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(54) **Detergent composition.**

(57) The stability of lipolytic enzymes in detergent compositions can be improved by addition of a stabilising material which is capable of reversibly forming a complex with the active site of the lipolytic enzyme. Preferred stabilising materials are boronic acid derivatives.

EP 0 478 050 A1

The present invention relates to detergent compositions comprising a lipolytic enzyme. In particular the present invention relates to liquid detergent compositions comprising a lipolytic enzyme.

It has previously been suggested to incorporate lipolytic enzymes into detergent compositions. For example US 4,566,985 (Applied Biochemists, Inc) describes the combined use of cellulase, protease, amylase and lipase type enzymes in liquid detergent compositions.

A problem in using lipolytic enzymes in detergent compositions is often the occurrence of enzyme instability, leading to a reduction in enzyme activity upon use of the compositions, for example in the washing of fabrics. These instability problems are especially apparent in detergent compositions comprising lipolytic enzymes in combination with proteolytic enzymes.

Surprisingly it has now been found that the stability of lipolytic enzymes in detergent compositions can markedly be improved by the use of a stabilising material which is capable of reversibly forming a complex with the active site of the lipolytic enzyme, said stabiliser-enzyme complex being less prone to de-stabilisation reactions.

Preferably the formation of a stabiliser-lipolytic enzyme complex prevents the destabilisation reaction i.e between lipolytic enzymes and proteolytic enzymes and/or improves the resistance against denaturation and against proteolytic breakdown of the lipolytic enzyme, such that the half-life time of the lipase in the composition at 37° C is increased by a least 25 %, more preferably at least 50 %, most preferably at least 100 %. For the purpose of this invention, the half-life time of the lipolytic enzyme is the time-period starting at the moment of preparation of the detergent formulation until the moment that only 50% of the enzyme-activity is left. Most preferably the stabilisation reaction is an inhibitory reaction, whereby the stabiliser-lipolytic enzyme complex is inactivated.

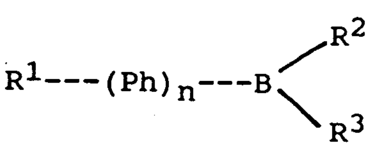
The reversibility of complex formation can generally be effected by adding the stabilisation material in a concentration of from 2 to 100 times the K_i, wherein the K_i is the concentration at which 50 % of the lipase enzymes have formed the complex. More preferably the concentration is from 5-25 times the K_i, most preferably from 8 to 15 times. In use the detergent composition will generally be diluted 50 to 500 times, especially about 100 times, providing a concentration of stabiliser such that the stabiliser-lipolytic enzyme complex will be dissociated, thus providing active lipolytic enzymes in the wash liquor.

Accordingly the present invention relates to a detergent composition comprising a lipolytic enzyme and a stabilising material capable of reversibly forming a complex with the active site of the lipolytic enzyme, therewith increasing the half-lifetime of the lipase material.

In a preferred embodiment of the invention the stabiliser material is added to the detergent composition in a concentration of from 2 to 100 times the K_i.

For the purpose of the present invention any material capable of reversible forming a complex with the active site of the lipolytic enzyme may be used. In a preferred embodiment of the invention, boronic acid materials are used.

Surprisingly it has now been found that the stability of lipolytic enzymes in detergent compositions can markedly be improved by specific boronic acid derivatives as stabilising materials. Boronic acid derivatives which are especially advantageous for stabilising lipase enzymes are of the following formula:



(I)

wherein:
Ph is a phenyl group, optionally substituted by halogen groups or NH_2 groups;
n is 0 or 1;
R^1 is Hydrogen, halogen, NH_2 or a C_{1-24} alkyl or alkenyl group, provided that when n is 0 then R1 contains at least 3, most preferably 8-20 carbon atoms;
R^2 and R^3 are independently selected from -OH, -Cl, -F, -Br and C_{1-8} alkyl or alkenyl groups.

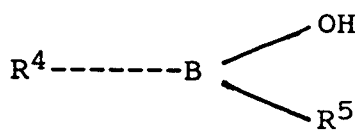
Accordingly a second embodiment of the invention relates to a detergent composition comprising a lipolytic enzyme in combination with one or more boronic acid derivatives of formula I as indicated above.

Although applicants do not wish to be bound by any theory, it is believed that the stabilisation of the lipase enzyme by the boronic acid derivatives works via an inhibitory mechanism, whereby the boronic acid derivative reacts with the active site of the lipolytic enzyme, therewith protecting the enzyme against destabilisation. In using the detergent composition, generally a dilution step will take place, for example by the addition of the detergent composition to the wash liquor. It is believed that due to this dilution, generally the protective boronic acid group will be detached form the active site of the lipolytic enzyme, therewith providing an active enzyme material under use conditions. This mechanism of stabilisation is believed to be especially advantageous if lipase enzymes are used in combination with proteolytic enzymes, because the boronic acid derivative tends to be bound to the active site of the lipase, therewith preventing the destabilisation reaction (= proteolytic breakdown) with the proteolytic enzyme.

Preferred boronic acid derivatives are of the formula I, whereby at least of the groups R^2 and R^3 is -OH, most preferably groups R^2 and R^3 both are -OH or one of these groups is -OH and the other is a C_{1-8} alkyl or alkenyl group.

For environmental reasons it is further preferred that the in formula I n is 0 and R^1 is a C_3-C_{24} group.

Especially preferred boronic acid derivatives are of the following formula:



(II)

wherein:

R^4 is a phenyl group or a C_{8-24} alkyl or alkenyl group;

R^5 is a -OH or a C_{1-8} alkyl or alkenyl group.

The level of boronic acid derivatives in the detergent compositions according to the present invention may be varied within a broad range, dependant on the level of lipolytic enzyme and the reactivity of the boronic acid derivative. Generally the level of boronic acid derivatives will be from 0.00001 to 3 % by weight of the composition, more preferred from 0.0001 to 1 %, most preferred from 0.001 to 0.5 %.

The added amount of lipolytic enzyme in the composition is preferably from 50-30,000 LU/g of detergent composition, whereby LU (lipase units) are defined as they are in EP 258 068. The level of lipolytic enzymes is often at least 100 LU/g, very usefully at least 250 LU/g, preferably less than 5000 LU/g, more preferably less than 2000 LU/g or less than 1000 LU/g.

The lipolytic enzyme can be chosen from among a wide range of lipases: in particular the lipases described in for example the following patent specifications, EP 214 761 (NOVO), EP 258 068 (NOVO) and EP 305 216 (NOVO), and especially lipolytic enzymes showing immunological cross-reactivity with antisera raised against lipases from Thermomyces lanuginosus ATCC 22070, EP 205 208 (UNILEVER) and EP 206 390 (UNILEVER), and especially lipases showing immunological cross-reactivity with antisera raised against lipase from Chromobacter viscosum var lipolyticum NRRL B-3673 , or against lipase from Alcaligenes PL-679, ATCC 31371 and FERM-P 3783, also the lipases described in specifications WO 87/00859 (Gist-Brocades), WO 89/09263 (Gist Brocades), EP 331 376 (AMANO), DE 39 08 131 (Toyo Jozo) and EP 204 284 (SAPPORO BREWERIES). Suitable in particular are for example the following commercially available lipase preparations: Novo Lipolase, Amano Lipase CE, P, B, AP, M-AP, AML and CES, and Meito lipases MY-30, OF, and PL, also esterase MM. lipozym, SP 225, SP 285, Siaken lipase, Enzeco lipase, Toyo Jozo lipase and Diosynth lipase.

These commercially available enzymes are preferably used at levels of from 0.01 to 10 % by weight of the detergent composition, more preferred 0.05 to 5 %, most preferred 0.1 to 2 %, whereby the levels relate to the enzyme preparation in the form as supplied.

Also lipase enzymes produced by genetic engineering may be used, such as for example described in WO 89-09263 (Gist-Brocades) and EP 218 272 (Gist-Brocades) as well as EP 258 068 (Novo) and EP 305 216 (Novo).

As stated above the use of boronic acid derivatives for the stabilisation of lipolytic enzymes is especially advantageous for detergent compositions comprising lipases in combination with proteolytic enzymes.

Protease can preferably be included in the composition in amounts of the order of about 1 to 100 GU/mg detergent composition. Preferably the amount ranges from 2-50 and more preferably from 5 to 20 GU/mg. A GU is a Glycine unit, defined as the proteolytic enzyme activity which, under standard conditions, during a 15 minute incubation at 40 °C, with N-acetyl casein as substrate, produces an amount of NH_2 groups equivalent to 1 micromole of glycine.

A preferred example of a protease enzyme which may be used in compositions according to the invention is the subtilisin variety sold as Savinase (TM of Novo-Nordisk A/S) or Maxacal (TM of Gist-Brocades/IBIS) or as Opticlean (ex MKC) or API22 (ex Showa Denko). Other useful examples of proteases include Maxatase, Esperase, Alcalase, proteinase K and subtilisin BPN', or variants obtained by enzyme engineering.

Commercially available proteolytic enzymes are preferably used at a level of from 0.01 to 10 % by weight of the composition, more preferably 0.05 to 2 %, most preferred 0.1 to 2 %, whereby the levels relate to the enzyme preparation in the form as supplied.

Protease with a high isoelectric point (e.g Savinase or Maxacal) have been found more stable under the conditions generally encountered in detergent compositions of the invention. Particularly good results have been obtained while using a high pI protease (ie pI above 9, say about 10) in a liquid detergent composition of pH less than about 9.

Detergent compositions of the invention may take any suitable physical form such as powders, pastes, tablets and liquids. A preferred embodiment of the present invention relates to liquid detergent compositions, in particular liquid detergent compositions comprising an aqueous phase.

Compositions of the present invention also comprise detergent active materials. In the widest definition the detergent active materials in general, may comprise one or more surfactants, and may be selected from anionic, cationic, nonionic, zwitterionic and amphoteric species, and (provided mutually compatible) mixtures thereof. For example, they may be chosen from any of the classes, sub-classes and specific materials described in "Surface Active Agents" Vol. I, by Schwartz & Perry, Interscience 1949 and "Surface Active Agents" Vol. II by Schwartz, Perry & Berch (Interscience 1958), in the current edition of "McCutcheon's Emulsifiers & Detergents" published by the McCutcheon division of Manufacturing Confectioners Company or in Tensid-Taschenbuch", H. Stache, 2nd Edn., Carl Hanser Verlag, Munchen & Wien, 1981.

Suitable nonionic surfactants include, in particular, the reaction products of compounds having a hydrophobic group and a reactive hydrogen atom, for example aliphatic alcohols, acids, amides or alkyl phenols with alkylene oxides, especially ethylene oxide either alone or with propylene oxide. Specific nonionic detergent compounds are alkyl (C_6-C_{18}) primary or secondary linear or branched alcohols with ethylene oxide, and products made by condensation of ethylene oxide with the reaction products of propylene oxide and ethylenediamine. Other so-called nonionic detergent compounds include long chain tertiary amine oxides, long chain tertiary phosphine oxides and dialkyl sulphoxides.

Also possible is the use of salting out resistant active materials, such as for example described in EP 328 177,

especially the use of alkyl poly glycoside surfactants, such as for example disclosed in EP 70 074.

Suitable anionic surfactants are usually water-soluble alkali metal salts of organic sulphates and sulphonates having alkyl radicals containing from about 8 to about 22 carbon atoms, the term alkyl being used to include the alkyl portion of higher acyl radicals. Examples of suitable synthetic anionic detergent compounds are sodium and potassium alkyl sulphates, especially those obtained by sulphating higher (C_8-C_{18}) alcohols produced for example from tallow or coconut oil, sodium and potassium alkyl (C_9-C_{20}) benzene sulphonates, particularly sodium linear secondary alkyl (C_{10}-C_{15}) benzene sulphonates; sodium alkyl glyceryl ether sulphates, especially those ethers of the higher alcohols derived from tallow or coconut oil and synthetic alcohols derived from petroleum; sodium coconut oil fatty monoglyceride sulphates and sulphonates; sodium and potassium salts of sulphuric acid esters of higher (C_8-C_{18}) fatty alcohol-alkylene oxide, particularly ethylene oxide, reaction products; the reaction products of fatty acids such as coconut fatty acids esterified with isethionic acid and neutralised with sodium hydroxide; sodium and potassium salts of fatty acid amides of methyl taurine; alkane monosulphonates such as those derived by reacting alpha-olefins (C_8-C_{20}) with sodium bisulphite and those derived from reacting paraffins with SO_2 and Cl_2 and then hydrolysing with a base to produce a random sulphonate; and olefin sulphonates, which term is used to describe the material made by reacting olefins, particularly C_{10}-C_{20} alpha-olefins, with SO_3 and then neutralising and hydrolysing the reaction product. The preferred anionic detergent compounds are sodium (C_{11}-C_{15}) alkyl benzene sulphonates and sodium or potassium primary (C_{10}-C_{18}) alkyl sulphates.

It is also possible, and sometimes preferred, to include an alkali metal soap of a fatty acid, especially a soap of an acid having from 12 to 18 carbon atoms, for example oleic acid, ricinoleic acid, and fatty acids derived from castor oil, alkylsuccinic acid, rapeseed oil, groundnut oil, coconut oil, palmkernel oil or mixtures thereof. The sodium or potassium soaps of these acids can be used.

In many (but not all) cases, the total detergent active material may be present at from 2% to 60% by weight of the total composition, for example from 5% to 50% and typically from 10% to 40% by weight. However, one preferred class of compositions comprises at least 20%, most preferably at least 25% and especially at least 30% of detergent active material based on the weight of the total composition.

Liquid detergent compositions of the invention may be un-structured (isotropic) or structured. Structured liquids of the invention may be internally structured whereby the structure is formed by the detergent active materials in the composition or externally structured, whereby the structure is provided by an external structurant. Preferably compositions of the invention are internally structured. Most preferably compositions of the invention comprise a structure of lamellar droplets comprising surfactant materials.

Some of the different kinds of active-structuring which are possible are described in the reference H.A. Barnes, "Detergents", Ch.2. in K. Walters (Ed), "Rheometry: Industrial Applications", J. Wiley & Sons, Letchworth 1980. In general, the degree of ordering of such systems increases with increasing surfactant and/or electrolyte concentrations. At very low concentrations, the surfactant can exist as a molecular solution, or as a solution of spherical micelles, both of these being isotropic. With the addition of further surfactant and/or electrolyte, structured (antisotropic) systems can form. They are referred to respectively, by various terms such as rod-micelles, planar lamellar structures, lamellar droplets and liquid crystalline phases. Often, different workers have used different terminology to refer to the structures which are really the same. For instance, in European patent specification EP-A-151 884, lamellar droplets are called "spherulites". The presence and identity of a surfactant structuring system in a liquid may be determined by means known to those skilled in the art for example, optical techniques, various rheometrical measurements, x-ray or neutron diffraction, and sometimes, electron microscopy.

When the compositions are of lamellar droplet structure then in many cases it is preferred for the aqueous continuous phase to contain dissolved electrolyte. As used herein, the term electrolyte means any ionic water soluble material. However, in lamellar dispersions, not all the electrolyte is necessarily dissolved but may be suspended as particles of solid because the total electrolyte concentration of the liquid is higher than the solubility limit of the electrolyte. Mixtures of electrolytes also may be used, with one or more of the electrolytes being in the dissolved aqueous phase and one or more being substantially only in the suspended solid phase. Two or more electrolytes may also be distributed approximately proportionally, between these two phases. In part, this may depend on processing, e.g. the order of addition of components. On the other hand, the term "salts" includes all organic and inorganic materials which may be included, other than surfactants and water, whether or not they are ionic, and this term encompasses the sub-set of the electrolytes (water soluble materials).

The selection of surfactant types and their proportions, in order to obtain a stable liquid with the required structure will be fully within the capability of those skilled in the art. However, it can be mentioned that an important sub-class of useful compositions is those where the detergent active material comprises blends of different surfactant types. Typical blends useful for fabric washing compositions include those where the primary surfactant(s) comprise nonionic and/or a non-alkoxylated anionic and/or an alkoxylated anionic surfactant.

In the case of blends of surfactants, the precise proportions of each component which will result in such stability and viscosity will depend on the type(s) and amount(s) of the electrolytes, as is the case with conventional structured liquids.

Preferably though, the compositions contain from 0% to 60%, especially from 1- 60 %, more preferably 10 to 45% of a salting-out electrolyte. Salting-out electrolyte has the meaning ascribed to in specification EP-A-79 646, that is salting-out electrolytes have a lyotropic number of less than 9.5. Optionally, some salting-in electrolyte (as defined in the latter specification) may also be included, provided it is of a kind and in an amount compatible with the other components and the composition is still in accordance with the definition of the invention claimed herein. Some or all of the electrolyte (whether salting-in or salting-out), or any substantially water insoluble salt which may be present, may have detergency builder properties. In any event, it is preferred that compositions according to the present invention include detergency builder material, some or all of which may be electrolyte. The builder material is any capable of reducing the level of free calcium ions in the wash liquor and will preferably provide the composition with other beneficial properties such as the generation of an alkaline pH, the suspension of soil removed from the fabric and the dispersion of the fabric softening clay material. Preferably the salting-out electrolyte comprises citrate.

Examples of phosphorus-containing inorganic detergency builders, when present, include the water-soluble salts, especially alkali metal pyrophosphates, orthophosphates, polyphosphates and phosphonates. Specific examples of inorganic phosphate builders include sodium and potassium tripolyphosphates, phosphates and hexametaphosphates. Phosphonate sequestrant builders may also be used.

Examples of non-phosphorus-containing inorganic detergency builders, when present, include water-soluble alkali metal carbonates, bicarbonates, silicates and crystalline and amorphous aluminosilicates. Specific examples include sodium carbonate (with or without calcite seeds), potassium carbonate, sodium and potassium bicarbonates, silicates and zeolites.

Examples of organic detergency builders, when present, include the alkaline metal, ammonium and substituted ammonium polyacetates, carboxylates, polycarboxylates, polyacetyl carboxylates and polyhydroxysulphonates. Specific examples include sodium, potassium, lithium, ammonium and substituted ammonium salts of ethylenediaminetetraacetic acid, nitrilitriacetic acid, oxydisuccinic acid, CMOS, TMS, TDS, melitic acid, benzene polycarboxylic acids and citric acid.

Preferably the level of non-soap builder material is from 0-50% by weight of the composition, more preferred from 5-40%, most preferred 10-35%.

In the context of organic builders, it is also desirable to incorporate polymers which are only partly dissolved, in the aqueous continuous phase as described in EP 301.882. This allows a viscosity reduction (due to the polymer which is dissolved) whilst incorporating a sufficiently high amount to achieve a secondary benefit, especially building, because the part which is not dissolved does not bring about the instability that would occur if substantially all were dissolved. Typical amounts are from 0.5 to 4.5% by weight.

It is further possible to include in the compositions of the present invention, alternatively, or in addition to the partly dissolved polymer, yet another polymer which is substantially totally soluble in the aqueous phase and has an electrolyte resistance of more than 5 grams sodium nitrilotriacetate in 100ml of a 5% by weight aqueous solution of the polymer, said second polymer also having a vapour pressure in 20% aqueous solution, equal to or less than the vapour pressure of a reference 2% by weight or greater aqueous solution of polyethylene glycol having an average molecular weight of 6000; said second polymer having a molecular weight of at least 1000. Use of such polymers is generally described in our EP 301,883. Typical levels are from 0.5 to 4.5% by weight.

The viscosity of compositions according to the present is preferably less than 1500 mPas, more preferred less than 1000 mPas, especially preferred between 30 and 900 mPas at 21 s^{-1}.

One way of regulating the viscosity and stability of compositions according to the present invention is to include viscosity regulating polymeric materials.

Viscosity and/or stability regulating polymers which are preferred for incorporation in compositions according to the invention include deflocculating polymers having a hydrophilic backbone and at least one hydrophobic side chain. Such polymers are for instance described in our co-pending European application EP 89201530.6 (EP 346 995).

Preferably the amount of viscosity regulating polymer is from 0.1 to 5% by weight of the total composition, more preferred from 0.2 to 2%.

Compositions of the invention may also comprise materials for adjusting the pH. For lowering the pH it is preferred to use weak acids, especially the use of organic acids is preferred, more preferred is the use of C_{1-8} carboxylic acids, most preferred is the use of citric acid.

Apart from the ingredients already mentioned, a number of optional ingredients may also be present, for example bleach materials such as percarbonates or perborates, bleach precursors such as TAED, lather boosters such as alkanolamides, particularly the monoethanolamides derived from palm kernel fatty acids and coconut fatty acids, fabric softeners such as clays, amines and amine oxides, lather depressants, inorganic salts such as sodium sulphate, and, usually present in very minor amounts, fluorescent agents, perfumes, germicides colorants and other enzymes such as cellulases and amylases.

Liquid compositions of the invention preferably comprise from 10 -80 % by weight of water, more preferably from 15- 60%, most preferably from 20-50 %.

Liquid detergent compositions according to the invention are preferably physically stable in that they show less than 2% by volume phase separation upon storage for 21 days after preparation at 25 °C.

In use the detergent compositions of the invention will be diluted with wash water to form a wash liquor for instance for use in a washing machine. The concentration of liquid detergent composition in the wash liquor is preferably from 0.05 to 10 %, more preferred from 0.1 to 3% by weight.

To ensure effective detergency, the liquid detergent compositions preferably are alkaline, and it is preferred that they should provide a pH within the range of about 7.0 to 12, preferably about 8 to about 11, when used in aqueous solutions of the composition at the recommended concentration. To meet this requirement, the undiluted liquid composition should preferably be of a pH above 7, for example about pH 8.0 to about 12.5. It should be noted that an excessively high pH, e.g. over about pH 13, is less desirable for domestic safety. If hydrogen peroxide is present in the liquid composition, then the pH is generally from 7.5 to 10.5, preferably 8 to 10, and especially 8.5 to 10, to ensure the combined effect of good detergency and good physical and chemical stability. The ingredients in any such highly alkaline detergent composition should, of course, be chosen for alkaline stability, especially for pH-sensitive materials such as enzymes, and a particularly suitable proteolytic enzyme. The pH may be adjusted by addition of a suitable alkaline or acid material.

Compositions according to the invention may be prepared by any method for the preparation of detergent compositions. A preferred method for the preparation of liquid detergent compositions of the invention comprises the addition of electrolyte materials -if any- to the water, followed by the addition of deflocculating polymers -if any-, the detergent active materials, the stabiliser material and the lipolytic enzyme as a premix and finally the remaining ingredients The lipolytic enzyme is preferably added as a premix with the stabiliser after addition of all ingredients (including the proteolytic enzyme if any).

The invention will now be illustrated by way of the following Examples. In all Examples, unless stated to the contrary, all percentages are by weight.

EXAMPLE I

Determination of the K_i for the stabilising reaction between several stabilising materials and lipolytic enzymes.

The K_i of the stabilisation reaction between Lipolase TM (ex NOVO) and the stabiliser materials as indicated below was calculated from the K_m values in the absence and presence of stabiliser. The K_m was determined by variation of the concentration of substrate (= olive oil emulsion, stabilised by gum arabic in a weight ratio of 1 : 1) both in the absence and presence of a stabiliser/Lipolase premix. If present the stabiliser was added as a premix with the lipolytic enzyme. The K_i was calculated from the K_m values.

The following results were obtained:

STABILISER	K_i (microM)
Butane boronic acid	67
Phenyl boronic acid	20
p-Bromophenyl boronic acid	0.4
p,o Dichlorophenyl boronic acid	0.36

These results indicate that a stabiliser/lipolytic enzyme complex can be formed at very low concentrations of stabiliser material (micromolar range)

EXAMPLE II

The capability of several stabilisers to form complexes with Proteolytic enzymes was tested by mixing a premix of Savinase TM and the stabiliser material to a 0.1 mg/ml solution of purified Lipolase TM (= substrate) in 2 g/l LAS, buffered with 10 mM Tris/HCl. While keeping the pH at 9.0 in a pH-stat apparatus, the alkali consumption was measured. From this the Savinase activity was calculated. A Savinase activity of 50 % was found at about the concentrations of stabilisers as indicated in the following table:

STABILISER	50 % Sav. Act at (mM)
Butane Boronic acid	9
Phenyl Boronic acid	5
p-Bromo phenyl Boronic acid	2
p,o Dichloro Boronic acid	1

These results indicate that although the stabiliser materials are capable of forming a complex with Savinase, this complex is only formed at relatively high concentrations (millimolar range).

EXAMPLE III

The stability of lipolytic enzymes in the presence of proteolytic enzymes at various levels of stabilising materials was determined as follows:

180 LU/ml Lipolase TN was incubated with 10,000 GU/ml Savinase TM in 2 g/l LAS and 20 mM Tris/HCl at a pH of 9.0 in the presence of various concentrations of stabilising materials. The residual Lipolase activity after 30 minutes at 30° C was determined.

When butane boronic acid was used (K_i = 67 micromolair) as the stabilising material a steep increase in stability of the Lipolytic enzyme was observed when raising the concentration of stabiliser from 0 to 2 times the K_i. By further increasing the concentration of stabiliser material a slight increase of the lipolytic enzyme stability could be observed.

When p,o Dichlorophenylboronic acid (K_i is 0.36 micromolair) was used as the stabilising material, again a steep increase in stability of the lipolytic enzyme was observed by raising the level of stabilising material from 0 to about 2 times the K_i value. By further increasing the stabiliser concentration a slight increase of the stability of the lipolytic enzymes could be observed.

These results indicate that if mixtures of Lipolytic and proteolytic enzymes are used, stabilisation take place at very low levels of stabiliser material, corresponding to the K_i of the formation of a stabiliser/lipolytic enzyme complex. The formation of this complex renders it possible to stabilise lipolytic enzymes by using only minor amounts of stabilising materials. Therefore if mixtures of Lipolytic enzymes and Proteolytic enzymes are used in combination with micromolar concentrations of stabilising materials, the stabilisation takes place via an inhibitory reaction on the active site of the Lipolytic enzyme and not via the formation of a Proteolytic enzyme/stabiliser complex.

EXAMPLE IV

The following compositions where prepared by adding the ingredients to the water in the following order: electrolytes, actives (as a premix), other ingredients except for lipase and stabiliser which were added as a premix as the final ingredient.

Ingredient (wt parts)	A
Water	50
LAS	6.8
LES	4.8
Nonionic	4.8
zeolite	19
Sokolan CP-5	3
NaOH	to pH 8.5
$CaCl_2$	0.15
Glycerol	8
Borax	5.7
Lipase	0.5 LU/mg
Savinase	var
stabiliser	var

Material specification:

LAS: C_{12-14} Linear alkyl benzene sulphonate(Dobanic 113)
LES: mixture Lauryl 3EO ether sulphate and Lauryl 7EO ether sulphate in a weight ratio of 1 : 1.
Nonionic: 1.2% synperonic A3, 3.6% synperonic A7.
Zeolite: Wessalith P
Lipase: Lipolase SP400 (ex NOVO)
Savinase: Savinase 16.0 L DX (ex NOVO)
Sample I contained lipase in the absence of savinase and stabilisers, sample II contained Lipase and Savinase, but no stabiliser, the remaining samples contained Lipase and Savinase and a stabiliser as indicated below. The level of Savinase in samples II-VIII was 10 GU/mg, the level of stabiliser in samples III-VIII was 0.25 %. The half life time of the Lipase enzyme at 37 °C was determined for each sample the results were as follows:

SAMPLE	STABILISER	$t_{0.5}$ (37 ° C, days)
I	--	21
II	--	11
III	A	11
IV	B	11
V	C	20
VI	D	25
VII	E	28
VIII	F	35

wherein:
A : iminodiacetic acid
B : picolinic acid
C : formula II, wherein R^4 is phenyl and R^5 is -OH;
D : formula I, wherein R^1 is -H, x = 1, Ph = meta NH_2 benzene, R^2 and R^3 are -OH;
E : formula I, wherein R^1 is -H, x = 1, Ph = para Br-benzene, R^2 and R^3 are -OH;
F : formula I, wherein R^1 is -H, x = 1, Ph = ortho, para diCl benzene, R^2 and R^3 are -OH;

These results indicate that the half-life time of lipase is markedly decreased by the addition of proteolytic enzymes (samples I and II). The addition of two carboxylic protease inhibitors, iminodiacetic acid and picolinic acid did not result in an enhancement of lipase stability (samples III and IV). If however boronic acid derivatives like phenylboronic acid, m-amino phenyl boronic acid, 2,4 dichloro phenyl boronic acid and p-bromo phenyl boronic acid are added a clear increase of the half life time of lipase can be observed.

Other useful boronic acid derivatives are: octadecane boronic acid and phenyl, butyl boronic acid.

EXAMPLE V

The following formulations were prepared by mixing the ingredients in the order listed to water.

INGREDIENTS (% wt)	A	B	C
Sodium-citrate 2aq	--	15	7
Borax	1.0	--	--
Glycerol	3.5	--	--
Propyleneglycol	12	--	--
Dobanic 113	7.5	18	16
Synperonic A7	19	18	8
Soap	15	9	16
Tinopal CBS-X	--	0.1	--
Polymer 1)	--	1.0	--
Silicone Q2-3300	--	0.1	--
Perfume	--	0.4	--
SXS	--	--	3
Triethanolamine	--	--	2
Monoethanolamine	--	--	2
Savinase TM (GU/mg)	10	10	10
Lipolase TM (LU/mg) 3)	0.4	0.5	0.5
Stabiliser 2)	0/0.25	0/0.25	0/0.25
Water	<---------balance-------->		

1) Polymer A11 of EP 346,995.

2) o,p dichlorophenyl boronic acid.

3) Added as a premix with the stabiliser.

The half life time of the lipase was determined at 37°C. For composition A the half life time in the absence of stabiliser was 1 day, in the presence of stabiliser 3 days. For composition B, the half life time in the absence of stabiliser was 14 days, in the presence of stabiliser 32 days. For composition C, the half-life time in the absence of stabiliser was 1 day, in the presence of stabiliser 2 days.

Example VI

To determine the enhancement of lipase stability in an isotropic liquid formulation using dichlorophenyl boronic acid DCPBA, the following isotropic composition was prepared

anionic surfactant	22.5%
nonionic surfactant	7.5%
Sodium hydroxide	2.0%
Propylene glycol	11.4%
Lipase	0.5 LU/mg
Savinase	(1)
DCPBA	(2)
Water	to 100%

(1) 0 or 10 GU/mg

(2) DCPBA incremental amounts 0.001% - 0.25%

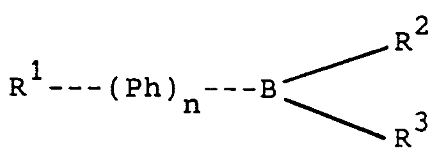
Upon storage at 37°C over 50 hours, it was found that concentrations as low as 0.001% give a measurable improvement in lipase stability. For the formulation contaning the protease, the stability is increased by at least a factor of three (50 hours, 0.25% DCPBA). In the absence of the protease, the improvement is less but still significant.

These results show that the lipolase stability enhancement is obtained not only in structured liquid compositions but also in isotropic liquids.

Claims

1. A detergent composition comprising a lipolytic enzyme and a stabilising material capable of reversibly forming a complex with the active site of the lipolytic enzyme, thereby increasing the half-life of the lipase material.

2. A detergent composition comprising a lipolytic enzyme in combination with a stabiliser material which is a boronic acid derivative of formula (I)

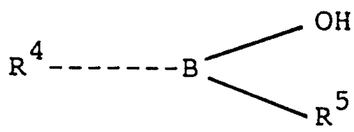


(I)

wherein:
Ph is a phenyl group, optionally substituted by at least one halogen group and/or NH_2 group;
n is 0 or 1;
R^1 is hydrogen, halogen, NH_2 or a C_{1-24} alkyl or alkenyl group, provided that when n is 0 then R^1 contains at least 3, most preferably 8-20 carbon atoms;
R^2 and R^3 are independently selected from -PH, -C1, -F, -Br and C_{1-8} alkyl or alkenyl groups.

3. A detergent composition according to claim 1 or 2, wherein the stabiliser material is added to the detergent composition in a concentration of from 2 to 100 times the K_i.

4. A detergent composition according to one or more of the preceding claims wherein the stabilising material is a boronic acid derivative of formula (II)



(II)

wherein:
R^4 is a phenyl group or a C_{8-24} alkyl or alkenyl group;
R^5 is an -OH or a C_{1-8} alkyl or alkenyl group.

5. A detergent composition according to one or more of the preceding claims comprising from 0.00001 to 3 % by weight of stabilising material.

6. A detergent composition according to one or more of the preceding claims comprising an amount of lipolytic enzyme of 50-30,000 LU/g of detergent composition.

7. A detergent composition according to one or more of the preceding claims also comprising a proteolytic enzyme, preferably in an amount of 1 to 100 GU/mg of detergent composition.

8. A detergent composition according to one or more of the preceding claims in liquid form, said liquid preferably comprising:
(a) 2-60 % by weight of detergent active materials;
(b) 0-60 % of salting out electrolytes;
(c) 0-50 % of non-soap builder materials;
(d) 10-80% of water.

9. A liquid detergent composition according to claim 8 having a viscosity of less than 1,500 mPas at 21 s^{-1}.

10. A liquid detergent composition according to claim 8 or claim 9, which composition comprises a suspension of lamellar droplets.

11. A liquid detergent composition according to claim 8 or claim 9, which composition is isotropic.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 20 2359

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.5)
X Y	AU-B-528 853 (P.M.J. BEDDING) * abstract; page 4, lines 32-40; page 5, line 1 * – – –	1 2-5	C 11 D 3/386
Y	US-A-3 912 595 (M.H.W. PHILIPP) * abstract; claims 1,5 * – – –	2-5	
X	FR-A-2 084 033 (KONINKLIJKE NEDERLANDSCHE GIST-EN SPIRITUSFABRIEKEN N.V.) * whole document; in particular page 16, example no. 8 * – – –	1	
A	US-A-4 668 630 (A.L. LOUDERBACK) * summary * – – –	1,2,4	
A	CHEMICAL ABSTRACTS vol. 104, no. 13, 31 March 1986, pages 309,310, abstract no. 104930k, Columbus, Ohio, US; L.D. SUTTON et al.: "Phenyl-n-butylboronic acid is a potent transition state analog inhibitor of lipolytic enzymes" & Biochem. Biophys. Res. Commun. 1986, vol. 134, no. 1, pages 386-392 * abstract * – – –	1,2,4	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
A	CHEMICAL ABSTRACTS vol. 99, no. 15, 10 October 1983, page 307, abstract no. 118436u, Columbus, Ohio, US; P. VANIO et al.: "N-(5-Dimethylaminonaphthalene-1-sulfony 1)-3-aminobenzeneboronic acid as an active-site-directed fluorescent probe of lipoprotein lipase" & Biochim. Biophys. Act 1983, vol. 746, no. 3, pages 217-219 * abstract * – – –	1,2,4	C 11 D
A	EP-A-0 385 526 (UNILEVER) * page 2, lines 36-44; page 3, lines 14-24; claims * – – – –/–	1,10	

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
Berlin	28 November 91	PELLI-WABLAT B

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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.5)
A	EP-A-0 381 431 (UNILEVER PLC) * claims * – – – – –	1,11	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

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Berlin	28 November 91	PELLI-WABLAT B

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