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(54) **Enzymatic detergent composition.**

(57) The invention relates to a detergent composition on the basis of nonionic detergents, which includes a mixture of certain lipolytic enzymes and proteases. In this composition, the lipases remain unaffected by the proteases.

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ENZYMATIC DETERGENT COMPOSITION

The present invention relates to an enzymatic detergent composition comprising as detergent active material solely a nonionic synthetic detergent, and a mixture of lipolytic and proteolytic enzymes.

5

Proteolytic enzymes are well known in detergent compositions. Lipolytic enzymes have been mentioned in the prior art as possible enzymes for detergent compositions, but there is relatively little prior art
10 directly concerned with lipases for inclusion in detergent compositions.

Insofar the compatibility of proteolytic and lipolytic enzymes with synthetic detergents is concerned, it is
15 well known that nonionic synthetic detergents in general do not negatively influence the activity of proteolytic and lipolytic enzymes, in contrast with e.g. anionic synthetic detergents, which often negatively influence the proteases and lipases.

20

However, since lipases are basically proteins, they would be susceptible to proteolytic action by the proteases if lipases were used in conjunction with proteases. It would therefore be expected that the
25 inclusion of proteases in a detergent composition comprising lipases and as detergent-active material solely a nonionic detergent surfactant (which does not affect either the lipase or the protease), would cause the lipase activity to be destroyed by the proteolytic
30 action of the proteases.

We have now surprisingly found that when using such compositions comprising a special class of lipases, hereinafter more specifically defined, this expected
35 loss of lipolytic activity does not occur, but that,

the activity of these lipases remains almost unaffected by the presence of the proteases in these compositions.

- 5 The class of lipases to be used according to the present invention embraces those lipases which show a positive immunological cross-reaction with the antibody of the lipase produced by the microorganism Chromobacter viscosum var. lipolyticum NRRL B 3673.
- 10 This lipase has been described in Dutch Patent Specification 154,269 of Toyo Jozo KK, and the microorganism is available to the public from the collection of the United States Department of Agriculture, Agricultural Research Service, Northern
- 15 Utilization and Development Division, Peoria, Illinois, under N° NRRL B 3673. This lipase will be referred to hereinafter as "Toyo Jozo" lipase. The lipases of the present invention should show a positive immunological cross-reaction with the Toyo Jozo lipase antibody,
- 20 using the standard and well-known immunodiffusion procedure according to Ouchterlony (Acta. Med. Scan., 133, pages 76-79 (1950)).

The preparation of the antiserum is carried out as follows:

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

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

The serum containing the required antibody is prepared

by centrifugation of clotted blood, taken on day 67.

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

10

All lipases showing a positive immunological cross-reaction with the Toyo Jozo lipase antibody as hereabove described are lipases according to the present invention. Typical examples thereof are the
15 lipase ex Pseudomonas fluorescens IAM 1057 (available under the trade name Amano-P), the lipase ex Pseudomonas fragi FERM P 1339 (available under the trade name Amano-B), lipase ex Pseudomonas nitroreducens var. lipolyticum FERM P 1338, the lipase
20 ex Pseudomonas sp. available under the trade name Amano-CES, lipase ex Pseudomonas cepacia, lipases ex Chromobacter viscosum, e.g. Chromobacter viscosum var. lipolyticum NRRL B 3673, commercially available from Toyo Jozo Co., Tagata, Japan; and further C. viscosum
25 lipases from US Biochemical Corp., U.S.A., and Diosynth Co., The Netherlands, and lipases ex Pseudomonas gladioli.

30 The lipases of the present invention are included in the detergent composition in such an amount that the final detergent composition has a lipolytic enzyme activity of from 100 to 0.005 LU/mg, preferably 25 to 0.05 LU/mg of the composition.

35 A Lipase Unit (LU) is that amount of lipase which produces 1 μ mol of titratable fatty acid per minute in a pH stat. under the following conditions: temperature 30°C; pH = 9.0; substrate is an emulsion of

3.3 wt.% of olive oil and 3.3% gum arabic, in the presence of 13 mmol/l Ca^{2+} and 20 mmol/l NaCl in 5 mmol/l Tris-buffer.

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

10

The proteases used according to the present invention are the well-known detergent proteases and mixtures thereof. Typical examples are the subtilisin type proteases, such as Alcalase, Esperase, Savinase, all
15 available from Novo Industri A/S, Maxatase and Maxacal, both available from Gist Brocades N.V., Kazuzase ex Showa Denko. (= API-21 = AP-1)

In general, the proteases are used in such an amount
20 that the final detergent composition has a proteolytic activity of 0.1 to 50 GU/mg, preferably 0.5 to 30 GU/mg. (GU = Glycine Unit; one GU is the amount of enzyme which under standard incubation conditions produces an amount of terminal NH_2 -groups equivalent
25 to 1 microgr./ml glycine.)

The detergent composition further comprises as detergent-active material solely a nonionic synthetic detergent. This type of detergent is well known in the
30 art. Nonionics usually consist of a hydrophobic moiety derived from fatty alcohols, fatty acid amides and alkylphenols, and a hydrophilic moiety consisting of alkylene oxide units. Typical examples are the condensation products of alkylene oxides such as
35 ethylene oxide, propylene oxide and butylene oxide and mixtures thereof with C_8 - C_{18} primary or secondary, branched or straight chain alcohols, C_8 - C_{18} fatty

acid amides, C₉-C₁₈ alkylphenols, etc. Further suitable examples are given in M. Schick "Nonionic surfactants" (1967).

- 5 In general, the composition contains from 1-50%, usually from 2-45%, and commonly from 5-30% by weight of the nonionic detergent. Mixtures of various nonionic detergents may also be used.
- 10 The detergent composition may furthermore include the usual detergent ingredients in the usual amounts. Thus, the composition may contain from 1-60%, preferably from 5-30% by weight of one or more organic and/or inorganic builders. Furthermore, it may contain from 1-35% of a
- 15 bleaching agent or a bleaching system comprising a bleaching agent and an activator therefor, e.g. sodium perborate with tetraacetyl ethylene diamine.

- The compositions may furthermore comprise lather
- 20 boosters, foam depressors, anti-corrosion agents, soil-suspending agents, sequestering agents, anti-soil redeposition agents, perfumes, dyes, solvents, stabilising agents for the enzymes and bleaching agents and so on. They may also comprise from 0.01-10% by
- 25 weight of enzymes other than lipases and proteases, such as amylases, oxidases and cellulases.

- The compositions of the present invention can be formulated in any desired form, such as powders, bars,
- 30 pastes, liquids, etc.

The invention will now further be illustrated by way of Examples.

Example 1

Washing experiments were carried out in a Tergotometer under the following conditions:

- 5 wash time and temperature: 10 minutes at 40°C;
 three rinses with cold water
 water hardness: 14 and 40°FH
 detergent composition concentration: 2 and 6 g/l
 number soil/wash cycles : 4
 10 protease concentration: 20 GU/ml
 lipase concentration: 1 LU/ml

As lipases were used: Amano-P, Diosynth and a lipase ex
Pseudomonas gladioli

- 15 test cloth: cotton] soiled with AS 8 /
 polyester/cotton] groundnut oil /
 polyester] milk powder

- The detergent composition used had the following
 20 formulation:

	<u>% by weight</u>
C ₁₂ -C ₁₄ linear primary alcohol condensed with 7 moles of ethylene oxide	9
sodium silicate	8
25 sodium carbonate	39
sodium sulphate	38
fluorescers, dyes, perfumes	0.3
water	q.s.

- 30 The reflectance of the test cloths and the residual
 weight percentage of fatty material on the test cloths
 were determined after the fourth wash cycle. The
 reflectance was measured in a Reflectometer at 460 nm
 with a UV filter in the light pathway, and the fatty
 35 matter by extracting the dried test cloths with
 petroleum ether, and determining the amount of fatty
 matter from the weight loss of the test cloth.

The following results were obtained:

		<u>Savin-</u>			<u>Cotton</u>	<u>PE/Cotton</u>	<u>Polyester</u>			
	<u>lipase</u>	<u>ase</u>	<u>g/l</u>	<u>°FH</u>	<u>R 460</u>	<u>% FM</u>	<u>R 460</u>	<u>% FM</u>	<u>R 460</u>	<u>% FM</u>
5										
	Amano-P	-	2	14	84.5	3.9	72.6	2.6	79.8	0.8
		+	2	14	87.1	3.6	75.4	2.4	80.5	0.7
		-	6	40	81.5	3.7	71.3	2.4	79.5	0.7
		+	6	40	86.5	3.0	75.4	1.9	81.7	0.4
10										
	Gladioli	-	2	14	81.1	4.6	68.5	4.5	74.5	4.3
		+	2	14	86.5	3.6	72.3	3.8	76.2	4.0
		-	6	40	76.6	4.7	68.0	3.7	75.9	3.4
		+	6	40	83.8	3.6	73.3	2.8	79.1	3.0
15										
	Diosynth	-	2	14	83.2	4.0	71.3	3.3	75.6	3.5
		+	2	14	86.8	3.6	74.3	2.8	77.2	3.0
		-	6	40	79.8	3.7	70.4	2.8	76.9	2.7
		+	6	40	85.4	2.9	74.7	2.1	79.8	2.1
20										
	Cepacia	-	2	14	83.8	4.1	71.2	3.4	78.3	2.8
		+	2	14	86.9	3.6	75.2	2.9	79.4	2.3
		-	6	40	79.6	4.1	70.0	3.2	78.8	1.9
		+	6	40	85.9	3.2	74.9	2.2	81.5	1.0

Example 2

The procedure of Example 1 was repeated, but under the following conditions:

- 5 detergent composition concentration: 2 g/l
 number of soil/wash cycles : 4
 wash time and temperature: 10 minutes at 40°C, three
 rinses with cold water
 water hardness: 14°FH
 10 agitation: 100 rpm
 lipase: ex Pseudomonas gladioli 1 LU/ml
 protease: 20 GU/ml

As proteases, Savinase, Alcalase, Maxatase, Maxacal, Kazuzase and Esperase were used.

15

The following results were obtained:

Protease	without lipase		with lipase	
	R460	% FM	R460	% FM
20 Savinase	69.3	5.6	87.2	2.8
Alcalase	69.3	5.4	87.2	2.8
Maxatase	70.0	5.3	87.5	2.8
Maxacal	68.9	5.7	87.1	2.8
25 Kazuzase	69.1	5.7	87.1	2.7
Esperase	68.6	5.7	87.1	2.7

Example 3

The stability of lipases according to the present invention in the presence of proteases and varying amounts of a nonionic detergent was tested by determining the residual lipase activity with the pH-stat. method. A composition comprising 0.7 g pentasodium triphosphate, 0.7 g sodium sulphate and 0.2 sodium silicate and varying amounts of C₁₃-C₁₅ alcohol, condensed with 7 and 3 moles of ethylene oxide, respectively (product A and product B) was used.

The temperature was 30°C, the water hardness 14°FH and the pH 9.5.

The lipase was either lipase ex Pseudomonas gladioli, or Amano-P, at 15 LU/ml, and the protease was Savinase, at 20 GU/ml.

The following results were obtained:

lipase	Product A			Product B		
	g/l non	% RA 10 min.	% RA 30 min.	g/l non	% RA 10 min.	% RA 30 min.
<u>Pseudomonas gladioli</u>	0	75	34	0	69	29
	0.25	98	103	0.25	98	100
	1.0	103	103	1.0	98	105
	2.0	99	102	2.0	95	90
	4.0	98	98	4.0	110	108
Amano-P	0	100	74			
	0.25	120	116			
	1.0	104	101			
	2.0	101	101			
	4.0	108	107			

% RA = % residual activity of the lipase

Example 4

Example 1 was repeated, using various lipases. The following results were obtained:

5

	<u>lipase</u>	<u>Savin-</u>		<u>°FH</u>	<u>Cotton</u>		<u>PE/Cotton</u>		<u>Polyester</u>	
		<u>ase</u>	<u>g/l</u>		<u>R 460</u>	<u>% FM</u>	<u>R 460</u>	<u>% FM</u>	<u>R 460</u>	<u>% FM</u>
10	No	-	2	14	72.1	5.9	60.6	6.6	67.9	4.7
		+	2	14	72.0	6.1	60.0	6.6	67.1	4.8
		-	6	40	71.8	5.3	59.7	5.4	69.9	3.5
		+	6	40	74.6	5.2	62.8	5.2	72.2	3.4
15	lipase MY	-	2	14	69.2	6.0	57.8	6.4	66.1	4.8
	(ex Cand.	+	2	14	72.7	5.9	60.1	6.5	67.1	5.0
	cyl.)	-	6	40	70.1	5.6	59.8	5.3	69.4	3.4
		+	6	40	73.7	5.3	62.4	5.4	69.6	3.7
20	lip. AP-6	-	2	14	69.0	6.0	57.4	6.5	66.4	4.8
	(ex Asp.	+	2	14	72.1	6.0	59.5	6.6	67.3	5.0
	niger)	-	6	40	71.3	5.5	59.5	5.4	69.8	3.5
		+	6	40	75.1	5.4	61.6	5.4	71.7	3.6
25	lip. SP285	-	2	14	77.8	4.5	65.8	4.1	70.2	3.6
	(ex	+	2	14	82.0	4.2	69.5	3.7	71.3	3.6
	Fusarium	-	6	40	74.8	4.8	64.1	4.4	71.8	3.2
	oxysporum)	+	6	40	75.0	5.0	64.8	4.4	72.0	3.4
30	lip. SP225	-	2	14	74.9	4.4	64.5	4.2	76.3	2.8
	(ex Mucor	+	2	14	79.3	3.5	67.4	3.8	76.6	2.5
	miehei)	-	6	40	71.7	4.9	61.3	4.4	75.5	1.7
		+	6	40	72.3	4.8	62.8	4.4	76.7	1.9
35	Toyo Jozo	-	2	14	86.2	2.7	71.3	2.4	78.6	1.7
	(lip. ex	+	2	14	85.8	2.7	71.9	2.4	78.2	1.7
	Chromobac.	-	6	40	84.9	2.8	69.9	1.8	77.4	1.4
	viscosum)	+	6	40	85.2	2.7	72.5	1.9	77.8	1.5

		<u>Savin-</u>			<u>Cotton</u>		<u>PE/Cotton</u>		<u>Polyester</u>	
	<u>lipase</u>	<u>ase</u>	<u>g/l</u>	<u>°FH</u>	<u>R 460</u>	<u>% FM</u>	<u>R 460</u>	<u>% FM</u>	<u>R 460</u>	<u>% FM</u>
5	Nagase	-	2	14	85.0	3.2	69.0	2.6	77.1	1.7
	(ex	+	2	14	86.4	2.4	71.9	2.3	78.3	1.1
	Rhizopus)	-	6	40	71.7	5.1	61.7	4.5	76.0	1.5
		+	6	40	72.6	5.2	62.1	4.3	76.1	1.8
10	C-4 (ex	-	2	14	72.1	5.5	60.8	5.3	74.4	3.4
	Candida	+	2	14	70.8	5.2	61.2	5.4	73.5	3.4
	cylin-	-	6	40	72.1	5.1	61.4	4.6	75.4	1.7
	dracea)	+	6	40	73.2	5.3	62.4	4.6	75.4	2.0
15	pancreas	-	2	14	72.8	5.2	62.4	5.2	74.5	3.5
	lipase	+	2	14	73.2	5.2	62.8	5.1	74.3	3.4
		-	6	40	72.9	5.3	62.3	4.6	75.9	1.7
		+	6	40	72.7	5.2	63.1	4.5	76.4	1.8
20	Esterase	-	2	14	72.6	5.3	61.9	5.2	75.3	3.3
	MM (ex	+	2	14	71.9	5.2	62.7	4.9	75.4	3.2
	Mucor	-	6	40	72.0	5.3	61.4	4.5	76.3	1.6
	miehei)	+	6	40	73.3	5.3	63.0	4.4	76.1	1.7
25	Amano-B	-	2	14	84.1	3.0	71.3	2.2	78.5	0.8
		+	2	14	85.8	3.1	71.5	2.7	79.2	0.9
		-	6	40	85.2	3.1	71.3	1.9	78.4	0.3
		+	6	40	86.3	3.0	72.8	1.9	79.4	0.2
30	Amano-CES	-	2	14	80.1	2.8	71.0	1.9	79.0	0.4
		+	2	14	81.4	2.9	73.0	1.8	79.7	0.4
		-	6	40	80.0	3.0	71.3	1.6	78.9	0.6
		+	6	40	82.0	3.0	73.3	1.7	79.0	0.8
35	Amano-CE	-	2	14	72.1	5.1	64.1	4.5	75.4	3.3
		+	2	14	72.7	5.1	66.6	4.1	75.8	3.3
		-	6	40	74.9	4.3	67.5	3.0	77.0	2.2
		+	6	40	77.5	4.0	69.0	2.9	77.2	2.4

	<u>lipase</u>	<u>Savin- ase</u>	<u>g/l</u>	<u>°FH</u>	<u>Cotton</u> R 460 % FM	<u>PE/Cotton</u> R 460 % FM	<u>Polyester</u> R 460 % FM			
5	lipase	-	2	14	68.9	5.8	61.8	5.2	74.7	3.5
	M-AP10	+	2	14	70.7	5.8	63.1	5.2	75.2	3.6
	(ex Mucor	-	6	40	71.4	5.1	66.3	3.7	76.6	2.3
	javanicus)	+	6	40	74.2	4.5	66.2	4.1	75.9	2.5
10	lipase	-	2	14	73.9	4.7	64.8	4.2	76.2	3.1
	F-AP (ex	+	2	14	72.1	5.2	65.5	4.7	75.7	3.4
	Rhizopus	-	6	40	74.7	4.6	69.4	2.4	77.1	1.9
	javanicus)	+	6	40	74.6	4.9	68.1	3.3	77.3	2.2
15	S80.000	-	2	14	72.1	5.1	66.0	4.3	76.1	3.1
	(ex	+	2	14	74.6	4.8	66.5	4.4	76.4	3.3
	Rhizopus	-	6	40	75.6	4.5	69.1	2.4	77.2	2.0
	arrhizus)	+	6	40	78.8	4.5	71.1	2.5	78.0	1.9
20	PS-54 (ex	-	2	14	76.0	4.3	67.0	3.6	75.3	3.0
	Thermom.	+	2	14	77.1	4.4	68.9	3.7	75.6	2.9
	lanu-	-	6	40	78.1	4.3	69.5	2.1	76.7	2.0
	ginosus)	+	6	40	79.6	4.2	72.1	2.4	78.1	1.8
25	ex Pseudo.	-	2	14	79.6	3.2	70.3	2.0	78.4	0.4
	gladioli	+	2	14	81.1	3.0	72.1	2.1	79.9	0.4
		-	6	40	79.6	3.5	69.6	1.9	77.8	0.1
		+	6	40	81.0	3.4	71.7	1.8	79.7	0.2
30	lipase ex	-	2	14	77.3	4.2	67.4	3.7	74.9	3.3
	Rhizopus	+	2	14	79.7	4.0	68.8	3.5	75.9	3.0
	sp.	-	6	40	77.4	3.7	68.1	2.4	77.8	1.7
		+	6	40	80.7	4.1	71.0	2.3	78.9	1.8

CLAIMS

1. A detergent composition comprising from 1-50%
by weight of one or more nonionic detergent surfactants
as the sole detergent surfactant, proteases in an
amount such that the detergent composition has a
5 proteolytic activity of 0.1-50 Glycine Units per
milligram, and lipases in an amount such that the
detergent composition has a lipolytic activity of
0.005-100 Lipase Units per milligram, the lipases being
those which show a positive immunological cross-
10 reaction with the antibody of the lipase produced by
Chromobacter viscosum var. lipolyticum NRRL-B 3673.

2. A composition according to claim 1, wherein
the lipase is obtained from Pseudomonas fluorescens,
15 Pseudomonas fragi, Pseudomonas nitroreducens var.
lipolyticum, Pseudomonas cepacia, Pseudomonas gladioli
and Chromobacter viscosum.
