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[54] **STABILIZED AQUEOUS ENZYME COMPOSITIONS**

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[63] Continuation of Ser. No. 786,432, Dec. 23, 1968, abandoned.

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[58] **Field of Search** ..... 195/63, 68; 424/94; 252/89, 132, 135, 546, 398, 403, DIG. 12, 153

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[57] **ABSTRACT**

Aqueous amylolytic enzyme-containing compositions comprising water, amylolytic enzyme, a water-soluble calcium salt, an organic co-stabilizing agent selected from aliphatic glycols and 1,3-propanediol and, optionally, a nonionic or zwitterionic detergent are disclosed. The compositions, useful as starch-degrading compositions, are stabilized substantially against loss of amylolytic enzyme activity during storage.

19 Claims, No Drawings

STABILIZED AQUEOUS ENZYME COMPOSITIONS

This is a continuation of application Ser. No. 786,432, filed Dec. 23, 1968, now abandoned.

FIELD OF THE INVENTION

This invention relates to aqueous amylolytic enzymecontaining compositions useful in the degradation of starchy materials and characterized by stabilization against loss of amylolytic activity.

The use of amylolytic enzymes in the alteration and/or degradation of starchy materials is known. For example, U.S. Pat. No. 2,607,359 (Aug. 19, 1962) describes compositions containing an amylolytic enzyme useful in facilitating the removal of porous materials such as wallpapers, labels and casein type pastes from surfaces to which the porous materials are held by a starch-containing adhesive. Similarly, Jaag in *Seifen, Ole, Fette, Wachse* 88, No. 24, pp. 789–793, (Nov. 1962) describes the use of amylolytic enzymes in laundry formulations. These enzymes aid in the laundry process by attacking starchy soils and stains found on soiled fabrics and decomposing and/or altering them so as to render them more removable during laundering. Enzymatic materials 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 during long periods of storage as evidenced by substantial loss in starch-degrading and/or soil- and stain-removing efficacy. The loss in amylolytic activity is particularly severe under conditions of high temperature. Furthermore, aqueous laundering solutions containing amylolytic enzymes often contain additional components desirable in the laundering process but which have an adverse effect on the amylolytic enzyme. Proteolytic enzymes, for example, while useful in providing proteinaceous soil- and stain-removing properties in laundering compositions, often tend to have such an adverse degrading effect on amylolytic enzymes.

Attempts have been made in the art to provide amylolytic enzyme-containing compositions wherein the enzymatic activity is preserved by the incorporation of a stabilizing agent. These attempts have generally involved incorporation in such compositions of water-soluble calcium salts. A trade bulletin describing bacterial amylases derived from Bacillus subtilis, published by Daiwa Kasei K. K. of Osaka, Japan, describes the stabilization of bacterial amylase with calcium and sodium ions. Similarly, Hamada et al., *Agr. Biol. Chem.*, 31, No. 1, pp. 1–6 (1967), describe the stabilizing effect of calcium ions on α-amylase. The employment of calcium salts to impede loss of amylolytic activity, particularly at high temperatures, has not been entirely satisfactory, particularly over extended periods of storage at high temperatures. Accordingly, there has been a need for amylolytic enzyme-containing compositions having improved amylolytic enzyme stability.

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

It is another object of this invention to provide aqueous amylolytic enzyme-containing compositions stabilized substantially against loss of activity by the presence of minor amounts of enzyme-stabilizing compounds.

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 re achieved by the provision of aqueous amylolytic enzyme-containing compositions containing minor amounts of calcium ion and certain organic costabilizing agents. The aqueous compositions of the present invention can additionally contain nonionic or zwitterionic detergent components to enhance the stability of the amylolytic enzymes in the aqueous compositions of this invention and to enhance the detergent properties of these compositions. The present invention is based in part on the discovery that extended periods of stabilization can be achieved by introducing into aqueous enzyme-containing compositions a source of calcium ion and an organic compound selected from the group consisting of aliphatic glycols and 1,3-propane diol.

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 percent amylolytic enzyme;
3. from about 0.001 to about 1 percent with respect to calcium ion of a water-soluble enzyme-stabilizing calcium salt;
4. from about 2 to about 27 percent of an organic compound selected from the group consisting of aliphatic glycols having the formula

$$HO[CH_2CH_2O]_xH$$

wherein x is from about 1 to about 200; and 1,3-propane diol; and

5. from 0 to about 15 percent of a detergent selected from the group consisting of nonionic detergents and zwitterionic detergents.

The amylolytic enzymes which can be stabilized in aqueous solution by the action of the hereinbefore described combination of calcium ion and organic compound are known materials and can be of fungal, plant, animal or bacterial origin. Suitable amylolytic enzymes include the α-amylases which are particularly well suited for breaking down starch molecules as they attack the $\alpha_{1,4}$-glycosidic linkages in starch. The degraded short chains are easily removed from their environment with water or aqueous solutions of detergents. Examples of suitable amylolytic enzymes include the α-amylases of mold origin including those derived from *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus alliaceus*, *Aspergillus wentii*, and *Pencillium glaucum*. The α-amylases derived from cereal grains, pancreatic sources and such bacteria as *Bacillus subtilis Bacillus macerans*, *Bacillus mesentericus* and *Bacillus thermophilus* are also useful herein. These enzymes are active in the pH range of from about 4.5 to about 10 and at temperatures from about 60°F. to about 150°F. Optimum activity of these α-amylases is generally exhibited in the pH range of from about 5.5 to about 7.5.

Preferred amylolytic enzymes herein are the α-amylases derived from the bacterial organism Bacillus subtilis. These amylases provide excellent desizing and starch digestive properties and are especially useful in the laundering of textile materials containing soils and stains of a starchy nature.

The amylolytic enzymes useful herein can be employed in a pure state. Generally they are employed in the form of a powdered commercially available preparation wherein the amylolytic enzyme is present in an amount of from about 2 to about 80 percent of the preparation. The remaining portion, i.e. about 20 to about 98 percent, comprises inert vehicle such as sodium sulfate, calcium sulfate, sodium chloride, clay or the like. In preparing the stabilized aqueous starch-degrading compositions of this invention, such commercial enzyme preparations are admixed with water and the remaining components of the compositions. The active enzyme content of these 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 hereinafter specified enzyme content. Insoluble inert materials are generally removed from the compositions of this invention to provide aqueous compositions which are clear and substantially free of precipitated deposits. Specific examples of commercial enzyme preparations suitable for use herein and the manufacturers thereof include: Diasmen α-amylase (Daiwa Kasei K. K. Tokyo, Japan); Rapidase α-amylase THC-25 (Rapidase, Seclin, France); Novo Bacterial α-amylase (Novo Industri, Copenhagen, Denmark); Wallerstein α-amylase (Wallerstein Company, Staten Island, New York); Rhozyme-33 and Rhozyme H-39 (Rohm & Haas, Philadelphia, Pennsylvania).

Preferred herein is a powdered enzyme preparation containing α-amylase and a mixture of alkaline and neutral proteases available as CRD-Protease (or Monsanto DA-10) from Monsanto Company, St. Louis, Missouri. This composition contains about 3 α-amylase and is useful herein to provide the compositions of the invention with amylolytic and proteolytic enzyme activity. Mixtures of proteases and α-amylases are preferred herein and include the enzyme preparations described in U.S. Pat. No. 3,031,380 to Minagawa et al. (Apr. 24, 1962).

The amount of amylolytic enzyme employed in the compositions of this invention can vary depending upon the activity of the enzyme or enzyme preparation, conditions of pH and the intended use of the compositions. When the stabilized aqueous compositions of this invention are employed as spot removers, they should contain an amount of amylolytic enzyme sufficient to remove the starchy soils and stains normally encountered in a laundering situation. Normally the compositions of this invention are prepared to contain from about 0.001 to about 1 percent enzyme by weight of the aqueous composition on a pure enzyme basis. For best results, the compositions preferably contain from about 0.01 to about 0.5 percent amylolytic enzyme. When a commercially available powdered enzyme preparation is employed as the source of amylolytic enzyme, the compositions of this invention contain from about 0.1 to about 4.0 percent of the powdered amylolytic enzyme-containing preparation as it is available in commercial form e.g., containing from about 2 to about 80 percent active enzyme. It will be appreciated that when such preparations are employed herein, the amount of the preparation required to provide aqueous compositions having desirable levels of amylolytic activity will depend on the activity level of the enzyme-containing preparation employed. The precise amounts of such materials employed can be readily determined by methods known in the art so long as the stabilized compositions of the invention provide an amount of amylolytic enzyme activity sufficient to provide desirable levels of starch degrading properties.

As used herein, amylolytic activity refers to the tendency of an amylolytic enzyme to perform the desired function of catalytic alteration and/or degradation of starchy materials. Stability, as used herein, refers to the tendency of an amylolytic enzyme to retain its enzymatic activity. The activity level of amylolytic enzyme suitable herein can be determined by numerous methods. A suitable method is the 3,5-dinitrosalicylate assay method. In accordance with this method, a sample of amylase is allowed to catalyze the hydrolysis of the 1,4-α-glycosidic bonds of starch and glycogens for five minutes at a temperature of 37°C. at a pH of 6.0. The reaction is terminated by the addition of buffered sodium 3,5-dinitrosalicylate, the color is developed and the amount of maltose determined by spectrophotometric response and comparison with solutions of analytical grade maltose hydrate. The amylase has one activity unit for each 0.4 mg. of maltose hydrate produced during hydrolysis under the specified conditions. The amylase activity method is well known and is described with particularly in P. Bernfeld, *Methods in Enzymol.* Vol I. p. 149 (1955).

As hereinbefore described, the present invention is based in part upon the surprising discovery that extended periods of enzyme stabilization can be achieved by incorporating into aqueous enzyme solutions a combination of water-soluble calcium salt and organic co-stabilizing compound hereinbefore described. The water-soluble salts of calcium include, for example, calcium chloride, calcium acetate, calcium citrate, calcium glycerol phosphate, calcium gluconate, calcium glucoheptanate, calcium lactate, calcium levulinate, calcium lactobionate, calcium malate, calcium lactophosphate, calcium succinate, calcium maleate, and calcium sulfate. The stabilized compositions of the invention are prepared to contain from about 0.001 to about 1 percent of the stabilized composition with respect to the calcium ion. Preferably from about 0.005 to about 0.05 percent with respect to the calcium ion, is employed for best stabilization. As described hereinbefore, certain of the commercially available enzyme preparations suitable herein contain, in addition to active enzyme, certain inert materials including for example, calcium chloride or calcium sulfate. When such an enzyme preparation is employed as the source of amylolytic enzyme, an amount of calcium ion is also incorporated thereby. Additional calcium ion is conveniently provided by the addition of one or more of the calcium salts hereinbefore described so as to provide a level of calcium ion within the hereinbefore described range. Preferred calcium salts include calcium acetate, calcium sulfate and calcium chloride.

The organic co-stabilizers which in concert with calcium ion provide enhanced amylolytic enzyme activity include the aliphatic glycols and 1,3-propanediol. The aliphatic glycols employed herein have the formula

$$HO[CH_2CH_2O]_xH$$

wherein x is from 1 to about 200, and include ethylene glycol and the polyethylene glycols. The polyethylene glycols useful herein are those wherein x in the hereinbefore described formula ranges from 2 to about 200 and include diethylene glycol, triethylene glycol and

the corresponding polymers of ethylene oxide wherein the average number of oxyethylene groups ranges upward from 4 (tetraethylene glycol) to about 200.

The aliphatic glycols useful herein range in consistency from light liquids to white waxy solids and dissolve in water to form clear solutions. Preferred aliphatic glycols herein include diethylene glycol and triethylene glycol. Also preferred are the polyethylene glycols wherein the average value of x is from 4 to about 80. These polyethylene glycols have average molecular weights of about 200 to about 3500 and are commercially available under the trade designation "Carbowax" with numerical designation referring to average molecular weight, e.g. 200, 400, 600, 1000, or the like. The upward numerical gradation corresponds to increasing molecular weight, increasing melting point and decreasing water-solubility. Mixtures of aliphatic glycols of the invention can be employed herein.

It has been found that 1,3-propanediol also provides an amylolytic enzyme-stabilizing effect as hereinbefore described. This compound is a preferred co-stabilizing agent herein and provides excellent stabilizing effects.

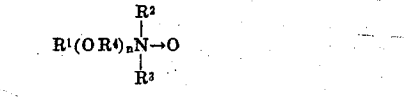
The organic amylolytic enzyme co-stabilizing compounds of this invention are employed in minor but effective amounts ranging from about 2 to about 27 percent by weight of the composition. Preferably the stabilized compositions are prepared to contain from about 5 to about 20 percent by weight of the co-stabilizing agent. The latter range is preferred from the standpoint of optimum stabilizing effects, particularly over long storage periods at high temperatures.

While the mechanism by which the calcium salts and organic co-stabilizing agent hereinbefore described coact to protect amylolytic enzymes against loss of activity is not precisely known, the combination of salt and organic compound provides levels of enzyme stability substantially greater than can be achieved by conventional calcium stabilization alone. This stabilization effect is observed even in the presence of proteases which tend to exert a harmful denaturing effect on α-amylases.

Water-soluble nonionic and zwitterionic detergents can be employed, as optional ingredients, in the compositions of this invention. These detergents enhance considerably the storage stability of the amylolytic enzymes employed herein and significantly improve the detergent characteristics of the composition. Because of these useful characteristics it is preferred to include nonionic and zwitterionic detergents in the aqueous enzyme compositions of the invention. The nonionics and zwitterionics can be utilized herein in amounts ranging from 0 to about 15 percent, and preferably from 4 to 10 percent, by weight of the enzyme-containing composition.

Examples of suitable nonionics for use herein include:

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 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. 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 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. 3. The condensation product of 1 mole of aliphatic alcohols having from eight to 22 carbon atoms, in either 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



wherein R^1 is an alkyl radical of from about eight to about 22 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 semi-polar bond. Specific examples of amine oxide detergents include: dimethyldodecylamine oxide and bis-(2-hydroxyethyl)-dodecylamine oxide.

6. Long chain tertiary phosphine oxides corresponding to the following general formula

$$RR'R''P \rightarrow O$$

wherein R is an alkyl, alkenyl or monohydroxyalkyl radical ranging from 10 to 22 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 semi-polar bond. Examples of suitable phosphine 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 sulfoxides having the formula

wherein R^5 is an alkyl radical containing from about 10 to about 22 carbon atoms, from 0 to about 5 ether linkages and from 0 to about 2 hydroxyl substituents, at least one moiety of R^5 being uninterrupted by ether linkages and containing from about 10 to about 18 car-

bon atoms, and wherein R^6 is an alkyl radical containing from one to three carbon atoms and from 0 to 2 hydroxyl groups. Specific examples of these sulfoxides are: dodecyl methyl sulfoxide and 3-hydroxy tridecl 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, 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-hydroxypropane-1-sulfonate. For more examples of zwitterionic synthetic detergents, see Diehl and Smith, "Laundering Fabrics in Cold Water Containing a Synthetic Detergent Composition," Canadian Patent No. 708,147 issued Apr. 20, 1965 at page 6, lines 1–22. This disclosure is specifically incorporated herein by reference.

Mixtures of various nonionic detergents or mixtures of nonionic detergents and zwitterionic detergents can be employed. 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-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 eight to 22 carbon atoms, e.g. 3-(N,N-dimethyl-N-tallowalkylammonio) propane-1-sulfonate. These compounds in addition to providing amylase stability per se enhance the stabilization of calcium and organic costabilizing compound. In addition they provide excellent detergency properties.

The stable 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 which might occur by adding the enzyme to water which does not contain the enzyme-stabilizing combination of the invention. 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 affects 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 stabilized aqueous compositions of this invention can also contain any of the usual detergent adjuvents, diluents and additives so long as they do not substantially interfere with the activity of the enzymatic components. For example, perfumes, anti-tarnishing agents, inert salts such as sodium sulfate, anti-redeposition agents, bacteriostatic agents, dyes, fluorescers, suds builders, suds depressors, and the like, can be utilized herein without detracting from the advantageous properties of these compositions. It is preferred that the compositions of the present invention contain in addition certain proteolytic enzymes. These enzymes include the alkaline proteases, neutral proteases, and acid proteases which aid materially the removal of proteinaceous soils and stains from laundered textiles. The employment of proteolytic enzymes in combination with the amylolytic enzymes 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. When proteolytic enzymes are included in the compositions of the present invention, it is desirable to include any of the known proteolytic enzyme-stabilizing materials known in the art to thereby enhance proteolytic enzyme activity upon storage. Suitable proteolytic enzyme stabilizing materials are described for example in Ser. No. 683,196, entitled "Stabilized Aqueous Enzyme Preparation" filed Nov. 15, 1967 by Charles Bruce McCarty.

The compositions of this invention can be employed as spot removers, detergent additives or as detergent cleaning compositions 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 any of the stains which these bleaches remove, do not weaken textile fibers, and do not attack or degrade fluorescers and whiteners. With the addition of optional nonionic and/or zwitterionic detergents, these compositions can be utilized per se as excellent cleaning compositions under a variety of washing conditions.

EXAMPLES

The following examples merely serve to illustrate the invention in specific detail and when read in conjunction with the foregoing description will aid in determining the full scope of the present invention. The examples are merely illustrative and are not intended to restrict this invention. All parts, percentages and ratios set forth herein are by weight unless otherwise indicated.

The following compositions were prepared and stored in closed glass bottles for the lengths of time indicated in Table I. Each composition containined 10 percent by weight of the organic co-stabilizing agent; 1 percent Monsanto CRD-Protease (a commercially available mixture of proteases and amylases derived from Bacillus subtilis); and 89 percent of an aqueous

stock solution containing 0.01 percent calcium acetate monohydrate and 0.29 percent sodium chloride. The amylolytic activity of each composition was measured at the stated intervals by the assay method hereinbefore described.

Control samples stored under identical conditions were also evaluated for retention of enzymatic activity. In Control-1, no organic co-stabilizing agent was employed. In the case of Control-2, no organic co-stabilizing agent was present and the stock solution was replaced with distilled water, i.e., no calcium acetate monohydrate or sodium chloride was present. The results are tabulated as follows:

Table I

Example	% Ca^{++}	Organic Co-Stabilizing Agent	Percent Remaining Activity After Storage at 100°F. for Weeks			
			2	4	6	8
Control-1	0.0023	None	54	34	29	17
Control-2	None	None	0*	—	—	—
1	0.0021	Ethylene Glycol	70	51	34	28
2	0.0021	Diethylene Glycol	71	64	44	37
3	0.0021	1,3-propanediol	60	47	43	35

*3 days

The following stabilized compositions, Examples 4to 29, were prepared. In each example, the water (containing calcium acetate monohydrate and sodium chloride) organic co-stabilizing agent and ethanol (lwhere employed) were thoroughly mixed, the nonionic or zwitterionic was added and the enzyme was added last. The enzymes employed were Alcalase (a proteolytic enzyme preparation having a crystalline enzyme content of about 6 percent and derived from Bacillus subtilis); and/or Monsanto CRD-Protease (a mixture of proteolytic and amylolytic enzymes derived from Bacillus subtilis). Ethanol, where present, was employed as a stabilizer for the proteolytic enzyme. In each example, an aqueous stock solution, hereinbefore described, was employed in an amount to bring the balance of the composition to 100 percent. The compositions were stored for the periods of time indicated in Table II at a temperature of 100°F. and their amylolytic activity evaluated as hereinbefore described. The compositions of Examples 4 to 29 perform well as spot removers, as additives to detergent compositions and as laundry detergents per se.

Table II

Ex.	% Ca^{++}	% Ethanol	% Organic Co-stabilizer	% Enzyme		% Surfactant*					% Remaining After Storage at 100°F. for Weeks			
				Alcalase	Monsanto CRD	A	B	C	D	E	2	4	6	8
4	0.0017	10	Diethylene Glycol (10%)	0.5	0.5		–	–	–	9	100	88	100	100
5	0.0019	5	Diethylene Glycol (5%)	0.5	0.5	5	–	–	–	–	100	79	50	50
6	0.0017	10	Triethylene Glycol (10%)	0.5	0.5	5	–	–	–	–	100	83	58	63
7	0.0019	5	Triethylene Glycol (5%)	0.5	0.5	5	–	–	–	–	98	70	53	30
8	0.0017	10	Polyethylene Glycol 380 (10%)	0.5	0.5	5	–	–	–	–	90	83	45	33
9	0.0019	5	Polyethylene Glycol 380 (5%)	0.5	0.5	5	–	–	–	–	82	61	31	11
10	0.0017	10	Polyethylene Glycol 4000 (10%)	0.5	0.5	5	–	–	–	–	70	42	18	7
11	0.0019	5	Polyethylene Glycol 4000 (5%)	0.5	0.5	5	–	–	–	–	79	32	18	4
12	0.0020	–	Triethylene Glycol (10%)	0.5	0.5	–	–	–	–	–	90	100	100	100
13	0.0020	–	Polyethylene Glycol 4000 (10%)	0.5	0.5	–	–	–	–	–	78	87	83	82
14	0.0019	10	Diethylene Glycol (10%)	0.5	0.5	–	5	–	–	–	68	75	59	59
15	0.0017	10	Diethylene Glycol (10%)	0.5	0.5	–	–	5	–	–	95	77	45	54
16	0.0017	10	Triethylene Glycol (10%)	0.5	0.5	–	–	–	5	–	100	85	75	74
17	0.0017	10	Polyethylene Glycol 380 (10%)	0.5	0.5	–	–	–	5	–	91	73	64	65
18	0.0017	10	Polyethylene Glycol 4000 (10%)	0.5	0.5	–	–	–	5	–	87	69	57	64
19	0.0020	–	Diethylene Glycol (10%)	0.5	0.5	–	–	–	–	–	96	73	73	75
20	0.0018	–	Diethylene Glycol (10%)	–	1.0	10	–	–	–	–	–	95	–	89
21	0.0018	–	Diethylene Glycol (10%)	–	1.0	–	10	–	–	–	–	87	–	72
22	0.0018	–	Diethylene Glycol (10%)	–	1.0	–	–	10	–	–	–	59	–	49
23	0.0018	–	Diethylene Glycol (10%)	–	1.0	–	–	–	10	–	–	51	–	36
24	0.0018	–	Diethylene Glycol (10%)	–	1.0	–	–	–	–	10	–	48	–	30
25	0.0018	–	1,3-Propanediol (10%)	–	1.0	10	–	–	–	–	–	89	–	81
26	0.0018	–	1,3-Propanediol (10%)	–	1.0	–	10	–	–	–	–	77	–	72
27	0.0018	–	1,3-Propanediol (10%)	–	1.0	–	–	10	–	–	–	86	–	65
28	0.0018	–	1,3-Propanediol (10%)	–	1.0	–	–	–	10	–	–	100	–	55
29	0.0018	–	1,3-Propanediol (10%)	–	1.0	–	–	–	–	10	–	100	–	74

*A—Tallow alcohol ethoxylated with 11 moles of ethylene oxide.
*B—Tallow alcohol ethoxylated with 30 moles of ethylene oxide.
*C—Coconut alcohol ethoxylated with 6 moles of ethylene oxide.
*D—HAPS - 3-(N,N-dimethyl-N-alkylammonio)2-hydroxy propane-1-sulfonate wherein the alkyl group is derived from middle-cut coconut alcohol: 2% C_{10}; 66% C_{12}; 23% C_{14} and 9% C_{16}.
*E—3-(N,N-dimethyl-N-tallowalkylammonio) propane-1-sulfonate.

EXAMPLE 30

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

Component	Weight %
α-Amylase (Diasmen, Daiwa Kasei KK)	1
Calcium Chloride	0.01
Polyethylene Glycol 4000	10
Alcalase (6% proteolytic enzyme)	1
Ethanol	10
Water	78

This composition can be employed without dilution as a soil- and stain-removing composition to remove or facilitate removal of starchy and proteinaceous matter from textile materials. The soil- and stain-removing efficacy is demonstrated even after extended periods of storage (8 weeks) at elevated temperature (120°F.). The composition of this example can be employed as an additive to commercial detergent formulations. When about **1.2** ml. of the composition is added per gallon of washing solution, excellent soil- and stain-removing properties are demonstrated.

EXAMPLE 31

Similar results are obtained when the following organic co-stabilizing compounds are employed in lieu of the co-stabilizing compounds employed in Examples 1 to 29 in that the amylolytic enzyme is stabilized in aqueous solution: ethylene glycol; diethylene glycol; triethylene glycol; tetraethylene glycol; polyethylene glycol 200; polyethylene glycol 300; polyethylene glycol 380; polyethylene glycol 600; polyethylene glycol 1000; polyethylene glycol 1500; polyethylene glycol 4000; polyethylene glycol 6000; and 1,3-propanediol.

Similar results are obtained when the following amylolytic enzymes are employed in lieu of those employed in Examples 1 to 29 in that the α-amylase is stabilized in aqueous solution: Diasmen α-amylase; Rapidase α-amylase THC-**25**; Novo Bacterial α-amylase; Wallerstein α-amylase; Rhozyme-**33** and Rhozyme H-**39.**

Similar results are obtained when the following nonionic and zwitterionic detergents are substituted for the tallow alcohol ethoxylates, coconut alcohol ethoxylate, 3-(N,N-dimethyl-N-middlecut-coconut-alkylammonio)2-hydroxypropane-1-sulfonate and 3-(N,N-dimethyl-N-tallowalkylammonio) propane-1-sulfonate employed in Examples 4 to 11, 14 to 18 and 20 to 29 in that the stabilization by calcium ion and organic co-stabilizing compound is enhanced and excellent cleaning properties are provided: decyl phenol ethoxylated with 20 moles of ethylene oxide per mole of decyl phenol, hexadecanoic amide, hexadecanoic diethanol amide, dimethyldodecylamine oxide, dimethyldodecylphosphine oxide, and dodecyl methyl sulfoxide, 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 and the total molecular weight of the compound being about 1700; the condensation product of ethylene oxide with the condensation product of propylene oxide and ethylene diamine wherein the product contains about 65 percent polyethylene oxide by weight and the total molecular weight of the compound is 6000.

Similar results are obtained when the calcium acetate monohydrate of Examples 1 to 29 is replaced with the following calcium salts in amounts providing an equal amount of calcium ion in that stabilization of amylolytic enzyme in aqueous solution is observed: calcium chloride; calcium citrate; calcium glycerol phosphate; calcium gluconate; calcium glucoheptanate; calcium lactate; calcium levulinate; calcium lactobionate; calcium malate; calcium lactophosphate; calcium succinate; calcium maleate; and calcium sulfate.

What is claimed is:

1. A stabilized aqueous enzyme composition consisting essentially of by weight of the composition:

1. from about 65 percent to about 97 percent water;
2. from about 0.001 percent to about 1 percent amylolytic enzyme;
3. from about 0.001 percent to about 1 percent with respect to calcium ion of a water-soluble enzyme-stabilizing calcium salt;
4. from about 2 percent to about 27 percent of an organic co-stabilizing compound selected from the group consisting of aliphatic glycols having the formula

$$HO[CH_2CH_2O]_xH$$

wherein x is from about 2 to about 200; and 1,3-propanediol; and

5. from 0 to about 15 % of a detergent selected from the group consisting of nonionic detergents and zwitterionic detergents.

2. The composition of claim **1** wherein the co-stabilizing compound is an aliphatic alcohol having the formula

$$HO[CH_2CH_2O]_xH$$

wherein x is from about 2 to about 200; and wherein the amylolytic enzyme is an α-amylase characterized by amylolytic activity in the pH range of from about 4.5 to about 10 and at a temperature of from about 60°F. to about 150°F.

3. The composition of claim **2** wherein the α-amylase is derived from *Bacillus subtilis.*

4. The composition of claim **3** wherein the α-amylase is present in an amount of about 0.01 to about 0.5 percent.

5. The composition of claim **4** wherein the calcium ion is present in an amount of about 0.005 to 0.05 percent and the organic co-stabilizing compound is present in an amount of from about 5 percent to about 20 percent.

6. The composition of claim **5** wherein the organic costabilizing compound is selected from the group consisting of diethylene glycol; triethylene glycol; and glycols of the formula

$$HO[CH_2CH_2O]_xH$$

wherein the average value of x is from 4 to about 80.

7. The composition of claim **6** wherein from about 4 to about 10 percent of a nonionic or zwitterionic detergent is present.

8. The composition of claim **7** 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.

9. The composition of claim **8** wherein the detergent is 3-(N,N-dimethyl-N-coconutalkylammonio)-2-hydroxypropane-1-sulfonate and the organic co-stabilizing compound is triethylene glycol.

10. The composition of claim **8** wherein the calcium salt is selected from the group consisting of calcium acetate, calcium sulfate and calcium chloride.

11. A stabilized aqueous enzyme composition consisting essentially of by weight of the composition:

1. from about 65 to about 97 percent water;
2. from about 0.001 to about 1 percent amylolytic enzyme;
3. from about 0.001 to about 1 percent with respect to caclium ion of a water-soluble, enzyme-stabilizing calcium salt;
4. from about 2 to about 27 percent of a 1,3-propanediol co-stabilizing compound; and
5. from 0 to about 15 percent of a detergent selected from the group consisting of nonionic detergents and zwitterionic detergents.

12. The composition of claim **11** wherein the amylolytic enzyme is an α-amylase characterised by amylolytic activity in the pH range of from about 4.5 to about 10 and at a temperature of from about 60°F. to about 150°F.

13. The Composition of claim **12** wherein the α-amylase is derived from Bacillus subtilis.

14. The composition of claim **13** wherein the α-amylase is present in an amount of from about 0.01 to about 0.5 percent.

15. The composition of claim **14** wherein the calcium ion is present in an amount of from about 0.005 to 0.05 percent and the organic co-stabilizing compound is present in an amount of from about 5 to about 20 percent.

16. The composition of claim **15** wherein from about 4 to about 10 percent of a detergent selected from the group consisting of nonionic and zwitterionic detergents is present.

17. The composition of claim **16** 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.

18. The composition of claim **17** wherein the detergent is 3-(N,N-dimethyl-N-coconutalkylammonio)-2-hydroxypropane-1-sulfonate.

19. The composition of claim **17** wherein the calcium salt is selected from the group consisting of calcium acetate, calcium sulfate and calcium choride.

* * * * *

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,819,528 Dated June 25, 1974

Inventor(s) Jim S. Berry

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 5, after invention delete "re" and insert therefor -- are --

Column 3, line 34, after "3" insert -- % --

Column 7, line 4, after "3-hydroxy" delete "tridecl" and insert therefor -- tridecyl --

Table II, first line, Column "A", insert -- 5 --

Table II, first line, Column "E", insert a hyphen

Table II, first line, Column "4", delete "88" and insert therefor -- 100 --

Table II, first line, Column "6", delete "100" and insert therefor -- 88 --

Table II, line 2, Column "E", delete "9"

Signed and Sealed this

ninth Day of *December 1975*

[SEAL]

Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents and Trademarks

PO-1050
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