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DETERGENT COMPOSITION CONTAINING PROTEOLYTIC ENZYMES

Christian Barrat, Brussels, Belgium, and Peter Robson, Farnham Common, England, assignors to The Procter & Gamble Company, Cincinnati, Ohio

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U.S. Cl. 252—545 7 Claims

ABSTRACT OF THE DISCLOSURE

A detergent composition containing a combination of surfactants and proteolytic enzymes which are produced by a strain of "*Bacillus alcalophilus*" deposited at the National Collection of Industrial Bacteria at Aberdeen, Scotland under NCIB No. 8772. The surface-active agents can be represented by zwitterionic surface-active agents, semipolar surface-active agents and mixtures thereof, and also by each of these possibilities in combination with nonionic surface-active agents and with certain maximum levels of anionic surface-active agents.

BACKGROUND OF THE INVENTION

The use of proteolytic enzymes in detergent compositions containing particularly anionic detergent actives alone or in admixture with other surface-active agents is old. These compositions have earned in recent years a wide commercial acceptability. The proteolytic enzyme preparations used in these compositions are mostly derived from *Bacillus subtilis* varieties or are structurally and functionally closely related enzymes. The proteolytic preparation which is produced by a *"bacillus alcalophilus"* strain has been known for a long time. Such preparations have found application in fields of technology outside of the detergent industry. Without having a scientifically precise explanation for this, it may well be that, the strong inhibition of the activity of these enzymes by detergent compositions containing anionic synthetic detergents has constituted the basic reason for not being incorporated into current detergent compositions.

It has now been found than an unexpected and unforeseeable increase in performance can be obtained from the use of detergent compositions having a well-defined surfactant system in combination with a very particular species of proteolytic enzymes. In more detail, the principal performance advantage, i.e. the enhanced stain removal during normal washing operations can be attained from the use of detergent compositions containing surface-active agents, enzymes and, if desired, other usual detergent composition ingredients, and which contains as essential ingredients from about 80, preferably about 90, to about 99.999% by weight of a zwitterionic and/or semipolar surface-active agent; and from 0.001 to about 20%, preferably to about 10% by weight of a proteolytic enzyme produced by the *Bacillus alcalophilus* strain No. NCIB 8772. The surface-active system of these unique detergent compositions can also contain a nonionic surface-active agent, whereby the ratio of zwitterionic and/or semipolar surface-active agents to nonionic surface-active agents can contain anionic detergents which would at most represent 70%, preferably less than 50% by weight, calculated on the total amount of surface-active agents present in the detergent composition.

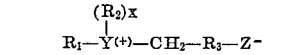
The preparation which is an essential ingredient in the context of the compositions of this invention, is produced by a micro-organism known as "*Bacillus alcalophilus.*" The strain of that micro-organism has been deposited at the National Collection of Industrial Bacteria at Aberdeen,

Scotland, No. NCIB 8772—and also at the National Collection of Type Cultures—No NCTC 4553—and is freely available. The preparation of these proteolytic enzymes from the micro-organism *Bacillus alcalophilus* is described by Dr. A. Vedder in Ned. Tijdschr. V. Hyg. Microbiol. En Serolog., pages 141 to 148 (1934).

The proteolytic preparation preferably is used in an amount from 0.001 to about 10%, most preferably from about 0.01 to about 2% by weight of the detergent composition. It may also be desirable to add optionally other enzyme species for example amylases and lipases. The function of the latters is as for the proteolytic enzymes, that is to aid and augment the soil and stain removal. This is currently attained thru the digesting and solubilizing activity the specific enzyme species exert on specific constituents of the dirt.

All zwitterionic ingredients which are known to be suitable for use in detergent technology fit the definition of zwitterionic detergent for the purpose of this development. The preferred zwitterionic surfactants are:

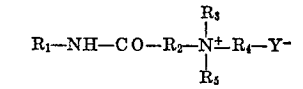
(a) Those corresponding to the general formula



wherein R_1 represents alkyl, alkenyl or a hydroxyalkyl radical with from about 8 to about 18 carbon atoms and containing if desired up to about 10 ethylene oxide moieties and also one glyceryl moiety, Y is selected from the group consisting of nitrogen, phosphorus and sulfur atoms, R_2 is an alkyl or monohydroxy alkyl group containing 1 to 4 carbon atoms, X is 1 when Y is S, 2 when Y is N or P, R_3 is an alkylene or hydroxyalkylene radical containing from 1 to about 4 carbon atoms and Z is a radical selected from the group consisting of carboxylate, sulfonate, sulfate, phosphate and phosphonate. Examples of this class of zwitterionic agents are:

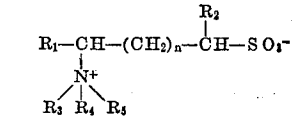
3-(N,N-dimethyl-N-hexadecyl ammonio)propane-1-sulfonate;
3-(N,N-dimethyl-N-hexadecyl ammonio)-2-hydroxy-propane-1-sulfonate;

(b) Those having the general formula:



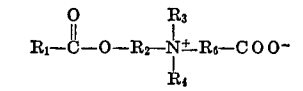
wherein R_1 represents a higher molecular weight alkyl group with 8 to 20 carbon atoms, R_2 an aliphatic bridging group, e.g. a CH_2-group or an amino alkyl group, R_3, R_4 and R_5 lower alkyl groups which contain maximum 4 and preferably maximum 2 carbon atoms, whereby the groups R_3 and R_5 can be connected to form a heretocyclic ring and Y one of the anions —COO^- or —SO_3^-;

(c) Sulfobetaines of the general formula:



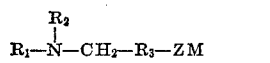
and which are disclosed in South African patent application 69/5,788; and

(d) The betaines of the general formula:



as described in U.S. Pat. 3,265,719;

(e) Those corresponding to the general formula

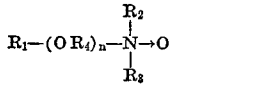


wherein R_1 is alkyl of about 8 to about 18 carbon atoms, R_2 is alkyl of 1 to about 3 carbon atoms or is H, R_3 is alkylene of 1 to about 4 carbon atoms, Z is carboxy, sulfonate, sulfate, phosphate or phosphonate and M is a salt-forming cation. Sodium 3-dodecyl amino propionate is an example of this class of zwitterionics;

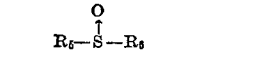
(e) The sodium salt of alkylbenzene-di C_{1-4} alkyl ammonio propane sulfonate; and

(f) The sodium salt of alkylbenzyl di-C_{1-4} alkyl ammonio propane sulfonate.

All semipolar surface-active agents which are currently used in detergent technology may be used within the compositions of this invention. Well-known examples of this class of surfactant ingredients include long chain tertiary amine oxides corresponding to the general formula:



wherein R_1 is an alkyl radical of from about 8 to about 18 carbon atoms, R_2 and R_3 are each methyl, ethyl or hydroxyethyl radicals, R_4 is ethylene, and n ranges from 0 to about 10. The arrow in the formula is a conventional representation of a semipolar bond. Specific examples of amine oxide detergents include dimethyldodecylamine oxide and bis-(2-hydroxyethyl) dodecylamine oxide; long chain tertiary phosphine oxides corresponding to the general formula $RR'R''P\rightarrow O$ wherein R is an alkyl, alkenyl or monohydroxyalkyl radical containing from 10 to 20 carbon atoms and R' and R'' are each alkyl or monohydroxyalkyl groups containing from 1 to 3 carbon atoms. The arrow in the formula is a conventional representation of a semipolar bond. Examples of suitable phosphine oxides are found in U.S. Pat. 3,304,263 and include: dimethyldodecyl-phosphine oxide and dimethyl-(2-hydroxydodecyl) phosphine oxide; and also the long chain sulfoxides having the formula



wherein R_5 is an alkyl radical containing from about 10 to about 28 carbon atoms, from 0 to about 6 ether linkages and from 0 to about 2 hydroxyl substituents, at least one moiety of R_5 being an alkyl radical containing 0 ether linkages and containing from about 10 to about 18 carbon atoms, and wherein R_6 is an alkyl radical containing from 1 to 3 carbon atoms and from one to two hydroxyl groups. Specific examples of these sulfoxides are: dodecyl methyl sulfoxide and 3-hydroxy tridecyl methyl sulfoxide.

The long chain tertiary amine oxides are, for reason of commercial availability, the preferred semipolar surfactants for use in the compositions of this invention.

From an overall detergency point of view, it may be desirable to use a combination of zwitterionic and semipolar surfactants.

Nonionic surface-active agents can be used as optional ingredients in the compositions of this invention. In principle, all nonionic surface ingredients can be used for that purpose. Obviously, the particular consistency of the finished detergent formulation and also the intended use are important deciding factors concerning the qualitative and quantitative choice of the nonionics. In general, however, the preferred nonionic are represented by the condensation products of alkylene oxides, preferably ethylene oxide with organic hydrophobic compounds, preferably fatty alcohols having from 10 to 20 carbon atoms in the alkyl chain. Typical hydrophobic groups include condensation products of propylene oxides with propylene glycol, alkylphenols, condensation products of propylene oxides and ethylene diamine, aliphatic alcohols having from 8 to 22 carbon atoms and amines of fatty acids. Examples of preferred nonionic detergents are represented by ingredients of the general formula AE_n wherein A represents an alkyl chain with from 8 to 30, preferably from 12 to 20 carbon atoms of an alkylphenyl radical with from 3 to 22 carbon atoms in the alkyl chain; E an alkylene oxy radical, preferably ethylene oxide and n an integer from 4 to 20. Particularly preferred are the condensation products of tallow or coconut alcohols with from 4 to 16 moles of ethylene oxide.

Also anionic surface-active agents can be used in the compositions of this invention. Qualitatively, all anionic detergents currently known to be suitable for use in detergent compositions, are compatible without adversely affecting the performance advantages resulting from the normal use of the compositions claimed. Quantitively, these anionic detergents should be restricted, however, as given hereinafter because of the inhibition of the enzymatic activity by the anionic surfactant if it exceeds a certain maximum limit by reference to the total amount of surface-active agents present in the detergent composition. This critical limit for anionic surface-active agents is unexpected indeed.

The compositions of this invention shall contain as essential ingredients from about 80, preferably about 90 to about 99.999% by weight of zwitterionic and/or surface-active agents; and from 0.001 to about 20%, preferably to about 10% by weight of the particular proteolytic enzyme. The preferred numbers of these class of detergents are listed hereinbefore. In the event a mixture of zwitterionic and semipolar detergents is used then the weight ratio of these ingredients shall preferably lie within the range from 4:1 to 1:4. It is also possible to add as optional surface-active ingredient a nonionic detergent. In that event, the weight ratio of zwitterionic and/or semipolar surface-active agent to nonionic surface-active agent shall be from about 4:1 to about 1:4. In another preferred embodiment in the context of this invention, anionic surface-active agents, can be added to the essential ingredients whereby the anionic surfactant shall represent less than 70, preferably less than 50% by weight calculated on the total amount of surface-active agents.

The detergent compositions of this invention can be present in solid, pasty or liquid state whatever is desired. Obviously, depending upon the particularities of the composition and also taking into account its intended use, the usual detergent additives can be added. Their qualitative and quantitative choice is again related to the type of composition and to the planned use. Examples of these detergent additives are detergent builders, peroxy bleach compounds, suds controlling agents (suds boosters and suds depressing agents), optical brighteners, dyes, perfumes, additional enzymes (amylases, lipases) and mixtures thereof.

The detergency builders can be employed in these compositions in an amount of up to 50% by weight calculated on the total detergent composition. Preferably the weight ratio of organic detergent to builders shall be from about 5:1 to about 1:20.

The builders can be inorganic or organic in nature and can be selected from a wide variety of known builder materials. Useful inorganic builders are alkali metal carbonates, phosphates, polyphosphates, and silicates. Specific examples of such salts are sodium and potassium tripolyphosphates, carbonates, phosphates and hexametaphosphates. Useful organic builders are alkali metal, ammonium and substituted ammonium polyphosphonates, polyacetates and polycarboxylates. The polyphosphonates specifically include the sodium and potassium salts of ethylene diphosphonic acid, sodium and potassium salts of ethane-1-hydroxy-1,1-diphosphonic acid and sodium and potassium salts of ethane-1,1,2-triphosphonic acid. Other examples include the water-soluble salts of ethane-2-car-

boxy-1,1-diphosphonic acid, hydroxymethanediphosphonic acid, carbonyldiphosphonic acid, ethane - 1,1,2-triphosphonic acid, ethane-2-hydroxy-1,1,2 - triphosphonic acid, propane-1,1,3,3-tetraphosphonic acid, propane-1,1,2,3-tetraphosphonic acid and propane-1,1,2,3-tetraphosphonic acid.

Polyacetate builder salts useful herein include the sodium, potassium, lithium, ammonium, and substituted ammonium salts of the following acids: ethylene-diaminetriacetic acid, N-(2-hydroxyethyl)-nitrilodiacetic acid, diethylenetriaminepentaacetic acid, 1,2 - diaminocyclo-hexanetetraacetic acid.

Peroxy bleach compounds can be incorporated in an amount of up to 30% by weight of the total detergent composition. All bleaching ingredients which are currently used in detergent compositions may fit within the compositions of this invention. Sodium perborate and sodium percarbonate are preferred because of their commercial availability. Also up to 20% by weight of the detergent composition of activators for peroxy bleach compounds may be added. They serve to take better profit of the oxybleach ingredient at lower temperature. As a rule, they form peracids with the active oxygen of the bleaching compounds; these peracids exert more efficiently and at lower temperature their bleaching activity. Well-known activators are maleic anhydride, phthalic anhydride, tetra-acetylmethylene-diamine, tetra-acetylethylenediamine, triacetylisocyanurate and benzoylimidazole.

The detergent compositions of this invention which do not contain peroxybleach compounds are particularly useful under normal washing conditions in the event their alkalinity is such that the pH of the washing solution containing from 2 to 10 g./l. of the finished detergent composition is at least 9.

Suds controlling agents in an amount of up to 10% of the finished detergent compositions can be added as well. Their amount and nature depend frequently upon the intended usage of the particular detergent composition. As an example, detergent compositions which are to be used for automatic (machine) laundry operations shall contain suds depressors such as for example saturated fatty acids having 16 to 22 carbon atoms or siloxanes.

Relatively minor quantities of other detergent additives such as optical brighteners, dyes, perfumes, and so on are incorporated in levels which normally do not exceed 5% by weight of the total detergent composition. Up to 5% of additional enzyme preparation such as amylases, lipases, or mixtures thereof, can be added as well. Minor quantities of nontoxic organic solvents with a relatively low volatility can be added as well. They aid to enhance the removal of greasy and oily soils. Examples of solvents suitable for use are diphenylethers, diphenylketones and dibutylphthalate.

The performance advantages derivable from the composition as claimed is illustrated by the following series of examples.

EXAMPLE I

The granular detergent composition having the formulation given hereinafter is prepared by conventional spray-drying of a slurry containing all but the spray-drying sensitive ingredients such as perborate which are blended with the granular detergent resulting from the spray-drying operation.

Ingredients:	Parts by weight
Surface active agents	See below.
Sodium tripolyphosphate	32.
Sodium perborate	26.
Silicate solids ratio $Na_2O/SiO=2.4$	6.5.
Carboxymethylcellulose	1.
Sodium sulfate	10.
Enzyme preparation	See below.
Minor ingredients and humidity	Balance to 100.

These detergent compositions are used for preparing laundering solutions with

a 0.7% product concentration;
a water-hardness of 20 grains/U.S. gallon.

Test swatches (cotton) stained with any of the following staining solutions are used for evaluation purposes:

milk-ink; egg-ink;
blood-ink-milk; water-cress.

These swatches are either commercially available, e.g. from EMPA, St. Gallen, Switzerland, or can be prepared by immersing the swatches in the corresponding staining solution, passing them through a hand-wringer, whereafter they are heated for 20 minutes in 70° C. water and dried.

The comparative testing procedure is as follows:

The stained swatches are washed in a "Launder-O-Meter" supplied by Atlas Electric Devices Company, Chicago, Ill., thereby using a heat-up cycle from room temperature to 60° C in 40 minutes. After having reached that latter temperature, the operation is interrupted, the swatches are rinsed, passed through a handwringer and dried for 30 minutes at 50° C.

The stain removal resulting from the washing procedure, as described hereabove, is measured with an EEL Spectrophotometer (Evans Electro-selenium Ltd. U.K.) thereby using filter number 603 for milk-ink, blood-milk-ink, and egg-ink stains and filter number 601 for cress stains. A reference (blank) operation is carried along with each series of tests, whereby, all testing parameters are identical to those given hereinbefore, except that no enzymes are present in the detergent compositions used.

The stain removal results are represented by the sum of the reflectances of the four differently stained swatches whereby the individual reflectance for a single swatch is represented by the reflectance for a single swatch is represented by the reflectance of the test swatch minus the reflectance of the reference swatch.

Additional testing parameters are:

	Surface-active agents	Proteolytic enzyme preparation
I	9 parts by weight linear dodecyl benzene sulfonate sodium salt.	Alcalase.*
II	Idem I	Produced by strain NCIB 8772.

*Proteolytic enzyme preparation sold by Novo Industries, A/S, Copenhagen.

The testing results are as follows:

Composition	Enzymatic* activity	Stain removal
I	10	34
	20	53
	30	65
II	10	10
	20	16
	30	19

*Expressed in mAU=milli-Anson unit/liter of washing solution. See also: Anson, Journ. Gen. Physiol. 22, 79 (1939).

The above data revealed the fairly complete inhibition of the proteolytic enzyme resulting from preparations produced by stain No. NCIB 8772 in the event they are used in current detergent compositions.

EXAMPLE II

The detergent composition of Example I has been used for comparative washing evaluations as described in Example I thereby considering the following additional variations.

	Surface active agents	Proteolytic enzyme preparation
I-A	9 parts by weight; coconut dimethyl amine oxide.	"Alcalase."
I-B	Idem I-A	Produced by strain No. NCIB 8772.
II-A	9 parts by weight: hexadecyl dimethyl ammonio propane sulfonate sodium salt.	"Alcalase."
II-B	Idem II-A	Produced by strain No. NCIB 8772.
III-A	9 parts by weight: C_{12-14} alkyl dimethyl ammonio propanol sulfonate sodium salt.	"Alcalase."
III-B	Idem III-A	Produced by strain No. NCIB 8772.
IV-A	8.5 parts by weight: linear alkyl benzene sulfonate sodium salt.	Produced by strain No. NCIB 8772.
IV-B	8.5 parts by weight: coconut dimethyl ammonio propanol sulfonate sodium salt.	Idem IV-A.
IV-C	4 parts by weight: coconut dimethyl ammonio propanol sulfonate sodium salt; plus 8 parts by weight linear alkyl benzene sulfonate sodium salt.	Do.

The testing results are as follows:

Composition	Enzymatic activity in mAU/l. wash solution	Stain removal
I-A	10	17
	30	44
I-B	10	83
	30	108
II-A	20	35
	40	54
II-B	20	100
	40	119
III-A	10	21
	30	44
III-B	10	82
	30	112
IV-A	20	20
	30	25
IV-B	20	96
	30	110
IV-C	20	68
	30	80

EXAMPLE III

The detergent compositions of Example I having an active system and a qualitative and quantitative enzyme content as referred to hereinafter, have been used for comparative washing-tests as described in Example I.

	Surface active agents	Proteolytic enzyme preparation
I-A	8.5 parts by weight linear alkyl benzene sulfonate sodium salt.	"Alcalase."
I-B	8.5 parts by weight coconut dimethyl ammonio propanol sulfonate sodium salt.	Do.
II-A	Idem I-A	Produced by strain No. NCIB 8772.
II-B	Idem I-B	Do.

The stain removal results are as follows:

Composition	Enzymatic activity in mAU/l. wash solution	Stain Removal
I-A	10	32
	30	65
I-B	10	23
	30	46
II-A	10	7
	30	17
II-B	10	78
	30	113

These data clearly bring forward the unusual enzymatic activity in detergent milieu derived from the enzyme preparations produced by strain No. NCIB 8772 versus what can be obtained from the incorporation of current proteases, i.e. Alcalase in identical compositions and under identical conditions.

The replacement of coconut dimethyl ammonio propanol sulfonate sodium salt by dimethyl hexadecyl ammonio propane sulfonate sodium salt, tallow di-C_{1-4}-alkyl-ammonio-propionate sodium salt, alkylbenzene ammonio propane sulfonate sodium salt, alkylbenzyl di-C_{1-4} alkyl ammonio propane sulfonate sodium salt, or mixtures thereof offers substantially the same performance advantages.

EXAMPLE IV

Comparative washing tests are carried out with the detergent compositions of Example I under the conditions as given there. The surface-active ingredients and the enzymes have been chosen as follows:

	Surface active agents	Proteolytic enzyme preparation
I-A	2 parts by weight coconut dimethyl ammonio propanol sulfonate sodium salt; 6 parts by weight coconut dimethyl amine oxide.	"Alcalase."
I-B	Idem I-A	Produced by strain No. NCIB 8772.
II-A	6 parts by weight condensation products of coconut alcohol with 6 moles of ethylene oxide; 2 parts by weight of coconut dimethyl ammonio propanol sulfonate sodium salt.	"Alcalase."
II-B	Idem II-A	Produced by strain No. NCIB 8772.
III-A	2 parts by weight condensation products of coconut alcohol with 6 moles of ethylene oxides; 6 parts by weight of C_{12-14} alkyl dimethyl amine oxide.	"Alcalase."
III-B	Idem III-A	Produced by strain No. NCIB 8772.

The stain removal results are as follows:

Composition	Enzymatic activity in mAU/l. wash solution	Stain removal
I-A	10	21
	20	38
	30	51
I-B	10	87
	20	104
	30	118
II-A	10	21
	20	30
	30	43
II-B	10	86
	20	100
	30	109
III-A	10	17
	20	35
	30	44
III-B	10	68
	20	92
	30	101

Anionic surface-active agents, particularly synthetic sulfonates and synthetic sulfates can be added to the compositions of this example without affecting substantially the cleaning performance as far as the content of these anionic surface-active agents is less than about 70% and preferably less than about 50% by weight calculated on the total amount of surface-active agents.

EXAMPLE V

Detergent compositions prepared as described in Example I and having the formulation given hereafter, are used for preparing wash solutions with a finished deter-

gent concentration of 0.6% by weight. The pH of the wash solutions is adjusted to 9.7.

Ingredient:	Parts by weight
Coconut dimethyl ammonio-propane sulfonate sodium salt	8.5.
Sodium tripolyphosphate	66.
Carboxymethylcellulose	1.4.
Sodium sulfate	15.
Enzymes	See below.
Minor ingredients and humidity	Balance to 100.

The wash solutions are used for carrying out comparative stain removal tests as described in Example I.

The testing results are as follows:

Proteolytic enzyme preparation	Enzymatic activity in mAU/l. wash solution	Stain removal
Alcalase	10	20
	30	50
Produced by strain No. NCIB 8772	10	70
	30	100

What is claimed is:

1. A detergent composition consisting essentially of:

(a) from about 80% to about 99.999% by weight of:

(i) a zwitterionic surface-active agent selected from the group consisting of coconut di-C_{1-4}-alkyl ammonio hydroxy propane sulfonate sodium salt, dimethyl hexadecyl ammonio propane sulfonate sodium salt, tallow di-C_{1-4}-alkyl ammonio propionate sodium salt, coconut dimethyl ammonio propane sulfonate sodium salt, alkylbenzene ammonio propane sulfonate sodium salt, and alkylbenzyl di-C_{1-4}-alkyl ammonio propane sulfonate sodium salt;

(ii) or a semipolar surface-active agent selected from the group consisting of C_{8-18}-alkyl di-C_{1-2}-alkyl amine oxide and C_{8-18}-alkyl dihydroxyethyl amine oxide;

(iii) or mixtures thereof; and

(b) from 0.001% to about 20% by weight of a proteolytic enzyme produced by *Bacillus alcalophilus* strain NCIB 8772.

2. The detergent composition of Claim 1 additionally containing a nonionic surface-active agent of the general formula AE_n wherein A represents an alkyl chain with from 8 to 30 carbon atoms or an alkyl phenyl radical with from 3 to 22 carbon atoms in the alkyl chain, E is an alkylene oxy radical, and n is an integer from 4 to 20; wherein the ratio of the zwitterionic and semipolar surface-active agents of (a) to the nonionic surface-active agent is from about 4:1 to about 1:4.

3. The detergent composition of Claim 1 additionally containing an anionic surface-active agent whereby said anionic detergent represents less than 70% by weight of the total amount of surface-active agents.

4. The detergent composition of Claim 1 consisting essentially of from 0.01 to about 2% by weight of the proteolytic enzyme.

5. The detergent composition of Claim 2 wherein the nonionic surface-active agent is the condensation product of coconut or tallow alcohol with from 4 to 16 moles of ethylene oxide.

6. The detergent composition of Claim 1 additionally containing a detergency builder selected from the group consisting of alkali metal carbonates, phosphates, silicates, and polyacetates; and ammonium polyphosphonates and polyacetates; wherein the ratio of surface-active agent to builder is from about 5:1 to about 1:20.

7. The detergent composition of Claim 1 wherein the weight ratio of the zwitterionic surface-active agent of (i) to the semipolar surface-active agent of (ii) is within the range of 4:1 to 1:4.

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LEON D. ROSDOL, Primary Examiner

E. L. ROLLINS, Assistant Examiner

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