(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 271 156**
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: **87202396.5**

(51) Int. Cl.⁴: **C11D 3/386**

(22) Date of filing: **02.12.87**

(30) Priority: **10.12.86 GB 8629537**

(43) Date of publication of application:
15.06.88 Bulletin 88/24

(84) Designated Contracting States:
CH DE ES FR GB IT LI NL SE

(71) Applicant: **UNILEVER NV**
Burgemeester s'Jacobplein 1 P.O. Box 760
NL-3000 DK Rotterdam(NL)

(84) **CH DE ES FR IT LI NL SE**

(71) Applicant: **UNILEVER PLC**
Unilever House Blackfriars P.O. Box 68
London EC4P 4BQ(GB)

(84) **GB**

(72) Inventor: **Van Dijk, Willem Robert**
Roerdompstraat 72
NL-3291 VM Strijen(NL)

(74) Representative: **Van Gent, Jan Paulus et al**
Unilever N.V. Patent Division P.O. Box 137
NL-3130 AC Vlaardingen(NL)

(54) **Enzymatic dishwashing composition.**

(57) The present invention relates to an enzymatic dishwashing composition comprising proteases and lipases. The lipases belong to a special class, i.e. those which show a positive immunological cross-reaction with the antibody of the lipase, produced by Chromobacter viscosum var. lipolyticum NRRL B 3683.

The use of the proteases and the lipases together provides for a synergistic cleaning effect of the dishwashing composition.

EP 0 271 156 A2

ENZYMATIC DISHWASHING COMPOSITION

The present invention relates to an enzymatic dishwashing composition comprising proteases and a special class of lipases.

The use of enzymes in dishwashing compositions, both for manual as well as mechanical dishwashing is generally well-known in the art. For that purpose, in particular amylases and/or proteases have been proposed.

Although lipases as a general class of enzymes have also been suggested, no specific proposals relating to the use of specific lipases in dishwashing compositions have been made as far as we know.

In dishwashing, the removal of egg-yolk, particularly dried-up or baked-on egg-yolk from the surface of the dishware is often a problem, and the satisfactory removal of this type of soil often requires special measures or special dishwashing compositions.

We have now surprisingly found that the inclusion of proteases and a special class of lipases in dishwashing compositions provide for a satisfactory removal of egg-yolk from the dishware. This removal is significantly superior to the removal, obtained with either the proteases or the lipases, and the combination of these two types of enzymes in fact produces a synergistic cleaning effect in this respect.

The proteases which can be used in the present invention can be any type of protease known for inclusion in detergent compositions. Most commercially available proteases are of the subtilisin type, and suitable examples of such proteases are Alcalase,

Esperase and Savinase, sold by NOVO Industri; Maxatase and Maxacal, sold by Gist Brocades; Optimase sold by Kali Chemie; Kazusase, sold by Showa Denka. Preferred are the so-called high alkaline proteases such as Savinase. Mixtures of various proteases can also be used. In general, the dishwashing compositions of the invention contain the proteases in such an amount, that the final composition has a proteolytic activity of 0.1 - 50, usually 1 - 40 and preferably 5 -30 GU/mg. A GU is a glycin unit, which is the amount of enzyme which under standard incubation conditions produces an amount of terminal NH_2-groups equivalent to 1 microgramme/ml glycin.

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. This lipase has been described in Dutch Patent Specification 154,269 of Toyo Jozo KK, and the 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 lipase will hereinafter be referred to as "Toyo Jozo" lipase. The 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 (1950)).

The preparation of the antiserum is carried out as follows:

Equal volume 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:

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 adjuvant

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

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 reaction with Toyo Jozo lipase antibody as hereabove described are lipases according to the present invention. Typical examples thereof are the 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 ex Pseudomonas sp., available under the trade name Amano-CES, the 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 Chromobacter viscosum lipases from US Biochemical Corp., U.S.A. and Diosynth Co., The Netherlands, and lipases ex Pseudomonas gladioli.

The lipases of the present invention are included in the dishwashing composition in such an amount that the final dishwashing composition has a lipolytic enzyme 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 µmol 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.

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 phenylsepharose adsorption techniques.

The dishwashing compositions of the present invention may furthermore comprise the usual ingredients of dishwashing compositions. Thus, they may comprise a small amount, in the order of 0.5 - 5 % by weight of a detergent surfactant, e.g. anionic or nonionic surfactants, such as a low or non-foaming nonionic surfactant. Such low or non-foaming nonionic surfactants are well-known in the art, and suitable examples can be found in M. Schick "Nonionic Surfactants" Vol. 1, (1967).

Furthermore, they may comprise organic and/or inorganic builder materials, usually in amounts of from 10 - 80 %, for most practical purposes from 20 - 60 % by weight. Such builder materials include alkali metal polyphosphates, -pyrophosphates, -hexametaphosphates, -orthophosphates, -carbonates, -bicarbonates, -borates,

-silicates; furthermore, alkali metal polycarboxylates and -polyhydroxysulphonates. Additional examples include alkali metal citrates, -nitrilotriacetates, -carboxymethyloxysuccinates, zeolites etc. Polyelectrolytes such as polymaleates and polyacrylates are also suitable examples.

Furthermore, peroxy-type bleaching agents may be included such as alkalimetal perborates, -percarbonates, -persulphates as well as organic peracids and salts thereof. Bleach precursors such as tetraacetylethylenediamine may also be included together with a peroxy type bleaching agents such as sodium perborate.

Buffers, perfumes, dyes, germicides, solvents, foam depressors, clays such as hectorites, corrosion inhibitors anti-tarnishing agents etc. may also be included if required.

Other enzymes such as amylases, cellulases, pectinases, pectin-esterases or oxidases may also be included. The compositions may be formulated in any desired form, such as powders, bars, cakes, blocks, pastes and liquids. The invention will further be illustrated by way of Example.

Example 1

Tests were carried out with an aqueous solution containing the following base formulation:

	g/l
pentasodium triphosphate	1.16
sodium carbonate 0 aq.	0.27
sodium disilicate	0.33
sodium sulphate 0 aq.	0.561

Comparisons were carried out with this formulation without enzymes, with Toyo Jozo lipase (0.25 g/l), with Savinase 6.0 CM (0.023 g/l) or with a mixture of these enzymes.

A set of 8 glass plates (4 x 4 cm) were soiled with 51.5 ± 1.8 mg baked-on egg yolk per glass plate. These were soaked in 1 liter water of 27°GH containing the above amounts of the dishwashing composition for a period of one hour at pH 10.0. After soaking the residual egg-yolk present on the plates was determined by weighing and measuring the weight difference of the soiled plates before and after soaking.

The difference (DW) is expressed as a percentage of the original amount of soil. The tests were repeated three times independently. The following results were obtained:

	DW (in %)		
no enzymes	96.4	95.5	95.2
only lipase	97.1	96.0	95.3
only Savinase	56.7	47.2	51.7
lipase + Savinase	22.3	20.5	22.9

At 35 and 45°C similar results were obtained. Replacing Savinase by Alcalase also produced similar results.

Example 2

Repeating Example 1, but using the Amano-P lipase, gave the following results:

	DW (in %)
no enzymes	97
only lipase	95
only Savinase	52
lipase + Savinase	20

Example 3

Glasses were cleaned in a Kenmore Sears dishwashing machine, using the normal wash programme at 60°C followed by a hot dry. The water hardness was 14.4°FH. The dishwashing composition was dosed in an amount of 30 g, and had the following formulation:

	% by weight
sodium tripolyphosphate	24
soda ash	20
sodium disilicate	11
linear C_{10} alcohol, condensed with 6 moles of ethylene oxide and 24 moles of propylene oxide	2.5
sodium sulphate	27.8
Amylase (4.8 MU/mg)	0.5
protease (Savinase®) (1544 GU/mg)	1.0
Toyo Jozo lipase	15 LU/ml.

The soiling was 6 g egg-yolk.

The glasses were washed consecutively several times, and the film formation was thereafter assessed using a scale of 0 - 5, 0 being no film at all and 5 being very severe film formation. These experiments were also carried out with the same formulation, but without lipase or without Savinase or without lipase and Savinase.

The following results were obtained:

	Number of consecutive washes	Film score
Base powder	3	0.5 *
Base powder + lipase	15	1.7
Base powder + Savinase	15	2.3
Base powder + lipase + Savinase	15	0.3

* severe spot formation occurred.

CLAIMS

1. An enzymatic dishwashing composition comprising from 0.5-5% by weight of a detergent surfactant, from 10-80% by weight of a builder, and proteases in an amount such that the composition has a proteolytic activity of 0.1-50 Glycine Units per milligram, characterised in that it further comprises from 0.005-100 LU/mg of a bacterial lipase which shows a positive immunological cross-reaction with the antibody of the lipase, produced by Chromobacter viscosum var. lipolyticum NRRL B 3673.

2. A composition according to claim 1, characterised in that the lipase is producible by Pseudomonas fluorescens, Pseudomonas fragi, Pseudomonas nitroreducens var. lipolyticum, Pseudomonas cepacia, Pseudomonas gladioli and Chromobacter viscosum.

****