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(54) Stabilized aqueous enzyme composition.

(57) α-Amylase derived from B. licheniformis is stabilized in aqueous liquid detergent compositions containing surfactants and sequestering agents by the inclusion of a calcium-containing component that provides at least about $1 \times 10^{-4.5}$ millimole of enzyme-accessible calcium per liter of composition.

Croydon Printing Company Ltd.

STABILIZED AQUEOUS ENZYME COMPOSITION

Roland G. Severson, Jr.

TECHNICAL FIELD

1. Field of the Invention

The present invention relates to stabilized aqueous enzyme compositions which contain detergent components.

2. Description of the Art

The formulation of enzyme-containing aqueous liquid detergent compositions is difficult due to the tendency toward rapid decrease in enzymatic activity in aqueous media during storage. U.S. Patent 4,111,855, Barrat et al, issued September 5, 1978, U.S. Patent 4,287,082, Tolfo et al, issued September 1, 1981, U.S. Patent 4,305,837, Kaminsky et al, issued December 15, 1981, and U.S. Patent 4,318,818, Letton et al, issued March 9, 1982, all disclose approaches to the stabilization of enzymes in aqueous media.

DISCLOSURE OF THE INVENTION

The stabilized aqueous enzyme compositions of this invention comprise: (a) from about 1% to about 75% of a non-soap detergent surfactant; (b) from about 0.01% to about 5% of an α-amylolytic enzyme, derived from Bacillus licheniformis; (c) from about 0.25% to about 40% of a sequestering agent; (d) a calcium-containing component to provide at least about $1 \times 10^{-4.5}$ millimoles of enzyme-accessible calcium ion per liter of said composition; and (e) from about 10% to about 80% water.

The stabilization of enzymes in aqueous media has been the subject of substantial study with particular effort directed to compositions such as liquid detergent compositions that face relatively prolonged storage and possible extremes of temperature before use. Proteolytic enzymes (protease) and amylolytic enzymes (α-amylase) have proven to be very useful detergency adjuvants because of their ability to solubilize or otherwise degrade soils containing, respectively, protein and carbohydrate components.

The use of calcium to stabilize enzymes in aqueous media is well known, but this method of stabilization is in conflict with the incorporation of detergency builder components into liquid detergent compositions. A major function of effective detergency builder components is to remove metal ions other than alkali metal ions from washing solutions by sequestration or precipitation. These undesirable metal ions, calcium and magnesium ions in particular, are generally designated water hardness. The incorporation of such detergency builder components in a liquid detergent composition clearly complicates enzyme stability considerations.

By practice of the present invention, it is now surprisingly found that an α-amylase can be stabilized in liquid detergent compositions containing calcium sequestering agents when said α-amylase is derived from Bacillus licheniformis (B. licheniformis) and a very low minimum level of "enzyme-accessible" calcium ion, as hereinafter defined, is provided by incorporation of a calcium-containing component in accordance with a mathematical formula involving the equilibrium constants of complexation of said calcium sequestering agents.

DETAILED DESCRIPTION OF THE INVENTION

Non-Soap Detergent Surfactants

The non-soap detergent surfactant can be selected from nonionic, anionic, cationic, zwitterionic, amphoteric and semi-polar nonionic surfactants and mixtures thereof. The surfactants preferably comprise from about 10% to about 65%, more preferably from about 20% to about 50% of the formula by weight.

Nonionic Surfactants

One useful type of nonionic surfactant is produced by condensing ethylene oxide with a hydrocarbon having a reactive hydrogen atom, e.g., a hydroxyl, carboxyl, amino, or amido group, in the presence of an acidic or basic catalyst. Such nonionic surfactants have the general formula $RA(CH_2CH_2O)_nH$ wherein R represents the hydrophobic moiety, A represents the group carrying the reactive hydrogen atom and n represents the average number of ethylene oxide moieties. R typically contains from about 8 to 22 carbon atoms, but can also be formed by the condensation of propylene oxide with a lower molecular weight compound. n can vary from about 2 to about 24 depending on the desired physical and detergency properties. The hydrophobic moiety of the nonionic compound is preferably a primary or secondary, straight or slightly branched, aliphatic alcohol having from about 8 to about 24, preferably from about 12 to about 20 carbon atoms.

Alkyl amine oxides and other semi-polar nonionic surfactants are hereinafter described. Another class of useful nonionic surfactants are alkylpolysaccharides having a hydrophobic group containing from about 8 to about 20 carbon atoms and a polysaccharide hydrophilic group containing from about 1.5 to about 10 saccharide units.

A more complete disclosure of suitable nonionic surfactants can be found in U.S. Patent 4,111,855 disclosed hereinbefore and incorporated herein by reference.

Anionic Surfactants

Synthetic anionic surfactants can be represented by the general formula R^1SO^3M wherein R^1 represents a hydrocarbon group selected from the group consisting of straight or branched alkyl radicals containing from about 8 to about 24 carbon atoms and alkyl phenyl radicals containing from about 9 to about 15 carbon atoms in the alkyl group. M is a salt forming cation which typically is selected from the group consisting of sodium, potassium, ammonium, monoalkanolammonium, dialkanolammonium, trialkanolammonium, and magnesium cations and mixtures thereof.

Preferred synthetic anionic surfactants include the water-soluble salts of alkylbenzene sulfonic acid containing from about 9 to about 15 carbon atoms in the alkyl group and water-soluble alkyl sulfates containing from about 10 to about 18 carbon atoms.

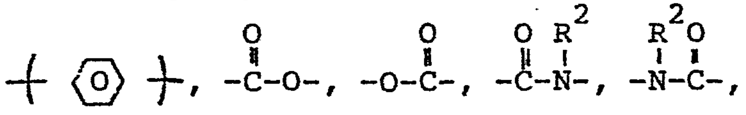
Another preferred synthetic anionic surfactant is a water-soluble salt of an alkyl polyethoxylate ether sulfate wherein the alkyl group contains from about 8 to about 24, preferably from about 10 to about 18 carbon atoms and there are from about 1 to about 20, preferably from about 1 to about 12 ethoxy groups. Other suitable anionic surfactants are disclosed in U.S. Patent 4,170,565, Flesher et al, issued October 9, 1979, incorporated herein by reference.

<u>Cationic Surfactants</u>

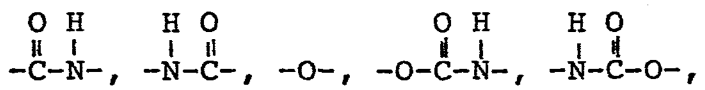
Suitable cationic surfactants have the general formula

$$R^2_m R^3_x Y_L Z$$

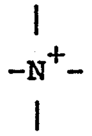
wherein each R^2 is an organic group containing a straight or branched alkyl or alkenyl group optionally substituted with up to three phenyl or hydroxy groups and optionally interrupted by up to four structures selected from the group consisting of

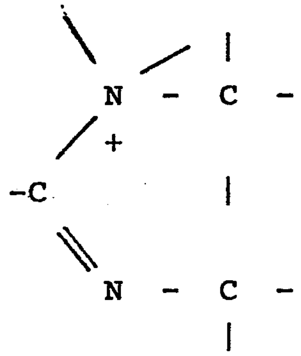


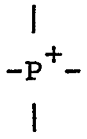
$-\!\bigcirc\!-$, $-\overset{\overset{O}{\|}}{C}-O-$, $-O-\overset{\overset{O}{\|}}{C}-$, $-\overset{\overset{O}{\|}}{C}-\overset{\overset{R^2}{|}}{N}-$, $-\overset{\overset{R^2}{|}}{N}-\overset{\overset{O}{\|}}{C}-$,

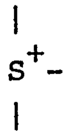


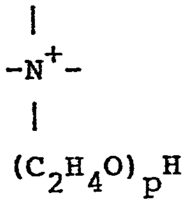
$-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-$, $-\overset{\overset{H}{|}}{N}-\overset{\overset{O}{\|}}{C}-$, $-O-$, $-O-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-$, $-\overset{\overset{H}{|}}{N}-\overset{\overset{O}{\|}}{C}-O-$, and mixtures thereof, each R^2 containing from about 8 to 22 carbon atoms, and which may additionally contain up to about 12 ethylene oxide groups, m is a number from 1 to 3, each R^3 is an alkyl or hydroxyalkyl group containing from 1 to 4 carbon atoms or a benzyl group with no more than one R^3 in a molecule being benzyl, x is a number from 0 to 11, the remainder of any carbon atoms positions being filled by hydrogens, Y is selected from the group consisting of:

(1)  $-\overset{|}{\underset{|}{N^+}}-$,

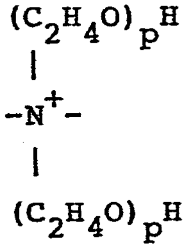
(2)  ,

(3)  $-\overset{|}{\underset{|}{P^+}}-$,

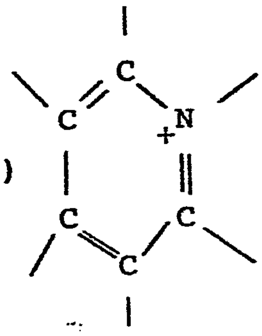
(4)  $\overset{|}{\underset{|}{S^+}}-$,

(5) 

$-\overset{|}{\underset{\underset{(C_2H_4O)_pH}{|}}{N^+}}-$, wherein p is from 1 to 12,

(6) 

$-\overset{\overset{(C_2H_4O)_pH}{|}}{\underset{\underset{(C_2H_4O)_pH}{|}}{N^+}}-$, wherein p is from 1 to 12,

(7) 

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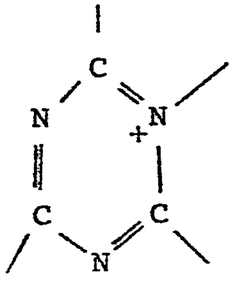
(8) 

, and

(9) mixtures thereof and Z is an anion such as halide, methyl sulfate or hydroxide.

One R_3 can also be a proton. The resultant tertiary amines can have characteristics similar to cationic surfactants at washing soultion pH values less than about 8.5.

A more complete disclosure of cationic surfactants can be found in U.S. Patent 4,228,044 by Cushman M. Cambre for Laundry Detergent Composition Having Enhanced

Particulate Soil Removal and Antiredeposition Performance, issued October 14, 1980, said patent being incorporated herein by reference.

When cationic surfactants are used in combination with anionic surfactants, compatibility must be considered. A type of cationic surfactant generally compatible with anionic surfactants is a C_{8-18} alkyl tri C_{1-3} alkyl ammonium chloride or methyl sulfate.

Zwitterionic Surfactants

Zwitterionic surfactants include derivatives of aliphatic quaternary ammonium, phosphonium, and sulfonium compounds in which the aliphatic moiety can be straight or branched chain and wherein one of the aliphatic substitutents contains from about 8 to 24 carbon atoms and one contains an anionic water-solubilizing group. Particularly preferred zwitterionic materials are the ethoxylated ammonium sulfonates and sulfates disclosed in U.S. Patents 3,925,262, Laughlin et al, issued December 9, 1975 and 3,929,678, Laughlin et al, issued December 30, 1975, said patents being incorporated herein by reference.

Ampholytic Surfactants

Ampholytic surfactants include derivatives of aliphatic heterocyclic secondary and ternary amines in which the aliphatic moiety can be straight chain or branched and wherein one of the aliphatic substituents contains from about 8 to about 24 carbon atoms and at least one aliphatic substituent contains an anionic water-solubilizing group.

Semi-Polar Nonionic Surfactants

Semi-polar nonionic surfactants include water-soluble amine oxides containing 1 alkyl or hydroxy alkyl moiety of from about 8 to about 28 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxy alkyl groups, containing from 1 to about 3 carbon atoms which can optionally be joined into ring structures; water-soluble phosphine oxides containing 1 alkyl or hydroxy alkyl moiety of from about 8 to about 28 and 2 moieties selected from the group consisting of alkyl groups and hydroxy alkyl groups, containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing 1 alkyl or hydroxy alkyl moiety of from about 8 to about 28 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxy alkyl moieties of from 1 to 3 carbon atoms.

For a more complete disclosure of compounds which are suitable for incorporation in detergent compositions, one can consult U.S. Patents 4,056,481, Tate (November 1, 1977); 4,049,586, Collier (September 20, 1977); 4,040,988, Vincent et al (August 9, 1977); 4,035,257, Cherney (July 12, 1977); 4,033,718, Holcolm et al (July 5, 1977); 4,019,999, Ohren et al (April 26, 1977); 4,019,998, Vincent et al (April 26, 1977); and 3,985,669, Krummel et al (October 12, 1976); all of said patents being incorporated herein by reference.

The Enzyme

The compositions of the invention contain from about 0.01% to about 5% by weight on a standard enzyme basis of an α-amylolytic enzyme (α-amylase) derived from B. licheniformis, preferably from about 0.05% to about 2% by weight and most preferably from about 0.1% to about 1.0% by weight. Commercial enzyme preparations, particularly those prepared for use in detergent compositions, are rarely pure enzyme and are, in any case, generally mixed with various inert materials. Commercial enzyme preparations will typically have a low calcium metal content complexed with the enzyme and as part of the inert portion of the preparation. As described hereinafter, this calcium content must be recognized for stability considerations. For purposes of describing the present invention and preferred enzyme levels, standard enzyme basis is defined as an enzyme preparation having an activity measured in Kilo Novo α-amylase Units (KNU) of 120 KNU/gram. One Kilo Novo α-amylase Unit (1 KNU) is the amount of enzyme which breaks down 5.26 grams of starch (Merck, Amylum Solubile Erg. B. 6, Batch 9947275) per hour at Novo's standard method for determination of α-amylase based upon the following standard conditions:

Substrate	soluble starch
Calcium content in solvent	0.0043M
Reaction time	7-20 min.
Temperature	37°C
pH	5.6

As described in British Patent Specification 1,296,839, incorporated herein by reference, the Novo method (Novo Industri A/S, Bagsvaerd, Denmark) is a modification of the SKB method performed at 37°C without the addition of β-amylase. The SKB method is described in Cereal Chemistry, 16, 712 (1939).

α-Amylases derived from B. licheniformis have been characterized as carbohydrases and more particularly 1,4-α-D-Glucan Glucanohydrolase. α-Amylases for use in detergent compositions, granule detergent compositions in particular, have been generally derived from Bacillus subtilis, but α-amylase derived from B. licheniformis suitable for use in the compositions of the present invention is available from a number of sources. Termamyl® 120L, Novo Industri A/S, Bagsvaerd, Denmark; Taka-Therm® L-340, Miles Laboratories, Elkhart, Indiana; Rohalase AT®, Rohm & Haas, West Philadelphia, PA; Maxamyl HT®, Gist Brocades, Delft, The Netherlands, are α-amylases derived from B. licheniformis and suitable for use in the compositions of the present invention. Reported deposit numbers for Bacillus licheniformis capable of producing α-amylase are NCIB 8061, NCIB 8059, ATCC 6634, ATCC 6598, ATCC 11945, ATCC 8480, and ATCC

9945a. British Patent Specification 1,296,839, published November 22, 1972, incorporated herein by reference, discloses methods of making α-amylases derived from B. licheniformis.

Sequestering Agent

The compositions of the invention contain from about 0.25% to about 40%, preferably from about 3% to about 30% and most preferably from about 5% to about 25% of a calcium ion sequestering agent. These sequestering agents act as detergency builders to improve cleaning, particularly when the washing solution will contain metal ions other than alkali metal ions. Sequestration involves the formation of a coordination complex of the sequestering agent and metallic ions in solution to reduce the interactions of calcium with other materials in the wash solution. As used herein, the term sequestering agent includes multidentate ligands which can act as chelating agents and can include some ion exchange materials, but not those detergency builder materials which remove calcium ions from solutions only by precipitation reactions.

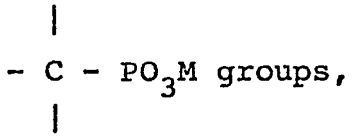
The sequestering agents used in the compositions of this invention include polyphosphates, polyphosphonates and polycarboxylates in soluble salt or acid form.

Polyphosphates which can sequester calcium ions are characterized by the general formula:

$$(\text{Monovalent Cation})_{n+2}P_nO_{3n+1}$$

and comprise the acid form and alkali metal, ammonium and substituted ammonium salts of pyrophosphoric acid and tripolyphosphoric acid and the water soluble polymetaphosphates. For use in aqueous liquid detergent compositions, pyrophosphoric acid and its salts are preferred because of their stability. Water solutions of tripolyphosphates and polymetaphosphates tend to degrade to a mixture of pyrophosphate and orthophosphate, the latter having the ability to precipitate but not sequester calcium.

Polyphosphonates comprise a large range of organic compounds having two or more



$$-\overset{|}{\underset{|}{C}}-PO_3M$$ groups,

wherein M is a hydrogen or a salt-forming radical. Suitable phosphonates include ethane-1-hydroxy-1,1-diphosphonates, ethanehydroxy-1,1,2-triphosphonates and their oligomeric ester chain condensates. Particularly suitable polyphosphonates for use in the compositions of the invention are nitrogen-containing polyphosphonates such as ethylenediaminetetramethylene phosphonic acid and diethylenetriaminepentamethylene phosphonic acid and alkali metal, ammonium and substituted ammonium salts thereof.

Suitable polycarboxylates include the acid form and alkali metal, ammonium and substituted ammonium salts of citric, ascorbic, phytic, mellitic, benzene pentacarboxylic, oxydiacetic, carboxymethyloxysuccinic, carboxymethyloxymalonic, cis-cyclohexanehexacarboxylic, cis-cyclopentanetetracarboxylic and oxydisuccinic acids. Also suitable are the polycarboxylate materials described in U.S. Patent 3,364,103 and polycarboxylate polymers and copolymers described in U.S. Patent 3,308,067, Diehl, issued March 7, 1967, both patents incorporated herein by reference.

With due regard to their stability in aqueous media, the polyacetal carboxylates disclosed in U.S. Patent 4,144,226 issued March 13,1979, to Crutchfield et al and U.S. Patent 4,146,495 issued March 27, 1979 to Crutchfield et al can be incorporated in the compositions of the invention.

Particularly suitable polycarboxylates are those containing nitrogen such as ethylenediaminetetraacetic acid, hydroxyethylenediaminetriacetic acid, diethylenetriaminepentaacetic acid and nitrilotriacetic acid and alklai metal, ammonium and substituted ammonium salts thereof.

As detailed by examples hereinafter, combinations of sequestering agents with different degrees of calcium ion sequestering power are particularly useful in the practice of this invention. The combinations of citric acid and a nitrogen-containing sequestering agent selected from the group consisting of ethylenediamine tetramethylene phosphonic acid, diethylenetriamine pentamethylenephosphonic acid, nitrilotriacetic acid, ethylenediaminetetraacetic acid, hydroxyethylenediaminetriacetic acid, diethylenetriaminepentaacetic acid or mixtures and suitable salts thereof are particularly preferred. Citric acid has a relatively lower equilibrium constant of complexation with calcium than the nitrogen-containing sequestering agents recited above. Preferred ratios of citric acid to the nitrogen-containing sequestering agent are from about 50:1 to about 1:2 by weight and, more preferably, from about 30:1 to about 2:1 by weight.

Enzyme-Accessible Calcium Ion

The stabilized aqueous enzyme compositions of this invention comprise a calcium-containing component to provide at least about $1x10^{-4.5}$ millimoles of enzyme-accessible calcium, as hereinafter defined, per liter of said composition. Preferably, sufficient calcium-containing component is incorporated to provide at least about $1x10^{-4}$ millimoles of enzyme-accessible calcium and more preferably at least about $1x10^{-3}$ millimoles. Most preferably the compositions contain at least about $1x10^{-2}$ millimoles of enzyme-accessible calcium per liter of said composition.

The calcium-containing component can be or can be part of other essential or optional components, for example, an anionic surfactant in the form of a calcium salt or a calcium-sequestering agent complex. More typically, water-soluble or solubilizable calcium salts such as calcium chloride are employed. In the aqueous liquid detergent compositions of the present invention, calcium in different forms, insoluble, sequestered and enzyme-accessible, will reach an equilibrium as a function of the composition components and pH without regard to the initial nature of the calcium-containing component.

A upper limit of enzyme-accessible calcium in compositions of the invention can be set by practical considerations not related to enzyme stability. There is little improvement in the stability of alpha-amylase derived from B.licheniformis in compositions of the invention at enzyme-accessible calcium levels above $1x10^{-2}$ millimoles per liter. In the preferred compositions of the invention this level of enzyme-accessible calcium is provided by total calcium levels of no more than about from 0.1% to about 0.3% of the composition by weight. At a recommended usage of 1/2 cup of the composition in a washing machine with a 18 gallon washing solution capacity, 0.18% total calcium (from 0.5% $CaCl_2$) increases water hardness of the washing solution by about 1/2 grain per gallon (as $CaCO_3$). Calcium is generally detrimental to detergency, but 1/2 grain/gallon is almost negligible. The calcium-containing component partially depletes the sequestering capacity of the sequestering agent or agents of the composition in so far as calcium is concerned. However, in washing solutions, a calcium-sequestering agent complex is nevertheless capable of sequestering heavy metal ions such as iron, manganese and copper which are associated with certain staining problems. This is a result of higher complexation and formation constants of said sequestering agents with heavy metal ions relative to the constants with calcium.

Enzyme-accessible calcium is analagous to free or ionic calcium in aqueous solutions of less complexity than the compositions of the invention.

For the purposes of this invention, enzyme-accessible calcium is determined by the method described in the following publication:

J. Morgan, F. Morel, Env. Sci. Tech. 6, 58-67 (1972), "A Numerical Method for Computing Equilibria in Aqueous Chemical Systems".

For the purposes of this invention said method is used to calculate enzyme-accessible calcium in compositions of the invention by use of the following assumptions:

1) ionic strength fixed at 0.5;
2) pH fixed at the pH of the composition;
3) only divalent (e.g., calcium) metals and multidentate ligands (the sequestering agents of this invention) are considered;
4) the free or uncomplexed calcium concentration is the enzyme-accessible calcium concentration.

Other methods of calculating equilibrium concentrations of all species in aqueous solutions provide essentially equivalent results and are also known to those skilled in the art.

It should be noted that alkaline earth metals other than calcium are substantially less effective for stabilizing the compositions of the invention.

Water

The compositions of this invention contain from about 10% to about 80% water, preferably from about 15% to about 60% water, by weight. Preferred compositions have a pH of from about 6.5 to about 9.0.

Optional Ingredients

The compositions of this invention can contain components other than those disclosed as essential.

The compositions of this invention can contain enzymes other than α-amylase derived from B.licheniformis, proteolytic enzymes in particular. Examples of suitable proteolytic enzymes include many enzyme preparations adapted for use in detergent compositions and, in fact, used in detergent compositions. Sources of the enzymes include commercial enzyme preparations such as Alcalase® sold by Novo Industries, A/S, Copenhagen, Denmark and Maxatase® sold by Gist-Brocades, Delft, The Netherlands. Other preferred enzyme compositions include those commercially available under the tradenames Esperase®, manufactured and sold by Novo Industries, A/S, Copenhagen, Denmark and "AZ-Protease" manufactured and sold by Gist-Brocades, Delft, The Netherlands.

It should be noted that levels of enzyme accessible calcium above about $1x10^{-2}$ millimoles per liter provide excellent stability to proteolytic enzymes (proteases) incorporated in the compositions of the invention and the inclusion of a proteolytic enzyme is a preferred embodiment. There is, however, no dramatic increase in proteolytic enzyme stability over any narrow range of enzyme-accessible calcium levels as is seen with α-amylase (derived from B.licheniformis) between $1x10^{-5}$ and $1x10^{-4}$.

A more complete disclosure of suitable enzymes can be found in U.S. Patent 4,101,457, Place et al issued July 18, 1978, incorporated herein by reference.

The compositions of this invention can contain solvents other than water. Low molecular weight primary or secondary alcohol exemplified by methanol, ethanol, propanol, and isopropanol are suitable. Monohydric alcohols are preferred for solubilizing the surfactant but polyols containing from 2 to about 6 carbon atoms and from 2 to about 6 hydroxy groups can be used and can provide improved enzyme stability. Examples of polyols include propylene glycol, ethylene glycol, glycerine and 1,2-propanediol. Ethanol is a particularly preferred alcohol. The compositions contain from 0% to about 30%, preferably from about 1% to about 15%, of alcohols.

A short chain carboxylic acid salt can be used to stabilize enzymes, particularly proteolytic enzymes, as disclosed in U.S. Patent 4,318,818, issued March 9, 1982. The short chain carboxylic acid salt preferably is a formate, e.g., formic acid and its salts. The formates are surprisingly much more effective than other short chain carboxylic salts such as the acetates and the propionates. The short chain carboxylic acid salt is used at a level from about 0.1% to about 10%, preferably from about 0.3% to about 3%, more preferably from about 0.5% to about 2.0% when the product pH is below about 8.5 and from about 3% to about 10%, preferably from about 4% to about 8%, when the product pH is from about 8.5 to about 10.

In a preferred embodiment, a fatty acid component is incorporated in an amount of from about 3% to about 25% by weight, preferably from about 5% to about 20% by weight. The fatty acids have from 10 to 22, preferably from 12 to 18 carbon atoms in the alkyl chain. Compositions containing fatty acids, will have a pH of from about 6.5 to about 9.0.

The compositions of the invention can contain such materials as fabric whiteners and brighteners, sudsing control agents, hydrotropes such as sodium toluene or xylene sulfonate, perfumes, colorants, opacifiers, anti-redeposition agents and alkalinity control or buffering agents such as monoethanolamine and triethanolamine. The use of these materials is well known in the detergent art.

Materials or anions that tend to precipitate calcium should preferably be restricted to minimum levels. Examples are carbonates, sulfates, and orthophosphates. Although the fatty acids of preferred compositions act to precipitate calcium in a washing solution, they have no such effect in the undiluted compositons of the invention if calcium levels are controlled carefully. The fatty acid content of the compositions of the invention, at least at pH values below 8.5, does not substantially effect the enzyme-accessible calcium level of said compositions and can be neglected when calculating the enzyme-accessible calcium level as disclosed hereinbefore.

The following examples illustrate the invention and facilitate its understanding.

All parts, percentages and ratios herein are by weight unless otherwise specified.

EXAMPLE I

Liquid detergent compositions were prepared by mixing the components listed hereinafter in the stated proportions.

	A	B
Surfactants		
C_{13} linear alkyl benzene sulfonic acid	9.25%	5.8%
Coco alkyl sulfate-acid form	-	8.8
C_{12-14} alkyl ether (ethoxy 1.0) sulfate-acid form	8.75	-
C_{12} alkyltrimethylammonium chloride	1.2	1.2
C_{12-13} alcohol ethoxy 6.5 condensate	6.5	10.0

Fatty Acids		
Lauric acid	10.1	7.5
Myristic acid	4.1	2.5
Oleic acid	-	5.0
Sequestering Agents		
Citric Acid	4.0	6.9
Sodium diethylenetriaminepentaacetate (DTPA)	(1)	0.6
Enzymes		
Maxatase®	.75	.75
Termamyl® 120L	0.37	0.37
Solvents		
Water	30.7	19.4
Ethanol	7.0	1.0
1,2-Propanediol	5.0	5.0
Other Components		
Monoethanolamine	2.0	12.0
Triethanolamine	4.0	6.7
NaOH & KOH	4.9	-
Sodium toluene sulfonate	-	5.0
Sodium formate	1.0	1.0
$CaCl_2$	(1)	(1)
Perfume, colorants, fabric whiteners and other miscellaneous optional ingredients	-----Remainder-----	
pH	8.4	8.4

(1) as indicated in tabulations below (quantity added is deducted from total water).

Composition A was prepared in thirteen variations with $CaCl_2$ and sodium diethylenetriaminepentaacetate (DTPA) levels as indicated. The variations were stored in airtight containers for 1 week at 100°F. α-amylase activity was then measured by Technicon Industrial Method #500-77P dated September 12, 1977, as revised September 1979, (Technicon Industrial Systems, Tarrytown, N.Y. 10591), but any suitable method such as the one described in U.S. Patent 4,284,722 issued August 18, 1981, to Tamuri et al, incorporated herein by reference, will give equivalent results. α-amylase activity at the end of the storage period was divided by activity directly after composition preparation to calculate percent retained activity.

Composition A - α-Amylase Stability

CaCl$_2$ added (2) %	DTPA %	% of composition DTPA complexed with calcium	%Ca sequested by DTPA	%Ca sequested by citrate	Enzyme accessible calcium (3)	% retained α-amylase activity 1 week 100°F
0.0	0.0	-	-	99.7	4.50	20
0.0	0.3	0.2	99.8	-	7.26	trace
0.0	0.6	0.1	99.9	-	7.56	trace
0.0	0.9	0.1	99.9	-	7.74	trace
0.1	0.0	-	-	99.7	1.61	93
0.1	0.3	99.7	66.0	33.9	2.09	90
0.1	0.6	75.2	99.7	-	4.08	47
0.1	0.9	50.3	99.9	-	4.56	trace
0.2	0.3	99.9	33.1	66.7	1.48	94
0.2	0.6	99.8	66.1	33.8	1.78	98
0.2	0.3	97.9	97.3	2.7	2.89	81
0.3	0.6	99.9	44.2	55.7	1.37	99
0.3	0.9	99.9	66.2	33.7	1.60	93

(2) in addition to low levels of calcium in enzyme preparations

(3) expressed as the negative log of the millimolar enzyme accessible calcium concentration

Composition B was prepared in seven variations with CaCl$_2$ levels as indicated. The variations were stored for 1 month at 90°F and at 70°F and evaluated for α-amylase stability in the same manner as the variations of Composition A.

Composition B - α-Amylase Stability

$CaCl_2$ added (2) %	% of composition DTPA complexed with calcium	%Ca sequested by DTPA	%Ca sequested by citrate	Enzyme accessible calcium (3)	% retained α-amylase activity 1 mo./ 90°F	% retained α-amylase activity 1 mo./ 70°F
0.0	-	99.9	-	7.56	0.0	6
0.125	93.1	98.6	1.4	3.44	56	79
0.25	99.9	52.9	46.9	1.60	89	93
0.5	100.0	26.5	73.3	1.07	90	93
1.0	100.0	13.3	51.6(4)	0.88	91	95

(2) in addition to low levels of calcium in enzyme preparation (quantity added is deducted from total water)

(3) expressed as negative log of millimolar enzyme-accessible calcium concentration

(4) plus 34.9% as precipitated calcium citrate

These results show the dramatic improvement in α-amylase (derived from B.licheniformis) stability in an aqueous liquid detergent composition when a calcium-containing component is incorporated that provides at least about $1x10^{-4.5}$ millimoles of enzyme-accessible calcium. The results also point out the advantage of mixtures of sequestering agents. A sequestering agent with high equilibrium constant of complexation, e.g., the nitrogen-containing sequestering agents disclosed hereinbefore, will tend to sequester all available calcium to the point of theoretical capacity, but can release such calcium in washing solutions to in turn sequester heavy metal ions such as copper, iron and manganese. A sequestering agent with a lower equilibrium constant of complexation such as alkali metal citrates or citric acid can be incorporated in the compositions of the invention without addition of calcium-containing components at a level to satisfy theoretical capacity. Thus, such sequestering agents are available to act as detergency builders effective in the presence of alkaline earth metal ions in washing solutions.

Apparently, α-amylase derived from B.licheniformis has, in effect, an equilibrium constant of complexation with calcium somewhat greater than citrate, or more precisely, an equilibrium constant that can hold sufficient calcium for stabilization in an aqueous solution containing at least about $1x10^{-4.5}$ millimoles of enzyme accessible calcium.

EXAMPLE II

The commercial enzymes Taka-Therm® L-340, Rohalase AT® and Maxamyl HT® are incorporated in the compositions of Example I replacing the Termamyl® 120L. Comparable α-amylase stability results are obtained at equivalent enzyme-accessible calcium levels.

EXAMPLE III

α-amylases derived by B.subtilis, Ban® from Novo Industri and Rapidase® from Gist Brocade, are incorporated in the compositions of Example I replacing the Termamyl R120L. It is not possible to add a sufficient amount of a calcium-containing component to stabilize said α-amylases derived from B.subtilis without precipitation of calcium citrate in the aqueous liquid detergent compositions.

EXAMPLE IV

Dodecyldimethyamine oxide replaces C_{12} alkyltrimethylammonium chloride in the compositions of Example I. Equivalent results are obtained.

EXAMPLE V

The following compositions are prepared by mixing the ingredients listed.

	A	B	C
C_{13} linear alkyl benzene sulfonic acid	12.1%	10.56%	-
C_{14-15} alkyl ethoxy-1 ether sulfate	3.9	-	10.0
Triethanolamine coco alkyl sulfate	-	4.0	-
C_{12-14} alkyltrimethyl-ammonium chloride	1.4	-	-
C_{14-15} alcohol ethoxylate-7	7.8	12.0	20.0
Lauric acid	9.08	7.5	-
Myristic acid	3.03	2.5	-
Oleic acid	2.4	5.0	-
Citric acid	5.3	0.2	0.05
Maxatase®	0.97	0.97	1.0
Termamyl® 120L	0.3	0.3	0.3
Diethylenetriaminepenta methylene phosphonic acid	0.29	0.3	0.3
Monoethanolamine	1.9	-	-
Triethanolamine	3.6	4.5	-
NaOH & KOH	4.19	1.65	-
Ethanol	4.8	8.63	7.0
1,2-Propanediol	7.2	3.0	-
Sodium formate	0.97	1.0	2.0
Calcium chloride	0.02	0.02	0.02
Water	29.2	35.5	58.0
Perfume, colorant, suds control agent fabric whiteners and other miscellaneous optional ingredients	----Remainder----		
pH	8.0	7.7	7.5

The incorporation of 0.02% calcium chloride in each composition provides an enzyme accessible calcium level of at least $1x10^{-4.5}$ millimoles per liter.

EXAMPLE VI

Potassium pyrophosphate, potassium carboxymethyloxymalonate and potassium carboxymethyloxysuccinate are each substituted for citric acid in Examples I, II and IV. Composition pH is adjusted to 8.0. A calcium chloride level of 0.3% by weight is sufficient to provide an enzyme-accessible calcium level greater than $1x10^{-4.5}$ millimoles and stabilize the α-amylase derived from B.licheniformis incorporated in said compositions.

CLAIMS

1. A stabilized aqueous enzyme composition comprising:
   (a) from about 1% to about 75% by weight of a non-soap detergent surfactant or mixtures thereof;
   (b) from about 0.01% to about 5% by weight on a standard enzyme basis of an α-amylolytic enzyme derived from B.licheniformis;
   (c) from about 0.25% to about 40% by weight of a sequestering agent or mixtures thereof;
   (d) a calcium-containing component to provide at least about $1 \times 10^{-4.5}$ millimoles of enzyme available calcium ion per liter of said composition; and
   (e) from about 10% to about 80% water by weight.

2. The composition of Claim 1 wherein said composition is an aqueous liquid detergent composition.

3. The composition of Claim 2 wherein said calcium-containing component provides at least about 1×10^{-3} millimoles of enzyme-available calcium ion per liter of said composition.

4. The composition of Claim 2 wherein said calcium-containing component provides at least about 1×10^{-2} millimoles of enzyme-available calcium ion per liter of said composition.

5. The composition of Claim 2 wherein said non-soap detergent surfactant comprises from about 10% to about 65% of said composition.

6. The composition of Claim 5 wherein said sequestering agent comprises from about 3% to about 30% of said composition by weight.

7. The composition of Claim 6 wherein said sequestering agent comprises citric acid or water soluble salts of citric acid or mixtures thereof.

8. The composition of Claim 7 wherein said sequestering agent comprises a nitrogen-containing sequestering agent.

9. The composition of Claim 2 wherein said composition comprises a proteolytic enzyme.

10. The composition of Claim 9 wherein said composition comprises formic acid or water soluble salts of formic acid or mixtures thereof.

11. The composition of Claim 6 wherein said composition has a pH of from about 6.5 to about 9.0.

12. The composition of Claim 11 wherein said composition comprises from about 3% to about 25% by weight of a fatty acid having from about 10 to about 22 carbon atoms.

13. The composition of Claim 12 wherein water comprises from about 15% to about 60% of said composition by weight.

14. The composition of Claim 2 wherein said calcium-containing component comprises a salt of calcium or mixtures thereof.

15. The composition of Claim 2 wherein said calcium-containing component comprises a calcium-sequestering agent complex or mixtures thereof.

16. The composition of Claim 15 wherein said calcium-sequestering agent complex comprises a nitrogen-containing sequestering agent.

EFG:sp(A39/A8)

European Patent Office

EUROPEAN SEARCH REPORT

0118933

Application number

EP 84 20 0157

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
Y	EP-A-0 028 866 (PROCTER & GAMBLE EUROPEAN TECHNICAL CENTER) * Page 7, line 25 - page 9, line 9; page 22; claims 1, 3, 8 * ---	1,2,5, 7-11, 14	C 11 D 3/386
D,Y	US-A-4 305 837 (G.J. KAMINSKY et al.) * Column 10, example 3; column 11, lines 40-59; claims 1, 2, 9 * ---	1,2,5, 7-11, 14	
D,A	US-A-4 111 855 (C.R. BARRAT et al.) * Column 13, lines 29-45; claim 1 * ---		
A	DE-A-2 025 748 (NOVO TERAPEUTISK LABORATORIUM A/S) * Claims 1-8 * -----		TECHNICAL FIELDS SEARCHED (Int. Cl. ³) C 11 D 3/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	15-05-1984	SCHULTZE D

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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