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(54) **Enzymatic liquid detergent composition.**

(57) A liquid detergent composition comprises a surfactant concentrate and a proteolytic enzyme derived from a microorganism: for improved storage stability in the liquid state the proteolytic enzyme is of the proteinase K type. Proteinase K is a known fungal alkaline serine protease from Tritirachium album Limber, and its use in liquid detergent compositions can provide enzymatic liquid detergent with improved enzyme storage stability in comparison with other proteolytic enzymes, thus reducing the amount of enzyme stabilisers usually required for proteolytic enzymes in liquid detergent compositions. Proteinase K also has improved performance in compositions with an increased ionic strength or molarity.

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Description

ENZYMATIC LIQUID DETERGENT COMPOSITIONS

Field of the Invention:

The present invention relates to enzymatic liquid detergent compositions. More particularly, it relates to enzymatic liquid detergent compositions which incorporate proteolytic enzyme.

Disclosure of Prior Art:

The use of proteolytic enzymes in liquid detergent compositions is well known; although these proteolytic enzymes can be of various types and sources, the proteolytic enzymes commonly used are those produced by Bacillus strains. Although with such proteolytic enzymes satisfactory results as regards performance can be achieved, it is frequently necessary to include enzyme-stabilizing systems in the liquid detergent compositions to provide a satisfactory enzyme stability during storage of the enzymatic liquid detergent composition.

We believe that representative examples of relevant prior art concerning proteases and stabilisation of proteases in liquid detergents are as follows.

Serine proteases from Bacillus subtilis are very widely known and used in detergent compositions.

The prior art also includes WO 88/03946 (Novo), which discloses, as detergent additives, combinations of Bacillus proteases with alkaline fungal or actinomycete proteases, e.g. those proteases obtainable from the genera Paecilomyces, Fusarium, and Nocardiopsis. The disclosure extends to the use of the detergent additive as a liquid, with a known enzyme stabiliser such as propylene glycol, for addition to a liquid detergent.

USP 3 707 504 (Procter & Gamble) discloses detergents for laundry and dishwashing, comprising protease from Thermoactinomyces vulgaris ATCC 15734, which are formulated as solid or liquid detergent compositions. This document mentions surprising stability of protease from Thermoactinomyces vulgaris in highly-alkaline detergent systems.

Proteinase K (E.C. 3.4.21.14) is a known alkaline serine protease. It is a fungal proteinase produced by the mould Tritirachium album (Limber). It has been the subject of several academic investigations, and relevant publications include Eur J Biochem 47 (1974), pages 91-97; and Hoppe-Seyler's Zeitschrift f Physiol Chemie 357 (1976), pages 937-947. In EMBO Journal 3(6), pages 1311-1314 (1984), A Pähler et al show the crystallographic 3D structure of proteinase K at a level of resolution that displays its secondary and tertiary protein structure. Furthermore, K-D Jany et al have published its full primary sequence in FEBS Letters 199(2) (1986) pages 139-144.

The use of a certain proteinase from Tritirachium album (Limber) for various purposes, including (generally) use in washing and cleaning compositions, has been mentioned in general terms in German patent application 1 965 281 (Merck), but this document makes no further specific proposals about the generally-mentioned washing and cleaning application. In particular, nothing is said or suggested in this document about any use of the material specifically in liquid detergent compositions. Moreover, DE 1 965 281 says, as regards the activity of the enzyme in relation to native (undenatured) proteins, that the Tritirachium enzyme breaks them down incompletely or not at all.

Representative examples of prior art as to enzyme stabilisation are as follows.

JP 47-35192 describes the use of glycerol or sorbitol with borax under certain conditions and proportions, to stabilise enzyme preparations including liquid washing materials.

DE 27 28 211 (Unilever) describes the use of polyols of 2 to 6 hydroxy groups together with boric acid or borate in ratios less than 1, particularly in unbuilt detergents.

GB 2 079 305 (Unilever) describes the use of polyols together with boric acid and/or borate and polyacrylate polymers as stabilising agents, while EP 0 080 223 (Unilever) describes the combined use of boric acid or borate and polyol or polyamino compounds with reducing salts, and EP 0 126 505 (Unilever) describes the use of boric acid or borate and reducing salts, together with succinic or other dicarboxylic acids. Other prior art deals with the use of stabilisers such as calcium formate/acetate.

Background, Aims and Summary of the Present Invention:

The prior art mentioned above includes a variety of enzyme-stabilising systems for use in connection with liquid detergent compositions, and these systems can indeed be effective, but the ingredients for them can be unacceptably expensive, and it is desirable to find a way to reduce or avoid their use.

Although the above-cited USP 3 707 504 mentions surprising stability of protease from Thermoactinomyces vulgaris in highly-alkaline systems, we have in practice experienced difficulty in formulating adequately stable liquid detergents with protease from this species among others.

Consequently, we believe there is still a need for protease-containing liquid detergent compositions of improved stability, and an aim of this invention is to satisfy this need.

A further aim of this invention is to provide liquid detergent compositions incorporating enzymes which need less than normal amounts of such enzyme stabilisers as those mentioned above, and/or which can be formulated without such stabilisers, for useful storage stability.

We have now found that enzymes of the Proteinase K type are of particular value as proteolytic enzymes in enzymatic liquid detergent compositions.

According to the invention there is provided a liquid detergent composition comprising a surfactant concentrate and a proteolytic enzyme derived from a microorganism, characterised in that the proteolytic enzyme is a fungal alkaline protease of the proteinase K type, for improved storage stability in the liquid state.

We have found that use of such proteolytic enzymes can provide liquid detergent compositions with an improved enzyme storage stability compared with the aforementioned Bacillus-originating proteases, and also in comparison with the above-mentioned alkaline protease from Thermoactinomyces, even in the absence (or presence in lower amounts than previously proposed) of enzyme-stabilizing systems.

Furthermore, proteinase K is especially effective in breaking down native keratin and other native proteins.

Further and Detailed description of the Invention:

For the purposes of this invention, equivalents of the proteinase K from Tritirachium album (Limber) are considered to be those fungal alkaline serine proteinases which show substantial homology with proteinase K itself, and possess the following characteristics: (i) presence of cysteine close to the protease active site; (ii) a content of tightly-bound calcium, bound with an affinity corresponding to dissociation pK (calcium) the order of about 5.5 to 8; (iii) presence of an SS (cystine) bridge in the protease tertiary structure; (iv) substantial resistance to inhibition of the protease activity by PCMB (parachloromercuribenzoate). It is believed that proteinase K itself also has a content of SS (cystine) bridges in the molar ratio 2:1 to its content of (free) cysteine, and a further content of weakly-bound calcium substantially equal to its content of tightly-bound calcium.

Also considered as equivalents of proteinase K for the purposes of this invention are proteases produced by rDNA manipulation on the basis of genetic material corresponding to a protease of the proteinase K type, with or without modifications.

Genetic engineering of the enzymes can be achieved by extraction of an appropriate alkaline serine protease gene, e.g. the gene for proteinase K from Tritirachium album Limber itself or from a mutant thereof, and introduction and expression of the gene or derivative thereof in a suitable producer organism. The technique described in WO 88/02775 (Novo) may be applied and adapted.

Also within the scope of the invention as equivalent to the use of the proteinases mentioned above is the use of analogues (e.g. analogues made by mutant organisms) and derivatives and conjugates of the proteinases.

The preferred protease for use in this invention is Proteinase K from Tritirachium album (Limber).

The proteinase K type enzyme can be used either alone or together with Bacillus or other common proteases, e.g. Savinase, Maxatase or Alcalase (Trade Marks) and/or other proteolytic enzymes, as well as with other types of enzymes such as lipases, amylases, cellulases and alcohol oxidases. Mixtures of the various other enzymes may also be present.

In general, our belief is that crude enzyme preparations of the type defined above perform better after storage than do the corresponding purified enzymes.

The proteinase defined above can preferably be included according to the present invention in an amount of 1 to 100 GU/mg liquid detergent. A GU is a Glycine Unit, which is defined as the proteolytic enzyme activity which, under standard conditions, during a 15-minute-incubation at 40 deg C, with N-acetyl casein as substrate, produces an amount of NH2-group equivalent to 1 micromole of glycine. Preferably, the amount ranges from 2 to 50 and particularly preferably from 5 to 20 GU/mg.

The liquid detergent compositions in which the proteinase is incorporated according to the present invention can be aqueous or non-aqueous, built or unbuilt liquid detergents which on their own are well known in the art. They have been amply described in the following patent specifications, hereby incorporated by reference : European patent 0 126 505 (Unilever) and European patent application 0 225 654.

Typically, aqueous liquid detergent compositions comprise from 1-60% by weight of one or more detergent-active compounds, from 0-60% by weight of one or more organic and/or inorganic builders, and optionally other conventional ingredients such as soil-suspending agents, hydrotropes, corrosion inhibitors, dyes, perfumes, silicates, optical brighteners, suds depressants, germicides, anti-tarnishing agents, opacifiers, fabric softening agents, oxygen-liberating bleaches such as hydrogen peroxide, sodium perborate, diperisophthalic anhydride, with or without bleach precursors, buffers and the like. The liquid medium is usually an aqueous medium.

The detergent-active compounds in the compositions can for example be anionic and/or nonionic surfactants, and the pH of the liquid detergent compositions can be chosen at will from a wide range, e.g. from about pH 7 upwards, e.g. a milder alkaline range from about pH 7.5 to about pH 9 or a stronger alkaline range from about pH 9 upwards.

For non-aqueous liquid detergent compositions the above ingredients and ranges also apply mutatis mutandis. Usually, these compositions contain a suspending medium for the other ingredients, the suspending medium comprising usually a nonionic detergent together with a suspending agent such as silica, a copolymer and the like.

Where the liquid detergent compositions contain inorganic or organic electrolyte salts, we have also found that the defined proteinase frequently gives an improved performance in liquid detergent compositions with an increased ionic strength or molarity.

Also included within the scope of the invention are liquid detergent compositions incorporating the defined protease as well as an enzyme-stabiliser, possibly in a lower amount than those amounts hitherto proposed.

The compositions may also comprise other detergent additives, for example without limitation polysaccharides such as pectinates and alginates chosen for compatibility with the pH and pI of the enzyme in use, and polycarboxylates, e.g. polyacrylates.

The invention is further illustrated by way of Example.

EXAMPLE 1

Storage experiments were carried out with a liquid detergent composition of the following formulation :

	% (w/w)
Dodecyl benzene sulphonic acid	9
C13-C13 primary linear alcohol condensed with 7 moles of ethylene oxide	2.25
Pentasodium triphosphate	27
Sodium hydroxide	1.1
Water	to 100

pH adjusted to 9

Such a formulation can if desired be prepared in accordance with EP 0 266 199 (Unilever).

Various proteases were included at 8-10 GU/mg liquid (at t = 0), and the protease stability was determined at regular intervals while storing the products at 37 deg C.

With Alcalase (Trade mark, Novo), a B. subtilis protease, there was found after 2 days a residual enzyme activity of only 8%; with Savinase (Trade Mark, Novo), a highly alkaline Bacillus protease, there was no more residual activity after only 1 day. With Proteinase K (from Sigma), there was found after 27 days still a residual activity of 22%.

Further storage stability testing was carried out using thermitase (TM) from Thermoactinomyces vulgaris, in a composition otherwise similar to that set out above. It was found that the Thermoactinomyces enzyme showed poor storage stability.

Alternative commercial sources of proteinase K essentially equivalent to that used in this example are Boehringer and Merck (Trade Marks).

EXAMPLE 2

With the formulation of Example 1 (pH = 9) washing tests with cotton test pieces were carried out in a Tergotometer (single wash) at a concentration of 3 g/l, at 30 deg C. The wash cycle was for 30 minutes at 60 rpm, the water hardness was 20 deg FH. The liquid/cloth ratio was 1:50.

The enzymes were dosed at 30 GU/ml wash liquor. The soils were AS 10, cocktail 1 and cocktail 2. The enzymes used were:

-- Savinase (Bacillus protease), from Novo;

-- an alkaline protease from Streptomyces griseus, from Calbiochem-Behring, which is reported to act on various keratinous proteins (as also applies to Proteinase K).

The following results were obtained :

	AS 10	Cocktail 1 (R 460)	Cocktail 2
Savinase	13.1	19.7	9.4
Strept.gris.	23.2	21.6	13.5
Proteinase K	14.3	21.0	8.7
Savinase + Proteinase K (1:1)	13.1	21.9	9.4
Savinase + Strept. gr.(1:1)	19.6	21.3	13.3

Cocktail 1 = Gelatin/BSA/milk powder 1:1:1

" 2 = Hemoglobin/BSA 2.3:1

EXAMPLE 3

Stability tests were performed in a liquid according to Example 1 with Savinase, Streptomyces griseus protease, a 1:1 mixture of Savinase with Strept. gris. protease and Proteinase K. The storage temperature was 37 deg C.

The following results were obtained (% residual activity) after the storage times indicated:

	Savinase	Strept. gris. protease	Savinase/ Strept. gris. protease (1:1)
5 hr	17	46	39
25 hr	1	6	6
96 hr	ND	ND	ND

(ND = not determined)

	Protei-nase K		Savinase/ Protei-nase K (1:1)
5 hr	79	5 hr	53
25 hr	56	24 hr	26
96 hr	46	48 hr	23

EXAMPLE 4

With the following formulation, stability and performance tests were carried out :

	% (w/w)
Dodecyl benzene sulphonic acid	16
C12-C15 alcohol condensed with 9 moles of ethylene oxide	7
Monoethanol amine	2
Citric acid	6.5
Sodium xylene sulphonate	6
Colouring agent	0.011
Fluorescer	0.078
Opacifier	0.11
Stearic acid	0.075
Perfume	0.15
Sodium hydroxide	4.10
Water	up to 100

The pH was adjusted to 10 with citric acid.

The stability at 37 deg C of the following enzymes (at 8-GU/mg liquid) was as follows:

	% RA
Savinase	10 after 1 day
Alcalase	15 after 29 hours
Proteinase K	26 after 9 days

In a miniwasher at 30 deg C for 30 minutes at 2 g/l in water of 6 deg FH, the following wash results were obtained with different proteases (This wash test was carried out with the above formulation which had a pH 10.8) :

AS 10	at 100 GU/ml :	Alcalase > Kazusase (TM ex Showa Denko) > Proteinase K abt. equal to Savinase
Cocktail 1 at 100 GU/ml:		Alcalase > Kazusase abt. equal to Proteinase K > Savinase
Cocktail 2 at 100 GU/ml :		Proteinase K abt. equal to Alcalase > Savinase > Kazusase.

EXAMPLE 5

The wash test of Example 4 was repeated, with the liquid detergent (pH v 10.8) as used in Example 4. By addition of NaCl the ionic strength was increased, and the wash performance was compared (expressed in % reflectance at 460 nm).

The following results were obtained :

Protease (dosed at 20 GU/ml wash liquor)	Formulation of Ex.4 (ionic strength 0.0044)	Formulation of Ex.4 + NaCl (ionic strength 0.026)
Savinase	62.5	70.5
Alcalase	68.5	71.5
Kazusase	64.5	71
Proteinase K	63	71

It is apparent that modifications and variations can be applied to the invention and to the several features mentioned and described herein, which can be applied in all combinations and subcombinations.

Claims

1. A liquid detergent composition comprising a surfactant concentrate and a proteolytic enzyme derived from a microorganism, characterised in that the proteolytic enzyme is a fungal alkaline protease of the proteinase K type.

2. A liquid detergent composition according to claim 1, characterised in that the liquid composition is an aqueous liquid composition.

3. A liquid detergent composition according to claim 1, characterised in that the liquid composition is a non-aqueous liquid composition.

4. A liquid detergent composition according to claim 1, 2 or 3, characterised in that the surfactant

consists essentially of anionic and/or nonionic surfactant.

5. A detergent composition according to any of claims 1 to 4, characterised in that the alkaline protease is introduced in crude form without prior extensive purification.

6. A detergent composition according to any of claims 1 to 5, wherein the alkaline protease comprises proteinase K derived from Tritirachium album Limber.

7. A detergent composition according to any of claims 1 to 6, wherein the proteolytic enzyme is present in an amount in the order of about 1 to 100 GU/mg liquid detergent.

8. A detergent composition according to any of claims 1 to 7, comprising glycerol borate stabiliser.

9. A detergent composition according to any preceding claim, characterised by any one or more of the features described in the foregoing description.