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(54) Stabilized enzymatic aqueous detergent compositions.

(57) The present invention provides a method for stabilizing enzymes in aqueous detergent compositions, which method comprises adding 3 to 50% by weight of a non-polymeric salt to said compositions to precipitate the enzymes into a separate phase and thereby protect the enzymes from other components left solubilized in the compositions. The invention further provides compositions having at least two phases provided in the process of the invention.

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BACKGROUND AND PRIOR ART

The invention relates to aqueous, detergent compositions containing enzymes and to methods of stabilizing the enzymes in these compositions via the use of simple, non-polymeric salts. More particularly, the invention provides compositions in which the enzymes have been stabilized by precipitating them into a separate phase using simple non-polymeric salts in order to protect the enzymes from other components left solubilized in the compositions.

The use of various enzymes in heavy duty liquid (HDL) aqueous detergent compositions is well known in the art. The stability of enzymes in these compositions is limited, however, due to the enzyme-denaturing properties common to all liquid detergents. For example, the pH of the solution (higher pH is generally associated with greater denaturation), the type of surfactant used (anionic surfactants are generally more harsh on enzymes), and type of builder used all can adversely impact on the stability of the enzyme. In addition, the stability of a protease is also affected by the process called autolysis in which the protease digests itself. Factors such as pH, ionic strength and the presence of protease inhibitors also affect autolysis.

Many attempts have been made in the art to help preserve the activity of enzymes in liquid detergents. For example, the art has attempted to minimize denaturation of enzymes by attempting to select surfactants, pH levels and builders which are less harsh and in which an acceptable level of enzyme storage stability can be maintained. Unfortunately, the selection of components or conditions which are less harsh (e.g. nonionics, lower pH) also compromises certain aspects of cleaning performance.

Attempts have also been made to stabilize enzymes in liquid detergents by using various protein stabilizers or stabilization systems (e.g. protease inhibitors). US-A-4 261 868 (Unilever) teaches the use of borax as a protease inhibitor and both US-A-4 243 546 (Shaer) and GB-A-1 354 761 (Henkel) teach the use of carboxylic acids as protease inhibitors. Various combinations of these protease inhibitors are also known in the art. US-A-4 305 837 to Kaminsky et al., for example, teaches the combination of carboxylic acids and simple alcohols and US-A-4 404 115 (Unilever) teaches the combination of borax and polyols as protease inhibitors. US-A-4,537,707 to Severson teaches the combination of borax and carboxylates as protease inhibitors.

Attempts have also been made to increase the life of enzymes in liquid detergent compositions using genetic engineering techniques, see e.g. US-A-4 760 025 (Genencor). However, these engineered enzymes are ultimately still subject to denaturation and autolysis (for proteases).

In all of the above-described references, the stability of the enzyme is directly linked to the composition of the detergent. This relationship is a direct result of the fact that the enzyme is itself a dissolved component of the liquid detergent and therefore exposed to all other co-solubilized formulation ingredients.

Attempts have also been made in the art to separate proteases from detergent compositions and thereby protect the protease from these components of the composition. US-A-4 863 626 to Coyne et al., for example, teaches the removal of enzyme from solution by encapsulation. Enzyme encapsulation is the process of surrounding the enzyme with an insoluble coating that forms a barrier between enzyme and solubilized formulation ingredients that are detrimental to enzyme activity (e.g. oxidants). Encapsulation involves the introduction of a separate component to the composition which can be very expensive. Enzyme encapsulation also requires the use of a release or trigger mechanism in order to free the enzyme from the capsule. Finally, because of the encapsulation, there is a loss of any enzymatic pre-treatment benefit which might be obtained otherwise.

EP-A-351,162 (Albright & Wilson and to NOVO) teaches a method for the preparation of a stabilized aqueous enzyme dispersion comprising (1) precipitating a water-soluble polymer from aqueous solution to form an aqueous dispersion and (2) before, during or after the precipitation of the polymer, contacting the polymer with an aqueous solution or fine aqueous dispersion of enzyme. It is clear from this application that stability of the enzyme is somehow dependent on contact with the polymer, either before, during or after precipitation of the polymer. No such polymer interaction is required by the subject invention. In addition, to the extent that an aqueous solution of enzyme may be added to the polymer even after the polymer has been precipitated, it is not clear that the enzyme taught in this reference even needs to be precipitated in the first place.

EP-A-298,654 (Hybrisens Ltd.) teaches the use of antibodies (protein) that are complimentary to proteases to protect the proteases during storage in liquid detergent formulation.

Thus, there appears to be no prior art which teaches that the precipitation of an enzyme may be accomplished with a simple non-polymeric salt, independent of interaction with other polymers or antibodies or independent of encapsulation, in order to cause a phase separation and protect the enzyme from other components remaining in the soluble phase.

SUMMARY OF THE INVENTION

The subject invention provides a method for stabilizing enzymes used in liquid detergent compositions. The method comprises separating or partitioning the enzyme into a separate phase (separate from other denaturing components left in the solubilized phase) via precipitation of the enzyme wherein the precipitation is affected by raising the level of simple, non-polymeric salt in the compositions to a level sufficient to cause the precipitation.

Precipitation of the enzyme is accomplished by raising the level of simple, non-polymeric salt until protein aggregation occurs. Eventually, the formed protein aggregates become so large as to precipitate from solution. The non-polymeric salt may be a simple salt, e.g. sodium sulphate, a builder salt, e.g. sodium citrate, a buffer salt such as sodium borate or a mixture thereof. Thus, the salt may have no apparent functionality other than to precipitate enzyme from solution or the salt may have a builder or other functionality. The salt must, however, be able to precipitate the enzyme into a separate phase. Typical examples of suitable precipitating salts are alkali metal or ammonium salts of -borate, - sulphate, -citrate, -carbonate and -nitrilotriacetate. When sodium salts are used, the lyotropic number must be less than 9.5 (see also US-A-4 530 780).

The exact amount of non-polymeric salt required to precipitate the enzyme varies with the enzyme which is to be precipitated, the pH of the solution, the nature of the cosolutes and the nature of the precipitating salt.

Preferably, a proteolytic enzyme is precipitated into a separate phase, and more preferably, the composition comprises one or more other enzymes, which are thereby protected against proteolytic degradation.

In another embodiment of the invention, the subject invention relates to compositions comprising at least 2 separate phases (i.e. precipitated and non-precipitated phases) made in accordance with the method described above. The levels of electrolyte used are generally higher than those previously known in the art for enzyme-containing liquid detergent compositions.

DETAILED DESCRIPTION OF THE INVENTION

The subject invention provides, in one aspect of the invention, a method for stabilizing enzymes in aqueous detergent compositions. More particularly, the invention provides a method for stabilizing enzymes in such compositions, which method comprises adding sufficient simple, non-polymeric salt to cause the enzyme to precipitate into a separate phase from the components left solubilized in the compositions. By "simple" is meant a non-polymeric salt. The salt used in the invention may be a monovalent, divalent or a multivalent salt.

The enzyme precipitation and phase separation results in the effective removal of enzyme from solution which in turn results in less enzyme denaturation and, in the case of proteases, less autolysis. While enzymes will still undergo some decay under these processes, the rate of decay is reduced because the enzymes are not free to diffuse as in solution. While not wishing to be bound by theory, the foregoing is what Applicants believe to be occurring. Whether the mechanism is correct or not, however, experimental support clearly shows the resulting increase in stability obtained by following the method.

In general, the precipitation approach to enzyme stabilization allows the use of harsher components (e.g. builders, actives, bleaches) than would otherwise be accommodated while maintaining acceptable enzyme stability. In addition, in the case of detergent proteases, a reduced level of inhibitor is required to reduce autolysis to an acceptable degree.

The precipitation and phase separation according to the subject invention is accomplished by adjusting the level of simple non-polymeric salt in the aqueous detergent composition to a level sufficient to cause the enzyme to become insoluble. This adjustment may be accomplished by raising the level of salt until protein aggregation occurs. Eventually, the formed protein aggregates become so large as to precipitate from solution.

The amount of insoluble enzyme is measured by determining how much enzyme is left soluble after centrifuging and assaying the enzyme-containing solution. Based on the enzyme activity in the supernatant of the centrifuged solution, it is possible to calculate how much enzyme is still soluble. Soluble enzyme is then defined as the per cent of supernatant enzyme activity after centrifugation divided by enzyme activity, which is the sum of the supernatant and the precipitate activity.

Any simple, non-polymeric salt which can cause enzyme aggregation and precipitation may be used. For example, the electrolyte may be a simple salt such as an alkali metal of sulphate. The salt may also have a functionality in addition to its functionality as a simple salt electrolyte. For example, the non-

polymeric salt may be a builder such as sodium citrate or trisodium nitrilotriacetate. The salt may also have a functionality as a buffer such as with an alkali metal borate. Other simple non-polymeric salts which may or may not have additional functionalities and which are known to those skilled in the art are also contemplated by the invention.

The amount of salt used is in the range from 3 to 50% by weight of the composition and will vary in part depending on the exact salt used. Preferably, the salt is used in an amount ranging from 10 to 50%, more preferably from 15 to 50%. In a preferred embodiment of the invention that salt is sodium sulphate and is used in an amount from 10 to 40%, preferably 15 to 20% of the solution.

The exact amount of electrolyte used will also depend on the pH of the detergent composition. In general, it is desirable to precipitate the enzyme at a point as close to the isoelectric point of the enzyme as possible. In practice, this is accomplished by adjusting the pH to as close to the isoelectric point as possible and then adding the simple, non-polymeric salt. In general, the closer to the isoelectric point, the less the amount of salt which is needed to cause precipitation.

The amount of non-polymeric salt may also depend on what other components are co-solubilized in the composition such as, for example, the types of actives or hydrotropes present in the composition. For example, it has generally been found that components which make the composition more polar might require the use of greater amounts of salt.

The method of the invention may be used to prepare compositions which are also contemplated by the invention. The compositions, after addition of electrolyte, are at least two-phase compositions. These compositions may comprise any of the detergent actives, builders, and optional ingredients well known to those skilled in the art of detergent compositions. More specifically, the various ingredients within these compositions are set forth below:

Detergent Active

The compositions of the invention may comprise a detergent active material usually incorporated in liquid detergent formulations.

The active detergent material may be an alkali metal or alkanolamine soap or a 10 to 24 carbon atom fatty acid, including polymerized fatty acids, or an anionic, nonionic, cationic, zwitterionic or amphoteric synthetic detergent material, or mixtures of any of these.

Examples of the anionic synthetic detergents are salts (including sodium, potassium, ammonium and substituted ammonium salts such as mono-, di- and triethanolamine salts of C_9-C_{20} alkylbenzenesulphonates, C_8-C_{22} primary or secondary alkanesulphonates, C_8-C_{24} olefinsulphonates, sulphonated polycarboxylic acids prepared by sulphonation of the pyrolyzed product of alkaline earth metal citrates, e.g., as described in GB-A-1 082 179, C_8-C_{22} alkylsulphates, C_8-C_{24} alkylpolyglycolether-sulphates, -carboxylates and -phosphates (containing up to 10 moles of ethylene oxide); further examples are described in "Surface Active Agents and Detergents" (Vol. I and II) by Schwartz, Perry and Berch. Any suitable anionic may be used and the examples are not intended to be limiting in any way.

Examples of nonionic synthetic detergents which may be used with the invention are the condensation products of ethylene oxide, propylene oxide and/or butylene oxide with C_8-C_{18} carbon alkylphenols, C_8-C_{18} primary or secondary aliphatic alcohols, C_8-C_{18} fatty acid amides; further examples of nonionics include tertiary amine oxides with one 8 to 18 carbon alkyl chain and two 1 to 3 carbon alkyl chains. The above reference also describes further examples of nonionics.

The average number of ethylene oxide or propylene oxide present in the above nonionics varies from 1 to 30. Mixtures of various nonionics, including mixtures of nonionics with a lower and a higher degree of alkoxylation, may also be used.

Also applicable are surfactants such as those described in EP-A-328 177 (Unilever), which show resistance to salting-out, the alkylpolyglycoside surfactants described in EP-A-070 074, and the alkyl monoglucosides described in WO88/10147 (Novo).

Anionic surfactants can be present for example in amounts in the range from about 5% to about 50% by weight of the liquid detergent concentrate. Preferably nonionic detergent is present in amounts greater than 1%, e.g. 2-20% by weight of the composition.

Examples of cationic detergents are the quaternary ammonium compounds such as alkyltrimethylammonium halogenides.

Examples of amphoteric or zwitterionic detergents are N-alkylamino acids, sulphobetaines, condensation products of fatty acids with protein hydrolysates; but owing to their relatively high costs they are usually used in combination with an anionic or a nonionic detergent. Mixtures of the various types of active detergents may also be used, and preference is given to mixtures of an anionic and nonionic detergent

active. Soaps (in the form of their sodium, potassium and substituted ammonium salts) of fatty acids may also be used, preferably in conjunction with an anionic and/or nonionic synthetic detergent.

Builders

Builders which can be used according to the method and compositions of this invention include conventional alkaline detergency builders, inorganic or organic, which can be used at levels from 0% to about 50% by weight of the composition, preferably from 1% to about 30% by weight, most preferably from about 20% to about 30%.

Examples of suitable inorganic alkaline detergency builders are water-soluble alkali metal phosphates, polyphosphates, borates, silicates and also carbonates. Specific examples of such salts are sodium and potassium triphosphates, pyrophosphates, orthophosphates, hexametaphosphates, tetraborates, silicates and carbonates.

Examples of suitable organic alkaline detergency builder salts are: (1) water-soluble amino polycarboxylates, e.g., sodium and potassium ethylenediaminetetraacetates, nitrilotriacetates and N-(2-hydroxyethyl)-nitrilodiacetates; (2) water-soluble salts of phytic acid, e.g., sodium and potassium phytates (see US-A-2 379 942); (3) water-soluble polyphosphonates, including specifically, sodium, potassium and lithium salts of ethane-1-hydroxy-1,1-diphosphonic acid; sodium, potassium and lithium salts of methylene diphosphonic acid; sodium, potassium and lithium salts of ethylene diphosphonic acid; and sodium, potassium and lithium salts of ethane-1,1,2-triphosphonic acid. Other examples include the alkali metal salts of ethane-2-carboxy-1, 1-diphosphonic acid hydroxymethanediphosphonic acid, carboxyldiphosphonic acid, ethane-1-hydroxy-1,1,2-triphosphonic acid, ethane-2-hydroxy-1,1,2-triphosphonic acid, propane-1,1,3,3-tetraphosphonic acid, propane-1,1,2,3-tetraphosphonic acid, and propane-1,2,2,3-tetraphosphonic acid; (4) water-soluble salts of polycarboxylate polymers and copolymers as described in US-A-3 308 067. The latter salts may be used as long as they are not precipitated in solution.

In addition, polycarboxylate builders can be used satisfactorily, including water-soluble salts of mellitic acid, citric acid, oxydisuccinic, tartrate mono- and disuccinic, and carboxymethyloxysuccinic acid and salts of polymers of itaconic acid and maleic acid. Certain zeolites or aluminosilicates can be used. One such aluminosilicate which is useful in the compositions of the invention is an amorphous water-insoluble hydrated compound of the formula $Na_x((AlO_2)_y.SiO_2)$, wherein x is a number from 1.0 to 1.2 and y is 1, said amorphous material being further characterized by an Mg + + exchange capacity of from about 50 mg. eq. $CaCO_3/g$ and a particle diameter of from about 0.01 micron to about 5 microns. This ion exchange builder is more fully described in GB-A-1 470 250.

A second water-insoluble synthetic aluminosilicate ion exchange material useful herein is crystalline in nature and has the formula $Na_z[(AlO_2)_y.(SiO_2)]xH_2)xH_2O$, wherein z and y are integers of at least 6; the molar ratio of z to y is in the range from 1.0 to about 0.5, and x is an integer from about 15 to about 264; said aluminosilicate ion exchange material having a particle size diameter from about 0.1 micron to about 100 microns; a calcium ion exchange capacity on an anhydrous basis of at least about 200 milligrams equivalent of $CaCO_3$ hardness per gram; and a calcium exchange rate on an anhydrous basis of at least about 200 grams/gallon/minute/gram. These synthetic aluminosilicates are more fully described in GB-A-1 429 143.

Enzymes

Enzymes which may be precipitated into a separate phase and thereby stabilized may be protease enzymes and/or lipase enzymes such as are known well in the art. There is, as is known, a tendency for lipase to be less stable in the presence of protease than in the absence of protease, however, in the presence of precipitated protease we observe that there is a relative stabilizing effect on the lipase which is also present.

Other enzymes which may be used include cellulases, oxidases, amylases or other stain and/or soil-removing enzymes. Mixtures of enzymes may be employed. For use in a liquid detergent, the enzyme is preferably selected for stability at alkaline pH.

Suitable proteolytic enzymes which may be used may be of vegetable, animal or microorganism origin. Preferably, it is of the latter origin, which includes yeasts, fungi, moulds and bacteria. Particularly preferred are bacterial subtilisin-type proteases, obtained from e.g. particular strains of **B. subtilis** and **B. licheniformis.** Examples of suitable commercially available proteases are Alcalase, Savinase, Esperase, Primase, all of NOVO Industri A/S; Maxatase and Maxacal of Gist-Brocades: Kazusase of Showa Denko. Genetically engineered proteases derived from the above-identified proteases may also be used. The amount of

proteolytic enzyme included in the composition ranges from 0.1 to 100,000 GU/g, based on the final composition. Naturally, mixtures of different proteolytic enzymes may be used.

A GU is a glycine unit, which is the amount of proteolytic enzyme which under standard incubation conditions produces an amount of terminal NH_2-groups equivalent to 1 microgram/ml of glycine.

Examples of suitable lipases which can be used include fungal lipases producible by **Humicola lanuginosa** and **Thermomyces lanuginosus,** or bacterial lipases which show a positive immunological cross-reaction with the antibody of the lipase produced by the microorganism **Chromobacter viscosum** var. **lipolyticum** NRRL B-3673. This microorganism has been described in Dutch patent specification 154,269 of Toyo Jozo K.K. and has been deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Tokyo, Japan, and added to the permanent collection under nr. KO Hatsu Ken Kin Ki 137 and is available to the public at the United States Department of Agriculture, Agricultural Research Service, Northern Utilization and Development Division at Peoria, Illinois, USA, under the nr. NRRL B-3673. The lipase produced by this microorganism is commercially available from Toyo Jozo Co., Tagata, Japan, hereafter referred to as "TJ lipase". These bacterial lipases should show a positive immunological cross-reaction with the TJ lipase antibody, using the standard and well-known immunodiffusion procedure according to Ouchterlony (Acta. Med. Scan., 133, pages 76-79 (1950).

The preparation of the antiserum is carried out as follows:

Equal volumes of 0.1 mg/ml antigen and of Freund's adjuvant (complete or incomplete) are mixed until an emulsion is obtained. Two female rabbits are injected with 2 ml samples of the emulsion according to the following scheme:

day 0 : antigen incomplete Freund's adjuvant
day 4 : antigen in complete Freund's adjuvant
day 32 : antigen in incomplete Freund's adjuvant
day 60 : booster of antigen in incomplete Freund's adjuvant

The serum containing the required antibody is prepared by centrifugation of clotted blood, taken on day 67.

The titre of the anti-TJ-lipase antiserum is determined by the inspection of precipitation of serial dilutions of antigen and antiserum according to the Ouchterlony procedure. A 2^5 dilution of antiserum was the dilution that still gave a visible precipitation with an antigen concentration of 0.1 mg/ml.

All bacterial lipases showing a positive immunological cross-reaction with the TJ-lipase antibody as hereabove described are lipases suitable in this embodiment of the invention. Typical examples thereof are the lipase ex **Pseudomonas fluorescens** IAM 1057 available from Amano Pharmaceutical Co., Nagoya, Japan, under the trade-name Amano-F lipase, the lipase ex **Pseudomonas fragi** FERM P 1339 (available under the trade-name Amano-B), the lipase ex **Pseudomonas nitroreducens** var. **lipolyticum** FERM P1338, the lipase ex **Pseudomonas sp.** available under the trade-name Amano CES, the lipase ex **Pseudomonas cepacia,** lipases ex **Chromobacter viscosum,** e.g. **Chromobacter viscosum** var. **lipolyticum** NRRL B-3673, commercially available from Toyo Jozo Co., Tagata, Japan; and further **Chromobacter viscosum** lipases from U.S. Biochemical Corp. USA and Diosynth Co., The Netherlands, and lipases ex **Pseudomonas gladioli.**

An example of a fungal lipase as defined above is the lipase ex **Humicola lanuginosa,** available from Amano under the tradename Amano CE; the lipase ex **Humicola lanuginosa,** as described in the aforesaid EP-A-258 068 (NOVO), as well as the lipase obtained by cloning the gene from **Humicola lanuginosa** and expressing this gene in **Aspergillus oryzae,** commercially available from NOVO Industri A/S under the trade-name "Lipolase". This lipolase is a preferred lipase for use in the present invention.

While various specific lipase enzymes have been described above, it is to be understood that any lipase which can confer the desired lipolytic activity to the composition may be used and the invention is not intended to be limited in any way by specific choice of lipase enzyme.

The lipases of this embodiment of the invention are included in the liquid detergent compositions in such an amount that the final composition has a lipolytic enzyme activity of from 100 to 0.005 LU/ml in the wash cycle, preferably 25 to 0.05 LU/ml when the formulation is dosed at a level of about 2 g/liter.

A Lipase Unit (LU) is that amount of lipase which produces 1/μmol of titratable fatty acid per minute in a pH stat under the following conditions: temperature 30 °C; pH = 9.0; substrate is an emulsion of 3.3 wt.% of olive oil and 3.3% gum arabic, in the presence of 13 mmol/l Ca^{2+} and 20 mmol/l NaCl in 5 mmol/l Tris buffer.

Naturally, mixtures of the above lipases can be used. The lipases can be used in their non-purified form or in a purified form, e.g. purified with the aid of well-known absorption methods, such as phenyl sepharose absorption techniques.

Stabilizer

Another component which may be optionally used in the compositions of the invention is a stabilizer or stabilizer system. Naturally, since the invention is concerned with a novel method of minimizing or possibly eliminating the amount of stabilizer used, this compound may not be required as an ingredient of the compositions. When present, however, the stabilization system comprises from about 0.1 to about 15% of the composition.

The composition may contain from about 0.01 to about 50, preferably from 0.1 to about 30, more preferably from about 1 to about 20 millimoles of calcium ion per liter. The enzyme stabilization systems, if present, may comprise calcium ion, boric acid, propylene glycol and/or short chain carboxylic acids.

When calcium ion is used, the level of calcium ion should be selected so that there is always some minimum level available for the enzyme after allowing for complexation with builders, etc. in the composition. Any water-soluble calcium salt can be used as the source of calcium ion including calcium chloride, calcium formate, calcium acetate, and calcium propionate. A small amount of calcium ion, generally from 0.05 to about 0.4 millimoles per liter, is often also present in the composition due to calcium in the enzyme slurry and formula water.

Another enzyme stabilizer which may be used, is propionic acid or a propionic acid salt capable of forming propionic acid. When used, the stabilizer may be used in an amount from about 0.1% to about 15% by weight of the compositions.

Other preferred enzyme stabilizers are polyols containing only carbon, hydrogen and oxygen atoms. Such stabilizers preferably contain from 2 to 6 carbon atoms and from 2 to 6 hydroxy groups. Examples include propylene glycol (especially 1,2-propanediol, which is preferred) ethylene glycol, glycerol, sorbitol, mannitol and glucose. The polyol generally represents from about 0.5% to about 15%, preferably from about 1.0 to about 8% by weight of the composition.

The compositions herein may also optionally contain from about 0.25% to about 5%, most preferably from about 0.5% to about 3% by weight of boric acid. The boric acid may be, but is preferably not, formed by a compound capable of forming boric acid in the composition. Boric acid is preferred, although other compounds such as boric oxide, borax and other alkali metal borates (e.g. sodium earth-, meta-, and pyroborate and sodium pentaborate) are suitable. Substituted boric acids (e.g. phenylboronic acid, butane boronic acid and bromo phenylboronic acid) can also be used in place of boric acid.

One especially preferred stabilization system is a polyol in combination with boric acid. Preferably, the weight ratio of polyol to boric acid added is at least 1, more preferably at least 1.3.

Optional Components

In addition to the ingredients described hereinbefore, the preferred compositions herein frequently contain a series of optional ingredients which are used for the known functionality in conventional levels. While the inventive compositions are premised on aqueous enzyme-containing detergent compositions, it is frequently desirable to use a phase regulant. This component, together with water, constitutes then the solvent matrix for the claimed liquid compositions. Suitable phase regulants are well known in liquid detergent technology and, for example, can be represented by hydrotropes such as salts of alkylarylsulphonates having up to 3 carbon atoms in the alkyl group, e.g., sodium, potassium, ammonium and ethanolamine salts of xylene-, toluene-, ethyl benzene-, cumene-, and isopropyl benzene sulphonic acids. Alcohols may also be used as phase regulants. This phase regulant is frequently used in an amount from about 0.5 to about 20%. The sum of phase regulant and water is normally in the range from 35 to 65%.

The preferred compositions herein can contain a series of further optional ingredients which are mostly used in additive levels, usually below about 5%. Examples of the like additives include: polyacids, suds-regulants, opacifiers, antioxidants, bactericides, dyes, perfumes, brighteners and the like.

The beneficial utilization of the claimed compositions under various usage conditions can require the utilization of a suds-regulant. While generally all deterrent suds- regulants can be utilized, preferred for use herein are alkylated polysiloxanes, such as dimethylpolysiloxane, also frequently termed silicones. The silicones are frequently used in a level not exceeding 0.5%, most preferably between 0.01% and 0.2%.

It can also be desirable to utilize opacifiers inasmuch as they contribute to create a uniform appearance of the concentrated liquid detergent compositions. Examples of suitable opacifiers include : polystyrene, commercially known as LYTRON 621, manufactured by MONSANTO CHEMICAL CORPORATION. The opacifiers are frequently used in an amount from 0.3% to 1.5%.

The compositions herein can also contain known antioxidants for their utility, frequently radical scavengers, in the art established levels, i.e. 0.001% to 0.25% (by reference to total composition). These

antioxidants are frequently introduced in conjunction with fatty acids.

Any stable polymer which can improve the physical stability of the compositions may also be incorporated. Among such polymers would be included the decoupling polymers disclosed in co-pending EP-A-346 995, or polycarboxylate polymers such as polyacrylic acids. These polymers are used in structured liquids. In isotropic liquids, stabilizing polymers which may be used include, for example, cross-linked polyacrylic acids.

Finally, water comprises the remainder of the compositions. Generally, the amount of water will vary from 30-80% of the composition, although this will depend on the amount of actives and the ingredients used.

Product pH

pH of the compositions of the invention generally will vary from about 6 to 13, preferably 8 to 11. As mentioned above, the closer the pH is to the isoelectric point of the enzyme, the easier it is to precipitate the enzyme.

The compositions of the present invention can be structured or unstructured. By structured is meant compositions in which a structure is formed from detergent active material, the detergent active existing as a separate phase dispersed within predominantly aqueous phase. This aqueous phase contains dissolved electrolyte. Four common product forms of this type are liquids for heavy duty fabrics washing, liquid abrasive, general purpose cleaners, and autodish liquids or gels. In the first class, the suspended solid can be substantially the same as the dissolved electrolyte, being an excess of same beyond the solubility limit. This solid is usually present as a detergency builder, i.e. to counteract the effects of calcium ion water hardness in the wash. In addition, it may be desirable to suspend substantially insoluble particles of bleach, for example diperoxy dodecanoic acid (DPDA). In the second class, the suspended solid is usually a particulate abrasive, insoluble in the system. In that case the electrolyte is a different, water-soluble material, present to contribute to structuring of the active material in the dispersed phase. In certain cases, the abrasive can, however, comprise partially soluble salts which dissolve when the product is diluted. In the third class, the structure is usually used for thickening products to give consumer-preferred flow properties, and sometimes to suspend pigment particles. Compositions of the first kind are described, for example, in our patent specification EP-A-038 101, whilst example of those in the second category are described in our specification EP-A-140 452. Those in the third category are, for example, described in US-A-4 244 840. Those in the fourth category are, for example, such as those described in US-A-4 836 948.

For those compositions which are active-structured, the dispersed structuring phase is generally believed to consist of an onion-like configuration comprising concentric bilayers of detergent active molecules, between which is trapped water (aqueous phase). These configurations of active material are sometimes referred to as lamellar droplets. It is believed that the close-packing of these droplets enables the solid materials to be kept in suspension. The lamellar droplets are themselves a sub-set of lamellar structures which are capable of being formed in detergent active/aqueous electrolyte systems. Lamellar systems, in general, are a category of structures which can exist in detergent liquids. The degree of ordering of these structures, from simple spherical micelles, through disc and rod-shaped micelles to lamellar droplets and beyond, progresses with increasing concentrations of the actives and electrolyte, as is well known, for example for the reference H.A. Barnes, 'Detergents', Ch. 2 in K.Walters (Ed.), 'Rheometry:Industrial Applications, J. Wilie & Sons, Letchworth 1980. The present invention is concerned with all such structured systems which are capable of suspending particulate solids, but especially those of lamellar droplet kind.

Compositions which are not structured are generally referred to as isotropic compositions.

It should be noted that, in the case of structured liquids, sufficient salt must be added to both structure the compositions and also precipitate the enzyme. The precipitating salt is salt which is generally in excess of that amount required only for structuring.

The enzyme may first be precipitated and then added to the liquid composition. There is no criticality in the order of addition of the components of the enzyme-precipitated compositions and how they are formed.

The following Examples are intended to illustrate the invention and facilitate into understanding and are not to limit the invention in any way.

Example 1

Stabilization of Alcalase (Novo) in a structured liquid detergent formulation

The enzyme was added separately as a precipitate to detergent samples containing various levels of salting-out electrolyte. In should be noted that the precipitated enzyme does not resolubilize when added to the composition.

The base formulation used is (wt%):

C_{11}-C_{12}-linear alkylbenzene sulphonate	9.5
C_{12}-C_{15} primary alcohol 9EO condensate	16.0
Fatty acid (Oleic:Coconut 6:4)	9.5
Sodium hydroxide	6.2
Citric acid	6.3
Water and minors to	100%
pH	8.7

Enzyme solubility was measured by centrifugation and assaying the resultant supernatant for enzyme activity. Per cent soluble enzyme is reported as the percentage of supernatant enzyme activity after centrifugation divided by the enzyme activity, which is the sum of the supernatant and the precipitate activity.

Alcalase		
Base formulation plus:	Half-life (hours, 37 ° C)	% Soluble Enzyme
no sodium sulphate	36	56
1.4% sodium sulphate	50	39
7% sodium sulphate	232	10
14% sodium sulphate	677	< 10

As is clear from this example, the greater the percentage of enzyme left insoluble (i.e. because it was insolubilized by phase precipitation), the greater the stability (i.e. greater half-life) of the enzyme.

Example 2

Utility of enzyme precipitation to protect non-proteolytic enzyme from degradation by detergent proteases when mixed together in the same formulation

This is desirable since enzymes such as lipases can provide enhanced cleaning ability to a detergent formula. A protease, Savinase (Novo), was added to a structured liquid detergent containing a detergent lipase, Lipolase (Novo). Lipolase half-life increased as a function of the amount of salting-out electrolyte that was present in the liquid formulation.

The base formulation used in the experiments is (wt%):

C_{11}-C_{12}-linear alkylbenzene sulphonate	6.7
C_{12}-C_{15} primary alcohol 9EO condensate	4.8
Zeolite	20.0
Sodium hydroxide	2.44
Citric acid	3.85
Water and minors	100%
pH	8.4

Base Formulation plus:	Lipolase Half-life (days, 37° C)
no sodium sulphate	3.6
1.4% sodium sulphate	9.7
7% sodium sulphate	> 24
14% sodium sulphate	> 24

This example shows that insolubilization of one enzyme by phase precipitation can protect various types of enzymes, even when mixed in the same formulation. In the example, lipase stays in solution while protease is precipitated.

Claims

1. A method for stabilizing enzymes in aqueous liquid detergent compositions comprising adding 3 to 50% by weight of a simple, non-polymeric salt to the composition to precipitate the enzyme into a phase, separate from the remaining solubilized composition.

2. A method according to Claim 1, wherein the salt is an alkali metal sulphate.

3. A method according to any one of the preceding Claims, wherein the salt has a functionality in addition to that of a simple electrolyte.

4. A method according to Claim 3, wherein the salt is a builder.

5. A method according to Claim 4, wherein the builder is an alkali metal citrate.

6. A method according to Claim 3, wherein the salt is a buffer salt.

7. A method according to Claim 6, wherein the buffer salt is an alkali metal borate.

8. A method according to any one of the preceding Claims, wherein the salt is added after the pH is adjusted to about the isoelectric point of the enzyme.

9. A method according to any one of the preceding Claims, wherein the enzyme is a proteolytic enzyme.

10. A method according to any one of the preceding Claims, whereby the composition comprises one or more other enzymes.