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(54) A STABILIZED LIQUID ENZYME COMPOSITION

STABILISIERTE FLÜSSIGE ENZYMZUSAMMENSETZUNG

COMPOSITION ENZYMATIQUE LIQUIDE STABILISÉE

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Description

FIELD OF THE INVENTION

[0001] The present Invention relates to a liquid composition, comprising a subtilisin and a subtilisin stabilizer.

BACKGROUND OF THE INVENTION

[0002] Storage stability problems are well known with liquids containing enzyme(s). Especially in enzyme-containing liquid detergents a major problem, in particular if the detergent contains protease, is that of ensuring enzyme activity over time

The prior art has dealt extensively with improving the storage stability, for example by adding a protease inhibitor.

Boric acid and boronic acids are known to reversibly inhibit proteolytic enzymes. A discussion of the inhibition of one serine protease, subtilisin, by boronic acid is provided in Molecular & Cellular Biochemistry 51, 1983, pp. 5-32.

Boronic acids have very different capacities as subtilisin inhibitors. Boronic acids containing only alkyl groups such as methyl, butyl or 2-cyclohexylethyl are poor inhibitors with methylboronic acid as the poorest inhibitor, whereas boronic acids bearing aromatic groups such as phenyl, 4-methoxyphenyl or 3,5-dichlorophenyl are good inhibitors with 3,5-dichlorophenylboronic acid as a particularly effective one (see Keller et al, Biochem. Biophys. Res. Com. 176, 1991, pp 401-405).

It is also claimed that aryl boronic acids which have a substitution at the 3-position relative to boron are unexpectedly good reversible protease inhibitors. Especially, acetamidophenyl boronic acid is claimed to be a superior inhibitor of proteolytic enzymes (see WHO 92/19707)

In WO 96/41859 substituted phenyl boronic acids are disclosed as suitable enzyme stabilizers.

It has been found that borates are reprotoxic in animals and therefore there is a demand to find alternatives which can be used to stabilize the enzymes in liquid compositions. In a particular embodiment of the present invention the liquid composition does not comprise borate or boric acid

In EP 0 378 261 B1 an enzyme stabilization system comprising carboxylic acids for aqueous liquid detergent compositions is disclosed.

[0003] US 5 919 313 A discloses a contact lens cleaning composition comprising an enzyme, an aromatic acid derivative and optionally surfactants. The aromatic acid derivative can be for instance benzoic acid, phenylacetic acid, phenylpropionic acid or phenylbutyric acid.

SUMMARY OF THE INVENTION

[0004] One object of the present invention is to provide a stable liquid composition.

It has surprisingly been found that certain aryl carboxylic

acids have extraordinary good capacities as subtilisin stabilizers in liquids, especially the stabilizers indicated below.

[0005] The present invention provides thus a liquid composition comprising a subtilisin and a subtilisin stabilizer as described below.

DETAILED DESCRIPTION OF THE INVENTION

The stabilizer

[0006] The present invention relates to stabilization of subtilisins in liquid compositions. The present invention provides a liquid composition comprising a subtilisin and a subtilisin stabilizer which is:

3-chlorobenzoic acid, 4-chlorobenzoic acid, 3,5-dichlorobenzoic acid, 3-chlorophenylacetic acid 3-chlorophenyl-3-propionic acid, and their corresponding salts

[0007] In a particular embodiment of the present invention the liquid composition comprises 0.001-20% w/w of the stabilizer. In a more particular embodiment of the present invention the liquid composition comprises 0.01-15% of the stabilizer. In an even more particular embodiment of the present invention the liquid composition comprises 0.1-10% w/w of the stabilizer. In a most particular embodiment of the present invention the liquid composition comprises 0.5-5 % of the stabilizer. In a further particular embodiment of the present invention the liquid composition comprises 1.5 to 5% w/w of the stabilizer.

In a particular embodiment of the present invention the liquid composition comprises 0.05-1000 mM of the stabilizer. In a more particular embodiment of the present invention the liquid composition comprises 0.5-750 mM of the stabilizer. In an even more particular embodiment of the present invention the liquid composition comprises 5-500 mM of the stabilizer. In a most particular embodiment of the present invention the liquid composition, comprises 25-250 mM of the stabilizer

In a particular embodiment the present invention provides a liquid detergent composition comprising a surfactant, a subtilisin and a subtilisin stabilizer of any of the formulas disclosed above.

[0008] It has surprisingly been found that the stabilizer also have a stabilizing effect at pH above 8 such as above 9.

Active compounds

[0009] According to the invention the liquid composition contains at least one subtilisin Mixtures of subtilisins are also included.

[0010] In a particular embodiment the liquid composition comprises more than 2 different enzymes. In a particular embodiment a liquid composition comprising two or more enzymes in which the first enzyme is a subtilisin and the second enzyme is selected from the group consisting of amylases, lipases, cellulases, lyases and oxi-

doreductases is preferred. In a more particular embodiment the second enzyme is a lipase.

[0011] It is to be understood that subtilisin variants (produced, for example, by recombinant techniques) are included within the meaning of the term subtilisin. Examples of such subtilisin variants are disclosed, e.g. in EP 251,446 (Genencor), WO 91/00345 (Novozymes), EP 525,610 (Solvay) and WO 94/02616 (Gist-Brocades NV).

[0012] Subtilisins are well-characterized physically and chemically. In addition to knowledge of the primary structure (amino acid sequence) of these enzymes, over 50 high resolution X-ray structures of subtilisins have been determined which delineate the binding of substrate, transition state, products, at least three different protease inhibitors, and define the structural consequences for natural variation (Kraut (1977) Ann. Rev. Biochem. 46 331-358).

One subgroup of the subtilases, I-SI, comprises the "classical" subtilisins, such as subtilisin 168, subtilisin BPN', subtilisin Carlsberg (ALCALASE®, Novozymes A/S), and subtilisin DY.

A further subgroup of the subtilases I-S2, is recognised by Siezen et al. (supra). Sub-group IS2 proteases are described as highly alkaline subtilisins and comprise enzymes such as subtilisin PB92 (MAXACAL®, Gist-Brocades NV), subtilisin 309 (SAVINASE®, Novozymes A/S), subtilisin 147 (ESPERASE®, Novozymes A/S), and alkaline elastase YaB.

Random and site-directed mutations of the subtilase gene have both arisen from knowledge of the physical and chemical properties of the enzyme and contributed information relating to subtilase's catalytic activity, substrate specificity, tertiary structure, etc (Wells et al. (1987) Proc. Natl. Acad. Sci. U.S.A. 84; 1219-1223; Wells et al. (1986) Phil. Trans. R. Soc. Lond. A. 317 415-423; Hwang and Warshel (1987) Biochem. 26 2669-2673; Rao et al., (1987) Nature 328 551-554).

More recent publications covering this area are Carter et al. (1989) Proteins 6240-248 relating to design of variants that cleave a specific target sequence in a substrate (positions 24 and 64); Graycar et al. (1992) Annals of the New York Academy of Sciences 672 71-79 discussing a number of previously published results; and Takagi (1993) Int. J. Biochem. 25 307-312 also reviewing previous results.

[0013] Examples of commercially available subtilisins include Kannase™, Evertase™, Esperase™, Alcalase™, Durazym™, Savinase™, Ovozyme™, Pyrase™, and Clear-Lens™ Pro (all available from Novozymes A/S, Bagsvaerd, Denmark). Other preferred include those described in WO 01/58275.

Other commercially available subtilisins include Maxatase™, Maxacal™, Maxapem™, Opticlean™, Propease™, Purafect™ and Purafect Ox™ (available from Genencor International Inc., Gist-Brocades, BASF, or DSM Nutritional Products).

[0014] Lipases: Suitable lipases include those of bacterial or fungal origin. Chemically or genetically modified

mutants are included.

Examples of useful lipases include a *Humicola lanuginosa* lipase, e.g., as described in EP 258 068 and EP 305 216, a *Rhizomucor miehei* lipase, e.g., as described in EP 238 023, a *Candida* lipase, such as a *C. antarctica* lipase, e.g., the *C. antarctica* lipase A or B described in EP 214 761, a *Pseudomonas* lipase such as a *P. pseudoalcaligenes* and *P. alcaligenes* lipase, e.g., as described in EP 218 272, a *P. cepacia* lipase, e.g., as described in EP 331 376, a *P. stutzeri* lipase, e.g., as disclosed in BP 1 372,034, a *P. fluorescens* lipase, a *Bacillus* lipase, e.g., a *B. subtilis* lipase (Dartois et al., (1993), Biochimica et Biophysica acta 1131, 253-260), a *B. stearothermophilus* lipase (JP 64/744992) and a *B. pumilus* lipase (WO 91/16422).

Furthermore, a number of cloned lipases may be useful, including the *Penicillium camembertii* lipase described by Yamaguchi et al., (1991), Gene 103, 61-67), the *Geotrichum candidum* lipase (Schimada, Y. et al., (1989), J. Biochem 106, 383-388), and various *Rhizopus* lipases such as a *R. delemar* lipase (Hass, M.J et al., (1991), Gene 109, 117-113), a *R. niveus* lipase (Kugimiya et al., (1992), Biosci. Biotech. Bio-chem. 56, 716-719) and a *R. oryzae* lipase.

Other types of lipolytic enzymes such as culinases may also be useful, e.g., a cutinase derived from *Pseudomonas mendocina* as described in WHO 88/09367, or a culinase derived from *Fusarium solani* pisi (e.g. described in WO 90/09446).

Examples of commercially available lipases include Lipex™, Lipoprime™, Lipopan™, Lipolase™, Lipolase™ Ultra, Lipozyme™, Palatase™, Resinase™, Novozym™ 435 and Lecitase™ (all available from Novozymes A/S). Other commercially available lipases include Lumafast™ (*Pseudomonas mendocina* lipase from Genencor International Inc.), Lipomax™ (*Ps. pseudoalcaligenes* lipase from Gist Brocades/Genencor Int. Inc.; and *Bacillus* sp. lipase from Solvay enzymes. Further lipases are available from other suppliers such as Lipase P "Amano" (Amano Pharmaceutical Co. Ltd.).

Amylases. Suitable amylases (α and/or β) include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. Amylases include, for example, α -amylases obtained from a special strain of *B. licheniformis*, described in more detail in British Patent Specification No. 1,296,839. Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™ and BAN™ (available from Novozymes A/S) and Rapidase™ and Maxamyl P™ (available from Gist-Brocades).

Cellulases: Suitable cellulases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. Suitable cellulases are disclosed in US 4,435,307, which discloses fungal cellulases produced from *Humicola insolens*. Especially suitable cellulases are the cellulases having color care benefits. Examples of such cellulases are cellulases described in European patent application No. 0 495 257.

Oxidoreductases: Any oxidoreductase suitable for use in

a liquid composition, e.g., peroxidases or oxidases such as laccases, can be used herein. Suitable peroxidases herein include those of plant, bacterial or fungal origin. Chemically or genetically modified mutants are included. Examples of suitable peroxidases are those derived from a strain of *Coprinus*, e.g., *C. cinerius* or *C. macrorhizus*, or from a strain of *Bacillus*, e.g., *B. pumilus*, particularly peroxidase according to WO 91/05858. Suitable laccases herein include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. Examples of suitable laccases are those obtainable from a strain of *Trametes*, e.g., *T. villosa* or *T. versicolor*, or from a strain of *Coprinus*, e.g., *C. cinereus*, or from a strain of *Myceliophthora*, e.g., *M. thermophila*.

[0015] Preferred oxidoreductases in the context of the invention are peroxidases (EC 1.11.1), laccases (EC 1.10.3.2) and glucose oxidases (EC 1.1.3.4)]. An Example of a commercially available, oxidoreductase is Gluzyme™ (enzyme available from Novozymes A/S). Further oxidoreductases are available from other suppliers. Preferred transferases are transferases in any of the following sub-classes:

- a Transferases transferring one-carbon groups (EC 2.1);
- b transferases transferring aldehyde or ketone residues (EC 2.2); acyltransferases (EC 2.3);
- c glycosyltransferases (EC 2.4);
- d transferases transferring alkyl or aryl groups, other than methyl groups (EC 2.5); and
- e transferases-transferring nitrogeneous groups (EC 2.6).

The Composition

[0016] The composition may be any composition, but particularly suitable composition are cleaning compositions, personal care compositions, textile processing compositions e.g. bleaching, pharmaceutical compositions, leather processing compositions, pulp or paper processing compositions, food and beverage compositions and animal feed compositions.

In a particular embodiment of the present invention the liquid composition is a liquid detergent, eg, laundry detergent or dishwashing detergent. In a more particular embodiment of the present invention the composition comprises a surfactant

The invention is further directed to the use of the liquid enzyme additive in liquid detergent composition.

The liquid composition may be a concentrated product to be added to liquid detergents. The amount of enzyme used in the liquid composition is thus very high. In a particular embodiment of the present invention the amount of enzyme present in the liquid composition is at least 1.5 g/L In a more particular embodiment of the present invention the amount of enzyme is at least 5 g/L. In an even more particular embodiment of the present invention the amount of enzyme present is at least 10 g/L. In

a most particular embodiment of the present invention the amount of enzyme present is at least 20 g/L such as even above 25 g/L. In a particular embodiment the amount of enzyme does not exceed 200 g/L. In a more particular embodiment of the present invention the amount of enzyme does not exceed 150 g/L. In a most particular embodiment of the present invention the amount of enzyme present in the liquid composition is less than 100 g/L.

In a particular embodiment of the present invention the liquid composition has a pH of more than 7.5 In a more particular embodiment the pH of the liquid composition is at least 7.7. In a most particular embodiment the pH of the liquid composition is at least 8.0.

In a most particular embodiment of the present invention the liquid composition contain one or more detergent builders. In a particular embodiment of the present invention the liquid composition comprises at least 1% w/w of detergent builders. In a more particular embodiment of the present invention the liquid composition comprises at least 2% w/w of detergent builders. In a most particular embodiment of the present invention the liquid composition comprises at least 5% w/w of detergent builders.

[0017] The present Invention is further described by the following examples which should not be construed as limiting the scope of the invention.

Detergent compositions

[0018] In a particular embodiment of the present invention the liquid composition is a liquid detergent composition. In a more particular embodiment of the present invention the liquid composition is a liquid detergent composition for dishwashing or for laundry.

[0019] The detergent composition of the invention may for example be formulated as a hand or machine laundry detergent composition including a laundry additive composition suitable for pretreatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations, or be formulated for hand or machine dishwashing operations.

[0020] Examples of subtilisins are, those derived from *Bacillus*, e.g., subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279).

Examples of useful are the variants described in WO 92/19729, WO 98/20115, and WO 98/20116.

Preferred commercially available subtilisins include Alcalase™, Savinase™, Primase™, Duralase™, Esperase™, and Kannase™ (Novozymes A/S), Maxatase™, Maxacal™, Maxapem™, Properase™, Purafect™, Purafect OxP™, FN2™, and FN3™ (Genencor International Inc.).

Lipases: Suitable lipases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Example of useful lipases include lipases from *Humicola* (synonym *Thermomyces*), e.g.

from *H. lanuginosa* (*T. lanuginasus*) as described in EP 258 068 and EP 305 216 or from *H. insolens* as described in WO 96/13580, a *Pseudomonas* lipase, e.g. from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), *P. cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012), a *Bacillus* lipase, e.g. from *B. subtilis* (Dartols et al. (1993), Biochemica et Biophysica Acta, 1131, 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422).

Other examples are lipase variants such as those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079 and WO 97/07202.

Preferred commercially available lipase enzymes include Lipolase™ and Lipolase Ultra™ (Novozymes A/S).

Amylases: Suitable amylases (α and/or β) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, α -amylases obtained from *Bacillus*, e.g. a special strain of *B. licheniformis*, described in more detail in GB 1,296,839.

Examples of useful amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424.

Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™ and BAN™ (Novozymes A/S). Rapidase™ and Purastar™ (from Genencor International Inc.).

Cellulases: Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g. the fungal cellulases produced from *Humicola Insolens*, *Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757 and WO 89/09259.

Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/108940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and PCT/DK98/00299.

Commercially available cellulases include Celluzyme™, and Carezyme™ (Novozymes A/S), Clazinas™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

Peroxidases/Oxidases: Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus*, e.g. from *C. cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and

WO 98/15257.

Commercially available peroxidases include Guardzyme™ (Novozymes A/S).

[0021] The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A liquid detergent may be aqueous, typically containing up to 70% water and 0-30 % organic solvent, or non-aqueous.

The detergent composition comprises one or more surfactants, which may be non-ionic including semi-polar and/or anionic and/or cationic and/or zwitterionic. The surfactants are typically present at a level of from 0.1% to 60% by weight.

When included therein the detergent will usually contain from about 1 % to about 40% of an anionic surfactant such as linear alkylbenzenesulfonate, alpha-olefinsulfonate, alkyl sulfate (fatty alcohol sulfate), alcohol ethoxysulfate, secondary alkanesulfonate, alpha-sulfo fatty acid methyl ester, alkyl- or alkenylsuccinic acid or soap. When included therein the detergent will usually contain from about 0.2% to about 40% of a non-ionic surfactant such as alcohol ethoxylate, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, polyhydroxy alkyl fatty add amide, or N-acyl N-alkyl derivatives of glucosamine ("glucamides"). The detergent may contain 0-65 % of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, carbonate, citrate, nitrilotriacetic add, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, alkyl- or alkenylsuccinic add, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst).

The detergent may comprise one or more polymers. Examples are carboxymethylcellulose, poly(vinylpyrrolidone), poly(ethylene glycol), poly(vinyl alcohol), poly(vinylpyridine-N-oxide), poly(vinylimidazole), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic add copolymers.

The detergent may contain a bleaching system which may comprise a H₂O₂ source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetylenediamine or nonanoyloxybenzenesulfonate. Alternatively, the bleaching system may comprise peroxyacids of e.g. the amide, imide, or sulfone type.

The detergent may also contain other conventional detergent ingredients such as e.g. fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil redeposition agents, dyes, bactericides, optical brighteners, hydrotropes, tarnish inhibitors, or perfumes.

EXAMPLES

Example 1

[0022] The inhibition constant $K_i = [E][I]/[EI]$ for the inhibition of Savinase was determined using standard methods under the following conditions:

Substrate: Succinyl-Alanine-Alanine-Proline-para-nitro-anilide = SAAPFpNA (Sigma S-7388).
Buffer: 0.1 M phosphate buffer pH 7.5
Temperature: 25°C
Enzyme concentration in assay $\approx 1 \times 10^{-8}$ M

The initial rate of substrate hydrolysis was determined with and without inhibitor at nine substrate concentrations in the range of 0.01 mM to 2 mM using an automated spectrophotometer.

The inhibition constant K_i was determined using Sigma Plot 9.0, Enzyme Kinetics Module 1.1

Inhibitor	K
Benzoic Acid	22 mM
Phenylacetic acid	10 mM
Phenylpropionic acid	9 mM
3,5-dichlorobenzoic acid	2 mM
4-formylbenzoic acid	25 mM
Terephthalic acid (4-carboxybenzoic acid)	99 mM

Example 2

[0023] The inhibition constant $K_i = [E][I]/[EI]$ for the inhibition of Savinase was determined using standard methods under the following conditions:

Substrate: Succinyl-Alanine-Alanine-Proline-para-nitro-anilide = SAAPFpNA (Sigma S-7388).
Buffer: 0.1 M phosphate buffer pH 7.5
Temperature: 25°C
Enzyme concentration in assay $\approx 1 \times 10^{-8}$ M

The initial rate of substrate hydrolysis was determined with and without inhibitor at nine substrate concentrations in the range of 0.01 mM to 2 mM using an automated spectrophotometer.

[0024] The inhibition constant K_i was determined using Sigma Plot 9.0, Enzyme Kinetics Module 1.1

Acids:

[0025]

Inhibitor	K_i
3-chlorobenzoic acid	4 mM
4-chlorobenzoic acid	5 mM
3-(3-chlorophenyl)propionic acid	5 mM

(continued)

Inhibitor	K_i
3-chlorophenylacetic acid	7 mM
3-(4-chlorophenyl)propionic acid	10 mM
4-chlorophenylacetic acid	11 mM
2-chlorophenylacetic acid	11 mM
2-chlorobenzoic acid	16 mM
2-aminobenzoic acid	16 mM
3-aminobenzoic acid	43 mM
4-aminobenzoic acid	82 mM
3-bromobenzoic acid	6 mM
3-iodobenzoic acid	9 mM
3-nitrobenzoic acid	9 mM
3-fluorobenzoic acid	17 mM
3-formylbenzoic acid	27 mM
3-(chloromethyl)benzoic acid	32 mM
3,5-dihydroxybenzoic acid	76 mM
3-hydroxybenzoic acid	29 mM
4-phenylbutyric acid	19 mM

Example 3 (Reference)

[0026] The inhibition constant $K_i = [E][I]/[EI]$ for the inhibition of Savinase was determined using standard methods under the following conditions:

Substrate: Succinyl-Alanine-Alanine-Proline-para-nitro-anilide = SAAPFpNA (Sigma S-7388).
Buffer: 0.1 M phosphate buffer pH 7.5
Temperature: 25°C
Enzyme concentration in assay $\approx 1 \times 10^{-8}$ M

The initial rate of substrate hydrolysis was determined with and without inhibitor at nine substrate concentrations in the range of 0.01 mM to 2 mM using an automated spectrophotometer.

The inhibition constant K_i was determined using Sigma Plot 9.0, Enzyme Kinetics Module 1.1

Aldehydes tested:

[0027]

Inhibitor	K_i
3-hydroxybenzaldehyde	16 mM
3,4-dihydroxybenzaldehyde	11 mM

Claims

1. A liquid composition comprising a subtilisin and a subtilisin stabilizer selected from the group consisting of 3-chlorobenzoic acid, 4-chlorobenzoic acid, 3,5-dichlorobenzoic acid, 3-chlorophenylacetic acid, 3-chlorophenyl-3-propionic acid and their corre-

sponding salts.

2. A liquid composition according to claim 1, wherein the enzyme is present in the liquid composition in an amount of at least 1.5 g/L.
3. A liquid composition according to any of claims 1-2, additionally comprising a second enzyme.
4. A liquid composition according to claim 3, wherein the second enzyme is an amylase, a lipase, a cellulase or an oxidoreductase, or a mixture thereof.
5. liquid composition according to any of claims 1-4, which has a pH of at least 8.
6. A liquid composition according to any of claims 1-5, wherein said stabilizer is 0.001-20% w/w of the liquid composition.
7. A liquid composition according to any of claims 1-6 which is a liquid detergent composition additionally comprising a surfactant.
8. A liquid composition according to any preceding claim which has a pH above 8, particularly above 9

Patentansprüche

1. Flüssige Zusammensetzung, umfassend ein Subtilisin und einen Subtilisinstabilisator, ausgewählt aus der Gruppe bestehend aus 3-Chlorbenzoesäure, 4-Chlorbenzoesäure, 3,5-Dichlorbenzoesäure, 3-Chlorphenyllessigsäure, 3-Chlorphenyl-3-propionsäure und ihren entsprechenden Salzen.
2. Flüssige Zusammensetzung nach Anspruch 1, wobei das Enzym in der flüssigen Zusammensetzung in einer Menge von mindestens 1,5 g/L vorliegt.
3. Flüssige Zusammensetzung nach einem beliebigen der Ansprüche 1-2, zusätzlich umfassend ein zweites Enzym.
4. Flüssige Zusammensetzung nach Anspruch 3, wobei das zweite Enzym eine Amylase, eine Lipase, eine Cellulase oder eine Oxidoreduktase, oder eine Mischung davon ist.
5. Flüssige Zusammensetzung nach einem beliebigen der Ansprüche 1-4, die einen pH von mindestens 8 aufweist.
6. Flüssige Zusammensetzung nach einem beliebigen der Ansprüche 1-5, wobei der Stabilisator 0,001-20% Gew./Gew. der flüssigen Zusammensetzung beträgt.

7. Flüssige Zusammensetzung nach einem beliebigen der Ansprüche 1-6, die eine flüssige Detergensenzymzusammensetzung ist, die zusätzlich ein oberflächenaktives Mittel umfasst.
8. Flüssige Zusammensetzung nach einem beliebigen der vorhergehenden Ansprüche, die einen pH oberhalb von 8, insbesondere oberhalb von 9, aufweist.

Revendications

1. Composition liquide comprenant une subtilisine et un stabilisateur de subtilisine choisi dans le groupe constitué de l'acide 3-chlorobenzoïque, l'acide 4-chlorobenzoïque, l'acide 3,5-dichlorobenzoïque, l'acide 3-chlorophénylacétique, l'acide 3-chlorophényl-3-propionique et leurs sels correspondants.
2. Composition liquide selon la revendication 1, dans laquelle l'enzyme est présente dans la composition liquide en une quantité d'au moins 1,5 g/L.
3. Composition liquide selon l'une quelconque des revendications 1-2, comprenant en outre une seconde enzyme.
4. Composition liquide selon la revendication 3, dans laquelle la seconde enzyme est une amylase, une lipase, une cellulase ou une oxydoréductase, ou un mélange de celles-ci.
5. Composition liquide selon l'une quelconque des revendications 1-4, qui a un pH d'au moins 8.
6. Composition liquide selon l'une quelconque des revendications 1-5, dans laquelle ledit stabilisateur est 0,001-20% p/p de la composition liquide
7. Composition liquide selon l'une quelconque des revendications 1-6, qui est une composition détergente liquide comprenant en outre un surfactant.
8. Composition liquide selon l'une quelconque des revendications précédentes, qui a un pH supérieur à 8, en particulier supérieur à 9.

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