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(54) Title: ALKALINE LIQUID DETERGENT COMPOSITION

(57) Abstract: Provided is a liquid detergent composition. The liquid detergent composition includes 1 to 60% by weight of a surfactant, 0.001 to 1.0% by weight of aryl boronic acid as an enzyme inhibitor, 0.1 to 20% by weight of one or more other enzyme inhibitor selected from the group consisting of formic acid, boric acid, and a salt thereof, 0.1 to 4.0% by weight of a protease, and the balance to 100% by weight being water, and has an alkaline pH. The liquid detergent composition has considerably improved enzyme storage stability by combination of aryl boronic acid with formic acid and/or boric acid.

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ALKALINE LIQUID DETERGENT COMPOSITION

Technical Field

5 The present invention relates to an alkaline liquid detergent composition, and more particularly, to an alkaline liquid detergent composition with improved enzyme storage stability by using the combination of aryl boronic acid as an enzyme inhibitor with other enzyme inhibitors.

10

Background Art

 A liquid detergent is easier to use than a granular detergent and has excellent solubility in cold water. However, a liquid detergent requires a high manufacturing cost and poor detergency, when
15 compared to a granular detergent. In order to solve these disadvantages, a liquid detergent contains a large amount of contaminant degradation enzymes. However, such enzymes used in a liquid detergent composition are dissolved in high water content, together with surfactants and other types of enzymes, and thus, may easily
20 experience the denaturation by the surfactant and autodigestion. In addition, it is difficult to preserve the storage stability of the enzymes from the pH of a liquid detergent composition.

 Conventionally, in order to stabilize an enzyme in a liquid detergent composition, a neutral or weak alkaline (pH of about 7 to 8.5)
25 liquid detergent composition including an enzyme stabilizer has been used. Due to such low pH, activity of an alkaline protease and detergency are lowered. Therefore, a liquid detergent has poor detergency relative to a granular detergent.

 Meanwhile, a boron compound has widely been known as a
30 reversible protease inhibitor. Inhibition of a subtilisin-based serine protease by boronic acid is disclosed in *Molecular & Cellular Biochemistry*, 1983, 51, pp. 5-32. In particular, *Biochem. Biophys. Res. Com.*, 1991, 176, pp. 401-405 discloses that boronic acid with an

aromatic group such as phenyl, 4-methoxyphenyl, and 3,5-dichlorophenyl has stronger protease inhibitory activity than boronic acid with an alkyl group such as methyl, butyl, and 2-cyclohexylethyl. In addition, PCT Publication WO 92/19707 discloses aryl boronic acid as a reversible subtilisin protease inhibitor and EP 0 478 050 A1 discloses a stabilization of lipase by inhibiting subtilisin protease with a boron compound.

However, the above-described documents are concerned with compositions capable of providing enzyme stabilities by using independent boron compounds with enzyme inhibitory effects. Unlike the present invention, there are no disclosures or discussions about compositions capable of providing synergistic enzyme stabilities by combination of a boron compound as an enzyme inhibitor with other enzyme inhibitors.

Therefore, while searching for a method of stabilizing an enzyme at a liquid detergent with a high pH, the present inventor found an alkaline liquid detergent composition capable of maintaining enzyme stability at a pH higher than in a conventional liquid detergent. This completed the present invention.

The alkaline liquid detergent composition of the present invention comprises the combination of aryl boronic acid as a conventional enzyme inhibitor with other enzyme inhibitors by a predetermined ratio for enzyme stabilization. In addition to these enzyme inhibitors, the alkaline liquid detergent composition of the present invention further comprises an anionic or non-ionic surfactant and the like.

Disclosure of the Invention

The present invention provides an alkaline liquid detergent composition capable of improving enzyme stability by using combinations of enzyme inhibitors.

5 According to an aspect of the present invention, there is provided a liquid detergent composition comprising: 1 to 60% by weight of a surfactant, 0.001 to 1.0% by weight of aryl boronic acid as an enzyme inhibitor, 0.1 to 20% by weight of one or more other enzyme inhibitor selected from the group consisting of formic acid, boric acid, and a salt thereof, 0.1 to 4.0% by weight of a protease, and the balance to 100% by weight being water. The liquid detergent composition has an alkaline pH.

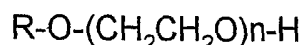
Best mode for carrying out the Invention**15 Surfactant**

A surfactant as used herein may be an anionic, cationic, or non-ionic surfactant. Preferably, the surfactant is a non-ionic surfactant, an anionic surfactant, or a mixture thereof. The non-ionic surfactant is the second widely used surfactant after anionic surfactant in the detergent industry and is a substance having a non-ionic hydrophilic group such as hydroxyl (OH) and carboxyl (RCOOR') groups. According to the shapes of a hydrophilic group, the non-ionic surfactant is classified as a polyethylene glycol type and a polyalcohol type. While the polyalcohol type surfactant is not used as a cleaning agent and a penetrating agent, the polyethylene glycol type surfactant is often used as a cleaning agent and a penetrating agent.

The polyethylene glycol type surfactant is prepared by adding hydrophilic ethylene oxide (CH₂CH₂O) to a hydrophobic raw material having a reactive hydrogen atom. As used herein, the term, reactive hydrogen atom denotes a hydrogen atom present in hydroxyl (OH), carboxyl (COOH), amine (NH₂), or amide (CONH₂) groups. Examples of the polyethylene glycol type surfactant to be used herein include higher alcohol-, alkyl phenyl-, fatty acid-, higher fatty acid-, aliphatic

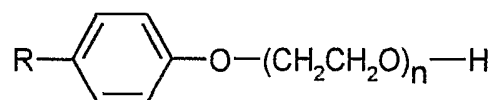
amide-, and alkanol amide-based polyethylene glycols. The general formulae of these compounds are as follows:

Higher alcohol-based polyethylene glycol:



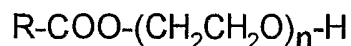
5 wherein, R is an alkyl group of C₈-C₁₆ and n is an integer of 5 to 25.

Alkylphenyl-based polyethylene glycol:



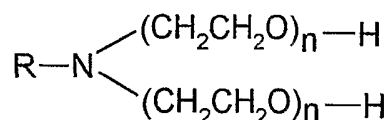
10 25. wherein, R is an alkyl group of C₈-C₁₆ and n is an integer of 5 to 25.

Fatty acid-based polyethylene glycol:



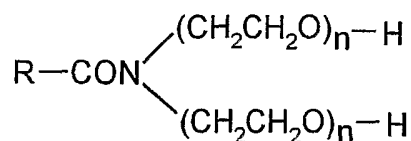
wherein, R is an alkyl group of C₁₀-C₁₈ and n is an integer of 1 to 3.

15 High aliphatic amine-based polyethylene glycol:



wherein, R is an alkyl group of C₁₀-C₁₈ and n is an integer of 1 to 3.

Aliphatic amide-based polyethylene glycol:



20 wherein, R is an alkyl group of C₁₀-C₁₈ and n is an integer of 1 to 3.

Alkanol amide-based polyethylene glycol:



25 wherein, R₁ is an alkyl group of C₁₂-C₂₀ and R₂ is an alkyl group of C₁-C₂.

The anionic surfactant may be alpha olefin sulfonate or its salt.

The alpha olefin surfactant has good biodegradability in natural state relative to a currently widely used anionic surfactant, straight alkyl benzene sulfonate and has excellent detergency and low raw material cost relative to alkyl sulfonate or alkylether sulfonate. Therefore, the alpha olefin sulfonate is the most widely used substance for a laundry detergent, together with the straight alkyl benzene sulfonate. The alpha olefin sulfonate includes long-chain alkene sulfonate($\text{RCH}=\text{CHCH}_2\text{SO}_3^-$) and its salt, long-chain hydroxyalkane sulfonate($\text{RCHOHCH}_2\text{CH}_2\text{SO}_3^-$) or its salt or a mixture of alkene sulfonate and hydroxyalkane sulfonate such as a mixture of alkene sodium sulfonate($\text{RCH}=\text{CHCH}_2\text{SO}_3\text{Na}$) and sodium hydroxyalkane sulfonate($\text{RCHOHCH}_2\text{CH}_2\text{SO}_3\text{Na}$). Preferred alpha olefin sulfonate contains from 10 to 18 carbon atoms in the R alkyl group. Preferably, the above mixture contains alkene sodium sulfonate and sodium hydroxyalkane sulfonate in a weight ratio of 7:3.

The anionic surfactant may also be straight alkyl benzene sulfonate, $\text{RC}_6\text{H}_4\text{SO}_3\text{M}$ wherein R is a linear alkyl group of $\text{C}_{12}\text{-C}_{18}$, preferably $\text{C}_{12}\text{-C}_{14}$, and M is a cation such as sodium, magnesium, ammonium, and substituted ammonium.

In addition, the anionic surfactant may be alkylsulfate, RSO_4M , alkylether sulfate, REnSO_4M wherein R is an alkyl group of $\text{C}_{12}\text{-C}_{20}$, E is ethylene oxide, $\text{CH}_2\text{CH}_2\text{O}$, n is an integer of 0 to 5, and M is a cation such as sodium, magnesium, ammonium, and substituted ammonium, and fatty acid soap, RCOOM wherein R is $\text{C}_{12}\text{-C}_{20}$ and M is a cation such as sodium, magnesium, ammonium, and substituted ammonium.

A mixture of the anionic and non-ionic surfactants may also be used.

Preferably, the surfactant is added in an amount of 1 to 60% by weight. If the content of the surfactant is less than 1% by weight, the function of detergency is poor, while if it exceeds 60% by weight, it is difficult to manufacture and maintain a liquid formulation.

Enzyme inhibitor

Generally, boron compounds such as boric acid, boronic acid, borate, and boric oxide are widely used as reversible protease inhibitors.

By way of examples, *Molecular & Cellular Biochemistry*, 1983, 51, pp. 5-32 discloses that boronic acid inhibits a subtilisin-based serine protease. In particular, *Biochem. Biophys. Res. Com.*, 1991, 176, pp. 401-405 discloses that boronic acid with an aromatic group such as phenyl, 4-methoxyphenyl, and 3,5-dichlorophenyl has stronger protease inhibitory activity than boronic acid with an alkyl group such as methyl, butyl, and 2-cyclohexylethyl. In addition, WO 92/19707 discloses aryl boronic acid as a reversible subtilisin protease inhibitor and EP 0 478 050 A1 discloses a boron compound as a storage stabilizer of lipase, which inhibits a subtilisin protease. In particular, 3,5-dichlorophenyl boronic acid is an excellent subtilisin protease inhibitor.

Enzyme inhibitory activity is conventionally represented as the K_i , the dissociation constant for binding of an inhibitor to an enzyme. If the K_i is low, the inhibitor has strong inhibitory activity. Therefore, the K_i is closely related with enzyme storage stability. However, the relationship between the K_i and the enzyme storage stability are not always directly proportional.

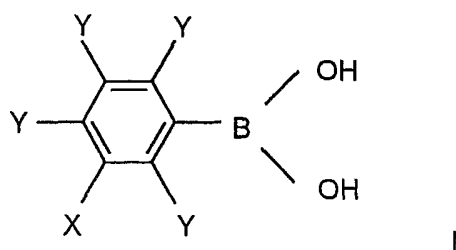
It is known that these boron compounds including boronic acid bind to an active site of protease to inhibit protease activity. These boron compounds inhibit activity of a protease, in particular serine protease, by covalent bonding with a serine group of the active site of the serine protease and by hydrogen bonding with a histidine group of the active site of the serine protease. In particular, it is known that a boron compound with an aryl group such as phenyl boronic acid has excellent serine protease inhibitory activity due to its strong covalent bonding with a serine group of the active site of the serine protease.

A liquid detergent composition of the present invention comprises 0.001 to 1.0% by weight of aryl boronic acid as an enzyme inhibitor and 0.1 to 20% by weight of one or more other enzyme inhibitor selected from the group consisting of formic acid, boric acid, and a salt thereof. That is, the enzyme inhibitor as used herein is the combination of the aryl boronic acid and one or more selected from the formic acid, the boric acid, and the salt thereof. Therefore, a synergistic enzyme stabilization

effect can be accomplished.

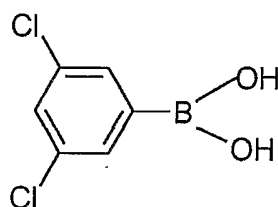
The aryl boronic acid may be that as represented by Formula I:

Formula I



5 wherein, X and Y are independently hydrogen, C₁-C₆ alkyl, aryl, substituted C₁-C₆ alkyl, substituted aryl, hydroxyl, hydroxyl derivative, amine, C₁-C₆ alkylated amine, amine derivative, halogen, nitro, thiol, thiol derivative, aldehyde, acid and its salt, ester, sulfonate, or phosphonate. Examples of the aryl boronic acid of Formula 1 include 3-nitrophenyl
10 boronic acid, phenyl boronic acid, and 3,5-dichlorophenyl boronic acid. Preferably, 3,5-dichlorophenyl boronic acid as represented by Formula 2 is used.

Formula II



15 Preferably, the aryl boronic acid is added in an amount of 0.001 to 1.0% by weight. If the content of the aryl boronic acid is less than 0.001% by weight, enzyme inhibitory activity is too insignificant to accomplish a desired enzyme inhibitory effect. On the other hand, if it
20 exceeds 1.0% by weight, an enzyme inhibitory efficiency relative to an added amount is lowered and increase of a raw material cost by excess use lowers an economical efficiency.

25 Preferably, one or more other type of enzyme inhibitor selected from the group consisting of formic acid, boric acid, and a salt thereof is added in an amount of 0.1 to 20.0% by weight, more preferably 0.5 to 10% by weight. If the content of the enzyme inhibitor is less than 0.1%

by weight, it is difficult to obtain enzyme inhibitory activity, while if it exceeds 20.0% by weight, it is difficult to maintain a liquid formulation due to insufficient dissolution.

Protease

5 The liquid detergent composition of the present invention comprises 0.1 to 5.0% by weight of an alkaline protease, and preferably, 0.1 to 4.0% by weight of an active protease. The active protease can be derived from animals, plants, or microorganisms. More preferably, the active protease is a commercially available subtilisin protease such
10 as alcalase, esperase, savinase, maxatase, maxacal, and maxapem, which is protein engineered Maxacal. Alcalase, esperase, and savinase are the trade names of Novo (Denmark) and maxatase, maxacal, and maxapem are the trade names of Genenco (Italy). In addition, an extracellular alkaline protease from alkalophilic *Vibrio metschnikovii* strain
15 RH530 (accession number KCTC 0088BP) isolated from Korean soil may be used.

Preferably, as used herein, the protease is added in an amount of 0.1 to 5.0% by weight. If the content of the protease is less than 0.1% by weight, detergency is lowered, while if it exceeds 5.0% by weight, the
20 function of detergency relative to an added amount is lowered. Furthermore, use of excess protease increases the added amount of the enzyme inhibitor and stabilizer therefore, thereby increasing a manufacturing cost. Therefore, use of excess protease is not preferable from an economical point of view.

Enzyme stabilizer

25 The liquid detergent composition of the present invention may further comprise various ingredients, except for the above-described surfactant and enzyme inhibitors such as the aryl boronic acid, formic acid, and boric acid. For example, the liquid detergent composition of
30 the present invention may comprise 1 to 30% by weight, preferably 5 to 30% by weight of an enzyme stabilizer. Generally, in the case of enzymes used in a liquid detergent composition, enzyme activities decrease by denaturation or autodigestion with time. The enzyme

stabilizer is used to structurally prevent the denaturation and autodigestion of enzymes and thus retard the decrease of enzyme activities. A representative enzyme stabilizer is propylene glycol, ethylene glycol, and polyoxyethylene glycol. The combination of propylene glycol and ethylene glycol by an appropriate ratio was used in the Examples of the present invention below.

Solubilizer

The liquid enzyme composition of the present invention may further comprise a solubilizer. The solubilizer may be a short chain alcohol such as ethanol, ethylene glycol, and propylene glycol, or hydrotrope such as sodium cumene sulfonate, sodium xylene sulfonate, and urea. Preferably, the solubilizer is added in an amount of 1 to 30% by weight.

Builder

The liquid detergent composition of the present invention may further comprise a builder. The builder is an important cleaning ingredient for a laundry detergent. Generally, the term, builder is a generic name for an alkalizing agent and a metal ion sequestrant. The builder may cause problems in terms of formulation and stability when used in a liquid detergent composition, and thus, the amount used is limited. The builder to be used herein is sodium carbonate, an alkalizing agent with excellent solubility, for example, sodium hydroxide, potassium hydroxide, triethanolamine, diethanolamine, and monoethanolamine. The builder may be added in an amount of 5% by weight or less, preferably 1 to 3% by weight to thereby adjust pH of the composition to a desired level.

Other than the above-described ingredients, the liquid detergent composition of the present invention may further comprise additives as conventionally used in the pertinent art. Examples of the additives include a hard water softening agent, a fluorescent whitening agent, and other types of enzymes. The hard water softening agent may be trisodium nitriloacetate, tetrasodium ethylenediamine tetraacetate (EDTA 4Na), disodium ethylenediamine tetraacetate (EDTA 2Na), or sodium

citrate. The liquid detergent composition of the present invention may further comprise a second detergent-compatible enzyme such as amylase and cellulase as a starch degrading enzyme, lipase as a lipid degrading enzyme, oxidoreductase, and a mixture thereof, except for the
5 above-described protease.

Preparation procedure

The liquid detergent composition of the present invention can be prepared as follows, for example.

First, surfactant bases are prepared and then enzyme inhibitors
10 such as sodium formate, and a boron compound including boric acid, and aryl boronic acid with varying concentrations and combination ratios are added to the surfactant bases. Next, alkalizing agents such as triethanolamine are added to adjust pH of a prepared mixture to alkaline and then proteases are added.

15 When an adjustment of the viscosity of the obtained mixture is required, short chain alcohols or hydrotropes as used in conventional liquid detergent compositions may be added. Herein, the short chain alcohols are those having carbon atoms of 1 to 5, such as ethyl alcohol, propylene glycol, isopropanol, and butanol. The hydrotropes are
20 substances generally used for viscosity adjustment or stability maintenance of detergent compositions, for example, sodium cumene sulfonate, xylene sulfonate, and urea.

Preferably, the final pH of the liquid detergent composition of the present invention is in the range of 8.5 to 11.0. If the pH is less than 8.5,
25 detergency is lowered, while if the pH exceeds 11.0, enzyme stability is not secured.

Examples

Hereinafter, the present invention will be described more specifically by examples. However, the following examples are provided
30 only for illustrations and thus the present invention is not limited to or by them. The alpha olefin sulfonate used in the Examples hereinafter was a mixture of alkene sodium sulfonate and hydroxyalkane sodium sulfonate which was commercially available.

Comparative Example 1: Preparation of liquid detergent base of the present invention and enzyme storage stability test with varying enzyme inhibitors

5 In this Example, liquid detergent compositions were prepared using compositional ratios as presented in Tables 1 and 2 according to the method disclosed in the above section "Preparation procedure" and then enzyme storage stabilities were tested.

1. Enzyme storage stability test

10 Enzyme storage stability was determined according to the azocasein method (Novo). Test samples of the prepared liquid detergent compositions were stored in an incubator at 40°C for 4 weeks and then the protease activities of the samples were measured at an interval of one week. In this case, initial measurement values were set to 100.

15 Particularly, the test procedure was as follows:

First, a predetermined amount of azocasein was dissolved in urea (50%) solution, 2M Tris (Tris(hydroxymethyl)aminomethane) buffer solution, and deionized water (D.I. water) to prepare an azocasein solution. Then, after aliquots of the test samples and the azocasein
20 solution were incubated in a 40°C water bath for 30 minutes, Absorbance was measured at 390 nm using a UV spectrometer. A protein used for protease activity measurement was azocasein (Sigma). Unless particularly specified otherwise, protease used in Examples was subtilisin protease with its optimal pH of 8 to 11.

25

Table 1: Type 1 of detergent compositions with varying enzyme inhibitors

Ingredients	Sample 1	Sample 2	Sample 3	Sample 4
Polyoxyethylene alkyl ether (EO=7 mole)	30.0	30.0	30.0	30.0
Straight alkylbenzene sulfonate	6.0	6.0	6.0	6.0
Alpha olefin sulfonate	3.0	3.0	3.0	3.0
Alkyl ether sulfate	5.0	5.0	5.0	5.0
Ethylene glycol	5.0	5.0	5.0	5.0
Propylene glycol	20.0	20.0	20.0	20.0
Ethyl alcohol	5.0	5.0	5.0	5.0
Sodium xylene sulfonate	2.0	2.0	2.0	2.0
Monoethanolamine	4.0	4.0	4.0	4.0
EDTA 4Na	0.2	0.2	0.2	0.2
3-Nitrophenyl boronic acid	0.1			
Phenyl boronic acid		0.1		
3,5-Dichlorophenyl boronic acid			0.1	
Protease	0.6	0.6	0.6	0.6
D.I. water	Balance*	Balance	Balance	Balance
Total	100	100	100	100

Unit: % by weight

*Balance means the amount of D.I. water to make up 100% and is used as the same meaning in the following Tables.

Table 2: Type 2 of detergent compositions with varying enzyme inhibitors

Ingredients	Sample 5	Sample 6	Sample 7	Sample 8
Polyoxyethylene alkyl ether (EO = 9 mole)	35.0	35.0	35.0	35.0
Propylene glycol	25.0	25.0	25.0	25.0
Ethyl alcohol	5.0	5.0	5.0	5.0
Sodium xylene sulfonate	2.0	2.0	2.0	2.0
Monoethanolamine	4.0	4.0	4.0	4.0
EDTA 4Na	0.2	0.2	0.2	0.2
3-Nitrophenyl boronic acid	0.1			
Phenyl boronic acid		0.1		
3,5-Dichlorophenyl boronic acid			0.1	
Protease	0.6	0.6	0.6	0.6
D.I. water	Balance	Balance	Balance	Balance
Total	100	100	100	100

Unit: % by weight

Table 3: Enzyme activities at high temperature with time

Sample	Initial	1 week	2 weeks	3 weeks	4 weeks
Sample 1	100	79	71	58	39
Sample 2	100	81	67	55	36
Sample 3	100	73	65	55	39
Sample 4	100	70	59	42	30
Sample 5	100	76	66	51	39
Sample 6	100	74	66	56	38
Sample 7	100	72	63	49	40
Sample 8	100	70	55	40	26

5

From the results as shown in Table 3, it can be seen that boronic acid-containing detergent compositions have excellent enzyme storage stabilities, when compared to boronic acid-free detergent compositions. However, after 4 weeks, protease activities of all the detergent compositions were reduced to less than 50%.

10

Comparative Example 2: Preparation of liquid detergent base of the present invention and enzyme storage stability test with varying

enzyme inhibitors

In this Example, liquid detergent compositions were prepared using compositional ratios as presented in Table 4 according to the method disclosed in the above section "Preparation procedure" and then
 5 enzyme storage stabilities were tested. Enzyme storage stabilities were determined in the same manner as in Comparative Example 1.

Table 4: Type 3 of detergent compositions with varying enzyme inhibitors

Ingredients	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13
Polyoxyethylene alkyl ether (EO=9mole)	26.0	21.0	35.0	28.0	30.0
Propylene glycol	10.0	5.0	25.0	17.0	21.0
Ethyl alcohol	5.0	3.0	5.0	3.7	3.0
Sodium xylene sulfonate	2.0	0.0	2.0	2.0	2.0
Monoethanolamine	3.3	4.0	3.0	3.0	3.0
EDTA 4Na	0.2	0.2	0.2	0.2	0.2
Boric acid	0.6	1.2			0.6
Sodium formate			1.0	1.5	1.0
Calcium chloride	0.07	0.07	0.07	0.07	0.07
Protease	0.6	0.6	0.6	0.6	0.6
D.I. water	Balance	Balance	Balance	Balance	Balance
Total	100	100	100	100	100

Unit: % by weight

Table 5: Enzyme activities at high temperature with time

Sample	Initial	1 week	2 weeks	3 weeks	4 weeks
Sample 9	100	76	56	37	29
Sample 10	100	70	49	32	20
Sample 11	100	73	53	35	24
Sample 12	100	73	50	36	30
Sample 13	100	75	61	42	33

5 As shown in Table 5, all the detergent compositions of samples 9 through 13 had lower enzyme storage stabilities than the boronic acid-containing detergent compositions of Table 3. Furthermore, there were little differences between the enzyme stabilities of all the detergent compositions of samples 9 through 13 and the boronic acid-free
10 detergent compositions of Table 3.

Example 1: Enzyme storage stability test with varying enzyme inhibitors

15 In this Example, enzyme storage stabilities of detergent compositions with combination of aryl boronic acid and other types of protease inhibitors were determined.

 For this, detergent compositions with combination of aryl boronic acid and boric acid or sodium formate were prepared according to compositional ratios of Tables 6 and 7 and then enzyme storage stabilities were determined at a high temperature.

20

Table 6: Detergent compositions with varying concentrations of
boric acid

Ingredients	Sample 14	Sample 15	Sample 16	Sample 17
Polyoxyethylene alkyl ether (EO=7 mole)	30.0	30.0	30.0	30.0
Straight alkylbenzene sulfonate	6.0	6.0	6.0	6.0
Alpha olefin sulfonate	3.0	3.0	3.0	3.0
Alkyl ether sulfate	5.0	5.0	5.0	5.0
Ethylene glycol	5.0	5.0	5.0	5.0
Propylene glycol	20.0	20.0	20.0	20.0
Ethyl alcohol	5.0	5.0	5.0	5.0
Sodium xylene sulfonate	2.0	2.0	2.0	2.0
Monoethanolamine	4.0	4.0	4.0	4.0
EDTA 4Na	0.2	0.2	0.2	0.2
3-Nitrophenyl boronic acid	0.1	0.1	0.1	0.1
Boric acid	0.5	1.0	1.5	2.0
Protease	0.9	0.9	0.9	0.9
D.I. water	Balance	Balance	Balance	Balance
Total	100	100	100	100

Unit: % by weight

Table 7: Detergent compositions with varying concentrations of sodium formate

Ingredients	Sample 18	Sample 19	Sample 20	Sample 21
Polyoxyethylene alkyl ether (EO=7 mole)	30.0	30.0	30.0	30.0
Straight alkylbenzene sulfonate	6.0	6.0	6.0	6.0
Alpha olefin sulfonate	3.0	3.0	3.0	3.0
Alkyl ether sulfate	5.0	5.0	5.0	5.0
Ethylene glycol	5.0	5.0	5.0	5.0
Propylene glycol	20.0	20.0	20.0	20.0
Ethyl alcohol	5.0	5.0	5.0	5.0
Sodium xylene sulfonate	2.0	2.0	2.0	2.0
Monoethanolamine	4.0	4.0	4.0	4.0
EDTA 4Na	0.2	0.2	0.2	0.2
Phenyl boronic acid	0.1	0.1	0.1	0.1
Sodium formate	0.5	1.0	1.5	2.0
Protease	0.9	0.9	0.9	0.9
D.I. water	Balance	Balance	Balance	Balance
Total	100	100	100	100

Unit: % by weight

5 Table 8: Enzyme activities at high temperature with time

Sample	Initial	1 week	2 weeks	3 weeks	4 weeks
Sample 14	100	80	72	67	52
Sample 15	100	83	75	67	52
Sample 16	100	85	77	71	55
Sample 17	100	90	77	69	59
Sample 18	100	85	80	70	50
Sample 19	100	90	84	65	53
Sample 20	100	93	87	70	53
Sample 21	100	91	85	71	52

From the results as shown in Table 8, it can be seen that the detergent compositions with combination of boronic acid and boric acid

or sodium formate have excellent enzyme storage stabilities, when compared to the compositions of Tables 3 and 5.

Example 2: Enzyme storage stability test with varying combinations of enzyme inhibitors

- 5 Detergent compositions containing all of the boronic acid, sodium formate, and boric acid as enzyme inhibitors were prepared according to the compositional ratios of Tables 9 and 10 and then enzyme storage stabilities were determined at a high temperature.

10 Table 9: Detergent compositions containing all of boronic acid, sodium formate, and boric acid, as enzyme inhibitors

Ingredients	Sample 22	Sample 23	Sample 24
Polyoxyethylene alkyl ether (EO=7 mole)	30.0	30.0	30.0
Straight alkylbenzene sulfonate	6.0	6.0	6.0
Alpha olefin sulfonate	3.0	3.0	3.0
Alkyl ether sulfate	5.0	5.0	5.0
Ethylene glycol	5.0	5.0	5.0
Propylene glycol	20.0	20.0	20.0
Ethyl alcohol	5.0	5.0	5.0
Sodium xylene sulfonate	2.0	2.0	2.0
Monoethanolamine	4.0	4.0	4.0
EDTA 4Na	0.2	0.2	0.2
Phenyl boronic acid	0.2	0.2	0.2
Boric acid	0.5	0.5	0.5
Sodium formate	1.0	1.5	2.0
Protease	0.9	0.9	0.9
D.I. water	Balance	Balance	Balance
Total	100	100	100

Unit: % by weight

Table 10: Detergent compositions containing all of boronic acid, sodium formate, and boric acid, as enzyme inhibitors

Ingredients	Sample 25	Sample 26	Sample 27	Sample 28
Polyoxyethylene alkyl ether (EO=7 mole)	30.0	30.0	30.0	30.0
Straight alkylbenzene sulfonate	6.0	6.0	6.0	6.0
Alpha olefin sulfonate	3.0	3.0	3.0	3.0
Alkyl ether sulfate	5.0	5.0	5.0	5.0
Ethylene glycol	5.0	5.0	5.0	5.0
Propylene glycol	20.0	20.0	20.0	20.0
Ethyl alcohol	5.0	5.0	5.0	5.0
Sodium xylene sulfonate	2.0	2.0	2.0	2.0
Monoethanolamine	4.0	4.0	4.0	4.0
EDTA 4Na	0.2	0.2	0.2	0.2
Phenyl boronic acid	0.2	0.2	0.2	0.2
Boric acid	0.3	0.6	0.9	1.2
Sodium formate	1.0	1.0	1.0	1.0
Protease	0.9	0.9	0.9	0.9
D.I. water	Balance	Balance	Balance	Balance
Total	100	100	100	100

Unit: % by weight

5 Table 11: Enzyme activities at high temperature with time

Sample	Initial	1 week	2 weeks	3 weeks	4 weeks
Sample 22	100	90	85	73	59
Sample 23	100	92	90	77	63
Sample 24	100	95	90	81	67
Sample 25	100	85	81	70	60
Sample 26	100	95	85	78	65
Sample 27	100	94	86	75	65
Sample 28	100	96	90	79	69

As shown in Table 11, detergent compositions with all of boronic acid, boric acid, and sodium formate, as enzyme inhibitors, maintained

average 60% or more of enzyme activities after 4 weeks, which are excellent results when compared to the above-obtained test results.

5 Example 3: Enzyme storage stability test with increase of boronic acid

Detergent compositions with increase of boronic acid were prepared according to the compositional ratio of Table 12 and then enzyme storage stabilities were determined at a high temperature.

10 Table 12: Detergent compositions with increase of boronic acid

Ingredients	Sample 29	Sample 30	Sample 31	Sample 32
Polyoxyethylene alkyl ether (EO=7 mole)	5.0	5.0	5.0	5.0
Polyoxyethylene alkyl ether (EO=9 mole)	13.0	13.0	13.0	13.0
Polyoxyethylene alkylphenyl ether (EO=9 mole)	2.0	2.0	2.0	2.0
Straight alkylbenzene sulfonate	6.0	6.0	6.0	6.0
Alpha olefin sulfonate	5.0	5.0	5.0	5.0
Alkyl ether sulfate	5.0	5.0	5.0	5.0
Ethylene glycol	5.0	5.0	5.0	5.0
Propylene glycol	20.0	20.0	20.0	20.0
Ethyl alcohol	5.0	5.0	5.0	5.0
Sodium xylene sulfonate	2.0	2.0	2.0	2.0
Monoethanolamine	4.0	4.0	4.0	4.0
EDTA 4Na	0.2	0.2	0.2	0.2
3-Nitrophenyl boronic acid	0.5	1.0		
Phenyl boronic acid			0.5	1.0
Boric acid	0.6	0.6	0.6	0.6
Sodium formate	1.3	1.3	1.3	1.3
Protease	0.9	0.9	0.9	0.9
D.I. water	Balance	Balance	Balance	Balance
Total	100	100	100	100

Unit: % by weight

Table 13: Enzyme activities at high temperature after 4 weeks

Sample	Initial	4 weeks
Sample 29	100	65
Sample 30	100	68
Sample 31	100	70
Sample 32	100	68

As shown in Table 13, enzyme storage stabilities were slightly changed depending on the concentration of boronic acid within a specific concentration range of boronic acid.

5 Example 4: Type 1 of enzyme storage stability test with varying types and contents of surfactants

10 Detergent compositions with varying types and contents of surfactants were prepared according to the compositional ratios of Table 14 and then enzyme storage stabilities were determined at a high temperature.

Table 14: Type 1 of detergent compositions with varying types
and contents of surfactants

Ingredients	Sample 33	Sample 34	Sample 35	Sample 36
Polyoxyethylene alkyl ether (EO=7 mole)				15.0
Polyoxyethylene alkyl ether (EO=9 mole)	13.0		7.0	
Polyoxyethylene alkylphenyl ether (EO=7 mole)	3.0			
Polyoxyethylene alkylphenyl ether (EO=9 mole)				20.0
Straight alkylbenzene sulfonate	15.0	20.0		2.0
Alpha olefin sulfonate	5.0	10.0	10.0	
Alkyl ether sulfate	5.0	10.0	10.0	
Alkyl sulfate	1.0			
Ethylene glycol	5.0	5.0	5.0	3.0
Propylene glycol	20.0	20.0	20.0	18.0
Ethyl alcohol	5.0	5.0	5.0	3.0
Sodium xylene sulfonate	2.0	2.0	2.0	
Monoethanolamine	4.0	3.0	3.0	4.0
Sodium carbonate		1.0	1.0	
Phenyl boronic acid	0.2	0.2	0.2	0.2
Boric acid	0.8	0.8	0.8	0.8
Sodium formate	1.3	1.3	1.3	1.3
Protease	0.9	0.9	0.9	0.9
D.I. water	Balance	Balance	Balance	Balance
Total	100	100	100	100

Unit: % by weight

Table 15: Enzyme activities at high temperature with time

Sample	Initial	1 week	2 weeks	3 weeks	4 weeks
Sample 33	100	93	82	77	69
Sample 34	100	85	76	70	63
Sample 35	100	80	71	65	60
Sample 36	100	90	85	75	66

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As shown in Table 15, detergent compositions with anionic surfactants exhibited slightly low enzyme activities, when compared to those with mainly non-ionic surfactants. However, all of the detergent compositions exhibited 60% or more of enzyme activities after 4 weeks.

10

Example 5: Type 2 of enzyme storage stability test with varying types and contents of surfactants

Detergent compositions with varying types and contents of surfactants were prepared according to the compositional ratios of Table 16 and then enzyme storage stabilities were determined at a high temperature.

Table 16: Type 2 of detergent compositions with varying types
and contents of surfactants

Ingredients	Sample 37	Sample 38	Sample 39	Sample 40
Polyoxyethylene alkyl ether (EO=9 mole)	13.0		7.0	27.0
Straight alkylbenzene sulfonate	20.0			
Alpha olefin sulfonate	5.0	10.0		
Alkyl ether sulfate	5.0	10.0	10.0	
Alkyl sulfate	1.0			
Ethylene glycol	5.0	5.0		
Propylene glycol	20.0	12.0	13.0	13.0
Ethyl alcohol	5.0	3.0		
Sodium xylene sulfonate	2.0	1.0	2.0	2.0
Monoethanolamine	4.0	3.0	3.0	3.0
Sodium carbonate		1.0		
Phenyl boronic acid	0.1	0.1	0.1	0.1
Boric acid	0.8	0.8	0.8	0.8
Sodium formate	1.3	1.3	1.3	1.3
Protease*	0.9	0.9	0.9	0.9
D.I. water	Balance	Balance	Balance	Balance
Total	100	100	100	100

Unit: % by weight

*Proteases: an extracellular alkaline protease from alkalophilic *Vibrio metschnikovii* strain RH530 (accession number KCTC 0088BP) isolated from Korean soil

Table 17: Enzyme activities at high temperature with time

Sample	Initial	1 week	2 weeks	3 weeks	4 weeks
Sample 37	100	92	85	75	65
Sample 38	100	83	72	63	59
Sample 39	100	83	71	60	53
Sample 40	100	94	87	70	63

5

As shown in Table 17, as the content of water decreases, enzyme stabilities increase, and vice versa. However, detergent compositions with low water contents exhibited still considerable enzyme stabilities.

10

Industrial Applicability

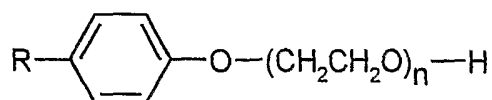
As apparent from the above description, the liquid detergent composition of the present invention has considerably improved enzyme storage stability by combination of aryl boronic acid with formic acid
5 and/or boric acid.

What is claimed is:

1. An alkaline liquid detergent composition comprising: 1 to 60% by weight of a surfactant, 0.001 to 1.0% by weight of aryl boronic acid as an enzyme inhibitor, 0.1 to 20% by weight of one or more other
 5 enzyme inhibitor selected from the group consisting of formic acid, boric acid, and a salt thereof, 0.1 to 5.0% by weight of a protease, and the balance to 100% by weight being water.

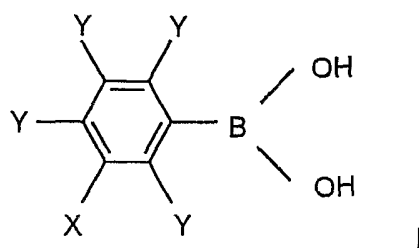
2. The liquid detergent composition according to claim 1,
 10 wherein the surfactant is a non-ionic surfactant, an anionic surfactant, or a mixture thereof.

3. The liquid detergent composition according to claim 2, wherein the non-ionic surfactant is polyoxyethylene alkyl ether,
 15 $R-O-(CH_2CH_2O)_n-H$ wherein R is an alkyl group of C_8-C_{16} and n is an integer of 5 to 25; or polyoxyethylene alkylphenyl ether,



wherein R is an alkyl group of C_8-C_{16} and n is an integer of 5 to 25; and the anionic surfactant is alpha olefin sulfonate; straight alkyl benzene sulfonate, $RC_6H_4SO_3M$ wherein R is a linear alkyl group of $C_{12}-C_{18}$ and M is a cation including sodium, magnesium, ammonium, and substituted ammonium; alkylsulfate, RSO_4M wherein R is an alkyl group of $C_{12}-C_{20}$ and M is a cation including sodium, magnesium, ammonium, and substituted ammonium; or
 20 alkylether sulfate, $REnSO_4M$ wherein R is an alkyl group of $C_{12}-C_{20}$, E is ethylene oxide, CH_2CH_2O , n is an integer of 0 to 5, and M is a cation including sodium, magnesium, ammonium, and substituted ammonium.

4. The liquid detergent composition according to claim 1, wherein the aryl boronic acid is that as represented by Formula I:



wherein, X and Y are independently hydrogen, C₁-C₆ alkyl, aryl, substituted C₁-C₆ alkyl, substituted aryl, hydroxyl, hydroxyl derivative, amine, C₁-C₆ alkylated amine, amine derivative, halogen, nitro, thiol, thiol
 5 derivative, aldehyde, acid and its salt, ester, sulfonate, or phosphonate.

5. The liquid detergent composition according to claim 4, wherein the aryl boronic acid is 3-nitrophenyl boronic acid, phenyl boronic acid, or 3,5-dichlorophenyl boronic acid.

10

6. The liquid detergent composition according to claim 1, wherein the protease is subtilisin protease, an extracellular alkaline protease from alkalophilic *Vibrio metschnikovii* strain RH530 under deposition number KCTC 0088BP, alcalaseTM, esperaseTM, savinaseTM,
 15 maxataseTM, maxacalTM, or maxapemTM, which is protein engineered Maxacal.

7. The liquid detergent composition according to claim 1, wherein the liquid detergent composition has pH of 8.5 to 11.0.

20

8. The liquid detergent composition according to claim 1, further comprising a second detergent-compatible enzyme selected from the group consisting of amylase, lipase, cellulase, oxidoreductase, and a mixture thereof; an enzyme stabilizer selected from the group consisting
 25 of propylene glycol, ethylene glycol, and polyoxyethylene glycol; an enzyme solubilizer selected from the group consisting of ethanol, ethylene glycol, propylene glycol, sodium cumene sulfonate, sodium xylene sulfonate, and urea; and a builder selected from the group

consisting of sodium carbonate, sodium hydroxide, potassium hydroxide, triethanolamine, diethanolamine, and monoethanolamine.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR03/01434

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C11D 3/386

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C11D, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patent and applicants for inventions since 1975. Korean Utility Models and applicants for inventions since 1975.
Japan Utility models and applicants for inventions since 1975.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
NPS, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO96/41859 A (NOVO NORDISK A/S) 27. Dec. 1996 See the whole document	1 - 8
Y	WO92/19707 A (The Procter & Gamble Company) 12.Nov.1992 See the whole document	1 - 8
Y	US 4842769 A (Colgate-Palmolive,.) 27. Jun. 1989 See the whole document	1 - 8
Y	US 5472628 A (The Procter & Gamble Company) 5. Dec. 1995 See the whole document	1 - 8

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR03/01434

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
W09219707A1	12.11.1992	AU2014892A	21.12.1992
		CA2109526A1	31.10.1992
		CN1067265A	23.12.1992
		HU65823A2	28.07.1994
		MX9202069A1	01.11.1992
		NZ242537A	27.06.1995
		PT100446A	31.08.1993
		TR26055A	15.12.1994
		W09219707A1	12.11.1992