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⑤④ **Enzymatic dishwashing and rinsing composition.**

⑤⑦ The inclusion of lipases in machine dishwashing and rinsing compositions provides for a significant reduction of the spot and film formation on the dishware. Particularly the use of bacterial lipases, which show a positive immunological cross-reaction with the lipase, produced by Chromobacter viscosum var. lipolyticum NRRL B 3673 provides for such significant reduction of spot and film formation.

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ENZYMATIC DISHWASHING AND RINSING COMPOSITION

The present invention relates to an enzymatic dishwashing or rinsing composition comprising lipolytic enzymes.

- 5 Enzymatic dishwashing compositions have been proposed in the art. As enzymes, mainly amylases and/or proteases have been proposed for inclusion in dishwashing compositions. Lipases have also been suggested, but have received far less attention than
- 10 the amylases and/or proteases. Dishwashing compositions, in particular machine dishwashing compositions which are used in the main wash step of a machine dishwashing operation, have in general a satisfactory cleaning performance. However, frequently
- 15 the articles cleaned with such products still do not have a satisfactory visual appearance after rinsing and drying, showing film or spots. Some main wash liquor is usually carried over from the main wash step to the rinse step, causing some deposition of soil resulting
- 20 in insoluble calcium salts on the articles to be rinsed, which results in visible film or spots on the articles when they are dry. In particular with glass articles, this causes an unsightly visual appearance.
- 25 We have now found that the addition of lipases to a main wash dishwashing composition or to a rinse composition significantly reduced the formation of film or spots on the articles cleaned or rinsed with such a composition. The lipases, used according to the present
- 30 invention, may be of any suitable origin such as yeasts, fungi and bacteria. Preferably, however, they are of bacterial origin, and particularly preferably they belong to the class of bacterial lipases which show a positive immunological cross-reaction with the
- 35 antibody of the lipase, produced by the microorganism

Chromobacter viscosum var. lipolyticum NRRL B-3673.

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

The preparation of the antiserum is carried out as follows:

20 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:
25 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
30 adjuvant.

The serum containing the required antibody is prepared by centrifugation of clotted blood, taken on day 67.

35 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.

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

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
25 activity of from 100 to 0.005 LU/mg preferably 25 to
0.05 LU/mg of the composition.

A Lipase Unit (LU) is that amount of lipase which
produces 1 μ mol of titratable fatty acid per minute
30 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.

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Naturally, mixtures of the above lipases can be used.
The lipases can be used in their nonpurified form, or

in a purified form, e.g. purified with the aid of well-known adsorption methods, such as a phenylsepharose-adsorption techniques.

5 The composition of the invention may furthermore comprise the usual ingredients of dishwashing or rinse compositions. Thus it may contain one or more alkali salts commonly used in dishwashing compositions. Thus, it may contain organic and/or inorganic builders such
10 as the alkali metal ortho-, pyro and tripolyphosphates and hexametaphosphates, silicates, carbonates, zeolites, borates, citrates, carboxymethyloxy-succinates, nitrilotriacetates and ethylenediamine-tetraacetates, polymeric polyelectrolytes such as
15 polyacrylates, polymaleates, and other known organic and inorganic builder compounds.

Usually, the amount of builders in the composition varies from 10-90% by weight, generally from 30-70% by
20 weight.

The composition may also contain a detergent-active compound. If a detergent-active compound is included, it usually is in an amount of from 0.5-10%, usually 1-
25 5%. Any well-known type of detergent active compound may be used, such as soaps, synthetic anionic, non-ionic, amphoteric detergent surfactant and mixtures thereof. Preferably, a nonionic detergent surfactant is used, especially a low-foaming one. Suitable examples
30 of such nonionic detergent surfactants can easily be found in M.Schick "Nonionic Surfactants" (1967).

The composition may furthermore contain other useful additives such as bleaching agents, bleaching agent
35 activators, hydrotropes, fillers, perfumes, colouring agents, germicides, soil-suspending agents, aminopolyphosphonic acids and alkali metal or alkaline earth

metal salts thereof, clays such as hectorites, anti-corrosion agents such as fatty acids, benztriazole and so on. Other enzymes such as proteases, amylases, oxidases may also be included.

5

As bleaching agents the peroxygen type bleaching agents, preferably with a bleach precursor such as TAED are suitable for inclusion in the machine dishwashing compositions.

10

A typical example of a conventional machine dishwashing composition usually contains an alkali metal tripolyphosphate in an amount of from 20-60%, an alkali metal silicate in an amount of from 40-80%, or an alkali metal disilicate in an amount of 5-30% by weight a peroxy type bleaching agent in an amount of from 1-15%, a low-foaming detergent surfactant in an amount of from 0.5-5%, and minor ingredients such as perfumes, colouring agents, hydrotropes, fillers, etc.

20

When formulated as a rinse composition, it may contain from 0.5-10% by weight of a nonionic surfactant, from 5-25% by weight of an inorganic or organic acid, from 1-5% by weight of a hydrotrope, dyes, germicides, clays, silica sols, etc., the balance being water. The products of the invention can be formulated in any desirable form, such as powders, granulates, cakes, bars, pastes, liquids etc. The invention will further be illustrated by way of example.

30

Example 1

In a commercial dishwashing machine tests were carried out under the following conditions:

detergent composition dosage: 30 g
 lipase dosage : 2.5 g (= 15 LU/ml)
 water hardness : main wash 27° GH
 flood 9° GH
 5 rinse 1 9° GH
 rinse 2 9° GH
 final rinse 9° GH
 wash temperature and time : 55°C - 60 minutes
 articles to be cleaned : clean dishes
 10 clean glasses
 test soil : 35 g egg-yolk, added at
 the beginning of the main
 wash.

15 The detergent composition had the following
 formulation:

	in gr.
sodium tripolyphosphate	11.61
sodium carbonate	2.7
20 TAED (59.7%)	1.81
sodium perborate	2.43
calcium-Dequest (47.6%)	0.397
sodium disilicate	3.3
low foaming nonionic	0.405
25 (Plurafac RA40)	
hectorite clay (Laponite XLS)	0.675
sodium sulphate	5.610
amylase (4.8 Maltose units/mg)	0.540

30 As lipase, the Toyo Jozo lipase was used.

The test was carried out in two runs, one without and
 one with lipase.

35 The glasses were appraised by an expert panel as to
 their visual appearance, and were given a score
 according to the following scale:

	<u>Film</u>	<u>Spots</u>
	1. no film	1. no spots
	2. slight film	2. 1-5 spots
	3. clear film	3. 5-10 spots
5	4. heavy film	4. 11-20 spots
		5. more than 20 spots

The following results were obtained:

10	run 1 (no lipase)	run 2 (with lipases)
	average film score : 2.3	2.3
	average number of spots : 250	100

Example 2

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

wash temperature : 65°C

- 20 soil : standard soil; at the end of the main wash, clean plates were put in the machine, so that no cleaning took place in the final rinse, but only a carry-over took place.
- 25 run 1 : no lipase added in the final rinse
- run 2 : 2.5 g Toyo Jozo lipase was added to the final rinse (15 LU/ml).

The main wash composition had the following

30 formulation:

		% by weight
	sodium tripolyphosphate	36
	sodium carbonate	9
	sodium disilicate	15
5	TAED	3.8
	sodium perborate tetrahydrate	8.8
	potassium metaborate	0.35
	sodium metaborate	0.85
	calcium-Dequest	0.63
10	Amylase (4.8 MU/mg)	1.8
	Esperase (a protease; 675 GU/mg)	1.8
	low-foaming nonionic (Plurafac RA 40)	1.35
	hectorite (Laponite XLS)	2.25
	benzotriazole	0.1
15	sodium sulphate	14.0
	water	9.5

The following spot and film scores of the glasses were obtained:

20

run 1 (no lipase)	run 2 (with lipases)
average film score : 1	1
average spot score : 5	2.7

25 Example 3

Replacing the Toyo Jozo lipase by the Amano-P lipase or Lipase MY (ex Candida cylindracea) or Amano AP-6 lipase (ex Aspergillus niger) in Example 2 gave similar

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Example 4

The following formulation is an example of a rinse aid

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composition to which lipases can be added:

	% by weight
low-foaming nonionic (Plurafac RA 30)	10
citric acid	18
sodium xylene sulphonate	3.0
5 dye	0.003
butylated hydroxytoluene	0.1
water	q.s.

CLAIMS

1. An enzymatic dishwashing or rinsing composition comprising from 0.5-10% by weight of a detergent surfactant and from 0-90% by weight of a builder, characterised in that it further comprises
5 from 0.005-100 LU/mg of a lipolytic enzyme.
2. A composition according to claim 1, characterised in that the lipase is a bacterial lipase which shows a positive immunological cross-reaction
10 with the antibody of the lipase, produced by Chromobacter viscosum var. lipolyticum NRRL B 3673.
3. A composition according to claim 2, characterised in that the lipase is producible by the
15 microorganism selected from the group consisting of Pseudomonas fluorescens, Pseudomonas fragi, Pseudomonas nitroreducens var. lipolyticum, Pseudomonas cepacia, Pseudomonas gladioli and Chromobacter viscosum.
- 20 4. A dishwashing composition according to claims 1-3, characterised in that it contains from 20-60% by weight of an alkali metal tripolyphosphate, from 40-80% by weight of an alkali metal silicate, from 1-15% by weight of a peroxy type bleaching agent and from 0.5-5%
25 by weight of a low-foaming nonionic detergent.
5. A rinsing composition according to claims 1-3, characterised in that it contains, in an aqueous medium, from 0.5-10% by weight of a nonionic
30 surfactant, from 5-25% by weight of an organic or inorganic acid, and from 1-5% by weight of a hydrotrope.
