
<td>A B C D E F</td>
<td>Alcalase</td>
</tr>
<tr>
<td>9</td>
<td>10 5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10 5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>10 5</td>
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<td>12</td>
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<td>13</td>
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<td></td>
</tr>
<tr>
<td>19</td>
<td>10 5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>10 5</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Wherein:
A. S-(N,N-dimethyl-N-alkylammonio)propylsulfone is used as the alkyl group.
B. S-(N,N-dimethyl-N-alkylammonio)-3-hydroxypropylsulfone wherein the alkyl group is derived from tallow alcohol.
C. S-(N,N-dimethyl-N-alkylammonio)propylsulfone wherein the alkyl group is derived from middle-cut coconut alcohol (8% C12, 66% C14, 29% C16 and 4% C18).
D. Tetrahydrofuran.
E. Tallow alcohol ethoxylated with 11 moles of ethylene oxide.
F. Tallow alcohol ethoxylated with 36 moles of ethylene oxide.
The following table, table IV, describes additional aqueous enzyme-containing compositions according to the invention enhanced enzyme activity and useful as spot removers, detergent additives and as detergent compositions per se.

<table>
<thead>
<tr>
<th>Example</th>
<th>Triethylene glycol dimethyl ether</th>
<th>Diethylene glycol dimethyl ether</th>
<th>Tetrahydrofuran</th>
<th>Furan</th>
<th>Acetone</th>
<th>Methyl ethyl ketone</th>
<th>Nonionic or zwitterionic detergent</th>
<th>Enzyme</th>
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<tbody>
<tr>
<td>21</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>10</td>
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<td>5</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1 See Example 9-20 of Table III. WALLERSTEIN bacterial a-amylase. 2 Deionized water containing 0.29% NaCl and 0.01% calcium acetate monohydrate.

### EXAMPLE 39

A stabilized aqueous enzyme composition is formulated according to this invention from the following components:

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcalase (1% crystalline enzyme)</td>
<td>1%</td>
</tr>
<tr>
<td>TA-66</td>
<td>1%</td>
</tr>
<tr>
<td>Acetone</td>
<td>10%</td>
</tr>
<tr>
<td>Water</td>
<td>84%</td>
</tr>
<tr>
<td>Phenyl Mercuro Acetate</td>
<td>0.025 p.p.m.</td>
</tr>
</tbody>
</table>

The pH of this preparation is adjusted to 7.0 with sodium hydroxide.

This composition can be employed without dilution as a soil- and stain-removing composition even after extended periods of storage. This composition sprayed directly onto stained or soiled areas of textile materials facilitates the removal of a variety of proteinaceous stains.

This composition can also be employed as an additive to commercial detergent formulations. When about 1.2 ml. of the composition of this invention is added per gallon of washing solution, excellent soil- and stain-removing properties are observed.

### EXAMPLE 40

Results substantially similar to those in the previous examples are obtained when the following enzymes or commercial enzyme compositions are substituted for Alcalase and Monsanto CRD Protease (DA-10) in the enzyme that the enzymes are stabilized in aqueous solutions: Subtilisin, BPN', elastase, keratinase, carboxypeptidase, aminopeptidase, asparaginopeptidase A, asparaginopeptidase B, collagenase, chymotrypsin, trypsin, pepsin, papain, bromelain, Maxatase, Protease B-4000, Protease AP, Alcalase, CRD Protease, Viokase, Pronase-P, Pronase-E, Pronase-AS, Pronase-AF, Bioprase, Rapiplase P-2000, Takamine, HI Proteolytic Enzyme 2000, Enzyme L-W, Thymol P-11 Concentrate, Pectinol, Rhozyme PF, Rhozyme J-25 and Ampoxyse 200.

Results substantially similar to those in the previous examples are obtained when the following stabilizing agents are substituted for those utilized in the previous examples that the enzymes are stabilized in aqueous solutions: ethylene glycol diethyl ether; ethylene glycol di-n-propyl ether; ethylene glycol di-n-butyl ether; diethylene glycol diethyl ether; triethylene glycol diethyl ether; triethylene glycol di-n-butyl ether; the methyl ether of pentaoxylethenated butanol; the methyl ether of octa oxygenated methanol; the butyl ether of decaoxylethenated butanol; pyran; 1,3-dioxane; 1,4-dioxane; dioxolan; di-n-propyl ketone; diisopropyl ketone; di-n-butyl ketone; and di-t-butyl ketone.

Results substantially similar to those in examples 9 through 38 are obtained when the following nonionic and zwitterionic detergents are substituted for the 3-(N,N-dimethyl-N-tallow alkylammonio)propane-1-sulfonate, the 3-(N,N-dimethyl-N-midlecuteo-coconut-alkylammonio)-2-hydroxypropane-1-sulfonate, dodecyldimethyl amine oxide and the coconuut- and tallow-alkyl ethoxylate compounds employed therein in that the stabilizing effects of the organic stabilizing agents are enhanced: decyl phenol ethoxylate with 20 moles of ethylene oxide per mole of decyl phenol, hexadecanoic amide, hexadecanol diethanol amide, dimethyldodecylamine oxide, dimethyldodecylphosphonic oxide and dodecyl methyl sulfosuccinate, the condensation product of ethylene oxide with the condensation product of propylene oxide with propylene glycol, the ethylene oxide portion of the compound being 50 percent of the total weight of the compound; about 1,700; the condensation product of ethylene oxide and ethylene diamine wherein the product contains about 65 percent polyethylene oxide by weight and the total molecular weight of the compound is 6,000.

Results substantially similar to those in examples 1 through 39 are obtained when the pH of the composition is maintained at 6.5, 8.0, and 8.5, in that the enzymes are stabilized for long periods of time. Enzyme stabilization is also obtained in examples 1 through 39 when the pH of the preparation is maintained at 5.0, 9.0, and 10.0.

The foregoing description of the invention has been presented describing certain operable and preferred embodiments. It is not intended that the invention should be so limited since variations and modifications thereof will be obvious to those skilled in the art, all of which are within the spirit and scope of this invention.

What is claimed is:

1. A stabilized aqueous enzyme composition consisting essentially of by weight of the composition:
   - from 65 to 97 percent water;
   - from about 0.001 to about 1.0 percent enzyme selected from the group consisting of proteases, a-amylases and mixtures thereof;
   - from about 2 to about 27 percent of a stabilizing agent selected from the group consisting of dialkyl glycol ethers having the general formula $R_1O[CH_2CH_2O]_xR_2$, wherein $R_1$ and $R_2$ are each alkyl of one to four carbon atoms and $x$ is 1 to 10.
   - from zero to about 15 percent of a detergent selected...
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11 from the group consisting of nonionic detergents and zwitterionic detergents.

2. The composition of claim 1 wherein the enzyme is an alkaline protease.

3. The composition of claim 2 wherein the alkaline protease is derived from Bacillus subtilis.

4. The composition of claim 1 wherein the enzyme is an α-amylase.

5. The composition of claim 1 wherein the enzyme is a mixture of protease and α-amylase.

6. The composition of claim 3 wherein the stabilizing agent is present in an amount of about 5 to about 20 percent.

7. The composition of claim 6 wherein the stabilizing agent is selected from the group consisting of ethylene glycol dimethyl ether and diethylene glycol dimethyl ether.

8. The composition of claim 7 wherein from about 4 to about 10 percent of a nonionic or zwitterionic detergent is present.

9. The composition of claim 8 wherein the detergent is selected from the group consisting of condensation products of 1 mole of aliphatic alcohol having from eight to 22 carbon atoms with from 5 to 40 moles of ethylene oxide; 3-(N,N-dimethyl-N-alkylammonio) propane-1-sulfonate wherein the alkyl has from eight to 22 carbon atoms; and 3-(N,N-dimethyl-N-alkylammonio)-2-hydroxypropane-1-sulfonate wherein the alkyl has from eight to 22 carbon atoms.

10. The composition of claim 9 wherein the pH is from about 5.0 to about 10.0 and wherein the enzyme is present in an amount of about 0.01 to about 0.5 percent.

11. The composition of claim 10 wherein the water is present in an amount of about 72 to about 95 percent.
It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 2, line 7, the formula should read \(- R_1\text{O}[\text{CH}_2\text{CH}_2\text{O}]_x\text{R}_2 \-.

Column 4, line 11, the formula should read \(- R_1\text{O}[\text{CH}_2\text{CH}_2\text{O}]_x\text{R}_2 \-.

Column 4, line 36, "di-N-propyl" should be -- di-n-propyl --.

Column 9, line 63, "HI Proteolytic Enzyme 2000" should read -- HT Proteolytic Enzyme 2000 --.

Column 10, line 1, before "enhanced" insert -- having --.

Column 10, line 1, delete "activity" and insert -- stability -- therefor.

Column 10, line 44, after "compound" insert -- and the total molecular weight of the compound --.

Column 10, Claim 1, line 73, the formula should read -- \(- R_1\text{O}[\text{CH}_2\text{CH}_2\text{O}]_x\text{R}_2 \-.

Signed and sealed this 11th day of July 1972.

(SEAL)
Attest:

EDWARD M. FLETCHER, JR.
Attesting Officer

ROBERT GOTTCHALK
Commissioner of Patents