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van Kampen et al.

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- [54] **GRANULAR PROTEOLYTIC ENZYME COMPOSITION**
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Related U.S. Application Data

- [63] Continuation-in-part of Ser. No. 20,019, March 16, 1970, abandoned.
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- [51] Int. Cl. **C11d 7/08, C11d 7/42, C11d 17/06**
- [58] Field of Search **195/63, 68; 252/89, 132, 135, 252/DIG. 12**

[56] **References Cited**

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[57] **ABSTRACT**

The invention is directed to an improvement in the storage stability of granular proteolytic enzyme compositions. The proteolytic enzymes are contained in granules to which an acidic substance has been added. The granules will comprise a neutral or alkaline carrier material, for example an alkali metal phosphate; a gluing agent, for example a nonionic surface active agent; a proteolytic enzyme and an acidic material, for example citric acid.

5 Claims, No Drawings

GRANULAR PROTEOLYTIC ENZYME COMPOSITION

Cross-Reference to Related Application

This application is a continuation-in-part of our earlier filed application Ser. No. 20, 019, filed 16th Mar., 5 1970 now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to granular proteolytic enzyme compositions, adapted to be formulated with a detergent composition, and to detergent compositions containing these granular proteolytic enzyme compositions.

2. Description of the Prior Art

Enzymic cleaning compositions are old. See, for example, German patent specifications 283,923 (Rohm, 1915) and 329,958 (Rohm, 1920). The enzymes aid in cleaning by attacking the soil and stains of the soiled objects to be cleaned. Proteinaceous soil and stains are attacked by proteolytic enzymes. Such proteolytic enzymes, suitable for cleaning purposes, are normally found in the form of fine powders. Such fine powders are difficult to handle and difficult to formulate. When such fine powders are incorporated in detergent compositions, their stability is often greatly impaired by several ingredients of the detergent composition, and by moisture, which may be taken up by the detergent composition during storage. It is believed that this moisture brings the proteolytic enzymes into contact with the other ingredients of the detergent composition, which are harmful to the proteolytic enzymes.

It has already been proposed in U.S. Pat. No. 3,519,570 to enhance the stability of proteolytic enzymes by bringing them in granular form. This is done by rendering the surfaces of base carried detergent granules glutinous with a low-melting, ordinarily solid, water-soluble nonionic surface-active agent which liquefies between 100°F and 200°F, and subsequently conglutinating with said glutinous surfaces the proteolytic enzymes in a powdered form. The granules thus obtained may be additionally coated with the nonionic surface-active agent to encapsulate the proteolytic enzymes and to protect them from moisture and other harmful ingredients.

Although such granular proteolytic enzyme compositions are much more stable than the fine, powdered proteolytic enzymes, their stability in detergent compositions is still often not satisfactory for detergent formulation purposes.

Object of the Invention

It is therefore an object of the present invention to further improve the storage stability of granular proteolytic enzyme granules. Further objects will become apparent from the detailed description of the invention given hereafter.

SUMMARY OF THE INVENTION

It has been found that incorporating an acidic substance in the nonionic layer of the granular proteolytic enzyme compositions of the type as heretofore described, greatly enhances the storage stability of said granular proteolytic enzyme compositions. The present invention therefore relates to a granular proteolytic enzyme composition, adapted to be formulated with a detergent composition, the granular proteolytic enzyme composition consisting essentially of:

1. a core of a non-acidic carrier material
2. a coating layer enrobing said core, the coating layer being a nonionic surface-active agent
3. proteolytic enzymes, attached to said coating layer
4. an acidic substance, dissolved or dispersed in said coating layer.

In another aspect of the invention, the granular proteolytic enzyme composition also contains an outer layer of a nonionic surface-active agent, in which an acidic substance is dissolved or dispersed.

DETAILED DESCRIPTION OF INVENTION

The granular proteolytic enzyme composition consists essentially of

1. a core of non-acidic carrier material
2. a coating layer of a nonionic surface-active agent enrobing said core, said coating layer containing an acidic substance dissolved or dispersed therein
3. proteolytic enzymes.

Core of non-acidic carrier material

The core of the granules consists of a non-acidic carrier material, i.e. a neutral or alkaline carrier material, from which granules can be made. The particle size of the carrier material should lie between 0.05 mm and 1.5 mm, preferably between 0.3 and 1.5 mm. The carrier material should have a porosity of lying between 0.15 ml/g and 1 ml/g preferably between 0.15 and 0.50 ml/g, determined by the mercury method. This method consists of adding mercury to the carrier material under vacuum and measuring the apparent mercury density in an apparatus as described by J.M. Vleeskens in his Thesis of Delft University, Holland 1959, using the equation $(\Delta Hg/\epsilon Hg) = \text{volume of pores} + (M/\epsilon s)$, where

ΔHg = difference in weight of mercury before and after addition to the sample

ϵHg = density of mercury

M = weight of sample of carrier material

ϵs = density of sample of carrier material

The carrier material must be sufficiently strong, i.e. must be non-friable, and may not be hygroscopic. Crystal water may be present, provided the carrier material does not melt in its own water of crystallization at temperatures below 40°C (as e.g. $Na_2SO_4 \cdot 10 aq$). Suitable examples of neutral and alkaline carrier materials are alkali metal phosphates, such as penta sodium and penta potassium triphosphate, tetrasodium pyrophosphate, sodium or potassium orthophosphate, sodium or potassium sulphate or mixtures thereof, alkalimetal borates and carbonates, alkalimetal silicates, gelatinized starch and starch derivatives. In general, those granulatable carrier materials are preferred that are desirable in a detergent composition, e.g. penta sodium or penta potassium tripolyphosphate.

Coating Layer

The coating layer consists of a nonionic surface-active agent that is liquid or semi-paste-like at room temperature. Higher melting liquefiable nonionic surface-active agents may also be used. Examples of suitable nonionic surface-active agents are alkyl phenols with eight to 18 carbon atoms in the alkyl chain, and condensed with 8 to 15 moles of ethylene oxide, such as nonylphenol condensed with 10 moles of ethylene oxide, C_8-C_{20} monohydric aliphatic alcohols condensed with 8 to 25 moles of ethylene oxide, such as tallow fatty alcohol condensed with 25 moles of ethylene ox-

ide, isotridecyl alcohol condensed with 11 moles of ethylene oxide secondary C_{11} - C_{15} alcohols condensed with 9 moles of ethylene oxide, fatty acid mono- and dialkylol amides with 10 to 20 carbon atoms in the fatty acid residue, and ethoxylated derivatives thereof. Other suitable nonionics can be found in Schick, M.J. "Nonionic Surfactants" 1967, which is hereby incorporated by way of Reference.

Acidic Substance

The acidic substances that are used in the present invention should have an acid pH value, preferably between 4 and 6, in a 1%-aqueous solution. Any suitable organic and/or inorganic acid substance may be used, provided it does not affect the enzyme negatively. Suitable examples are acid salts, such as sodium or potassium acid pyrophosphate, alkali metal acid orthophosphate, inorganic acids such as phosphoric acid, and organic acids like citric acid and the sodium salt thereof, adipic acid and the like.

Particularly preferred are acidic substances which are soluble or dispersible in the nonionic surface-active agent, such as citric acid.

Proteolytic enzymes

The proteolytic enzymes which can be used in the present invention may be of animal and vegetable origin or may be obtained from micro organisms, including bacteria and fungi. Examples of such proteolytic enzymes are pepsin, trypsin, papain, aspergillus peptidase. The preferred proteolytic enzymes are those produced by micro organisms, such as the proteases produced by *Bacillus subtilis*. The commercial proteolytic enzymes are normally in the form of a fine powder containing the enzymes and an inert carrier material, such as sodium or calcium sulphate or clay. The preferred proteolytic enzymes are of the subtilisine type, commercially available under the trade names Alcalase and Maxatase. Alcalase is a serine protease, produced by Novo Industrie A/S Copenhagen, by submerged fermentation of a special strain of *Bacillus subtilis*. Maxatase is also a protease, produced from *Bacillus subtilis*, manufactured by the Royal Dutch Fermentation Industries at Delft, Holland.

The granular proteolytic enzyme composition of the present invention can be prepared in various ways. It is preferred to incorporate the acidic substance in the nonionic surface-active agent first, admixing this coating material with the granular carrier material and subsequently affixing the proteolytic enzyme powders thereon.

The granular proteolytic enzyme compositions are usually further coated with a suitable coating material. Suitable coating materials are those, which are also suitable as coating agents for the core of the granular proteolytic enzyme compositions as described above, but other film-forming agents such as polyvinylalcohol, polyvinylpyrrolidone, shellac, fatty acids, esters, waxes, etc. can be used.

The outer coating layer thus formed may also comprise the acidic substance, and it constitutes a preferred embodiment of the present invention when the inner and outer layers contain the acidic substance. The layer that contains the acidic substance forms, as it were, an acidic barrier against the harmful influences of e.g. particular ingredients of the detergent composition as well as a barrier to moisture present therein.

The amount of acidic substance required in the present invention is dependent upon the activity of the proteolytic enzymes as well as upon the amount of proteolytic enzyme present. In general, the granular proteolytic enzyme composition should contain an amount of acidic substance such that the weight ratio between the proteolytic enzyme, calculated to a standard activity of 1.5 Anson unit/g, and the acidic substance is from 10:1 to 1:10. Preferably the weight ratio is 2:1 to 1:2. The ratio between the nonionic surface-active agent and acidic substance is from 10:1 to 1:1. The acidic substance is from 0.5 to 10 percent, preferably from 2 to 8 percent by weight of the granule. The granular proteolytic enzyme composition is adapted to be particularly formulated with a detergent composition. Such a detergent composition may comprise one or more detergent surfactants, such as anionic and nonionic detergent surfactants, soap and mixtures thereof.

In general, the detergent composition may contain from 2 to 20 percent by weight of an anionid detergent surfactant. Examples thereof are alkylbenzenesulphonates with 12 to 18 carbon atoms in the alkyl group, alkylsulphates with 10 to 24 carbon atoms in the alkyl group, olefinsulphonates prepared by sulphonating straight-chain α -olefins with 10 to 20 carbon atoms and subsequently neutralizing and hydrolyzing the sulphonation reaction product, acylisethionates with 12 to 18 carbon atoms in the acyl-group and acyltaurates with 12 to 18 carbon atoms in the acyl group. The composition may furthermore contain 1 to 10 percent by weight of a nonionic detergent surfactant. Examples thereof are the condensation products of alkylene oxide, such as ethylene oxide and propylene oxide with alcohols having from 12 to 24 carbon atoms in the alkyl group, with alkylphenols having from eight to 18 carbon atoms in the alkyl group, with fatty acid amides with a C_{10} - C_{24} fatty acid residue, with polyalkyleneglycols and mixed alkylene oxide condensation products.

The compositions may also contain as alkali metal soap in an amount of 1 to 10 percent by weight. Examples thereof are the alkali metal soaps of C_{12} - C_{22} fatty acids, such as palm oil-, hardened fish oil, coconut oil, and tallow fatty acids.

For medium sudsing compositions the amount of the different detergent surfactants are

8 to 15 percent by weight of the anionic detergent surfactant

1 to 6 percent by weight of the nonionic detergent surfactant

1 to 5 percent by weight of the alkali metal soap whereas for low sudsing compositions the amounts are:

2 to 9 percent by weight of the anionic detergent surfactant

2 to 10 percent by weight of the nonionic detergent surfactant

5 to 10 percent by weight of the alkali metal soap.

The remaining part of the composition may consist of the normal constituents of a detergent composition. They may contain from 20 to 50 percent of a condensed phosphate, such as pentasodium triphosphate. If desirable, part of the condensed phosphate may be replaced by an organic builder, such as trisodium nitrilotriacetate.

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Furthermore, from 2 to 10 percent of sodium silicate may be present, 0.5 to 3 percent of sodium carboxymethylcellulose or other suitable soil-suspending agent may be present. If desired, up to 45 percent by weight of a bleaching agent, such as sodium perborate may be added for heavy-duty purposes.

The compositions may furthermore contain alkali metal salts, such as sodium sulphate, sodium carbonate, sodium borates, and furthermore other adjuvants, such as tarnish inhibitors, perfumes, germicides, coloring agents, lather modifiers, fluorescers, solvents, bleach precursors.

The granular proteolytic enzyme compositions of the invention may be present in an amount of 0.5–15 percent, preferably 5–10 percent, by weight of the detergent composition.

Examples of detergent compositions according to the invention will now be given by way of illustration only and in a non-limitative manner.

EXAMPLE I

Granules of the composition as given in Table I were prepared by dry-mixing the granular anhydrous carrier materials having a particle size of 50–250 μ with a commercially available powdered proteolytic enzyme, manufactured by submerged fermentation with a strain of *Bacillus subtilis* known under the trade name Alcalase (with an activity of 1.5 Anson unit/g), in a mixing apparatus. Subsequently this dry mixture was granulated by quickly dropwise adding liquid nonylphenol condensed with 10 moles of ethylene oxide, to which citric acid had been added first. The granules obtained were then sieved with a sieve having apertures of 0.5–1 mm and added in an amount of 5 percent to detergent composition of the following formula:

sodium salt of straight chain dodecylbenzenesulphonate	5.5
sodium soap of commercial stearic acid	8.0
nonylphenol condensed with 14 moles of ethylene oxide	3.0
sodium silicate	5.0
sodium sulphate	9.9
sodium carboxymethylcellulose	1.0
pentasodium triphosphate	36.0
sodium perborate	30.0
water, perfume, etc.	rest

The residual activity of the proteolytic enzyme was determined after 7 days' storage of the detergent composition with the enzyme granules at 30°C and 80 percent R.H. The results are given in Table I.

TABLE I

Number of granule	Granulatable carrier material		Enzyme, alcalase (proteolytic enzyme)	Acidic substance, citric acid	Gluing agent, nonylphenol condensed with 10 moles of ethylene oxide	Percent residual activity after 7 days
	Anhydrous pentasodium triphosphate (powder)	Anhydrous disodium acid pyrophosphate (powder)				
1	55	11	16.5	6	17.5	37
2	49	11	15	6	19	52
3	49	11	15.5	8.5	16	45
4	53	11	16.5	6.5	13	49
5	52	11	17.5	4	15.5	54

EXAMPLE II

Granules were prepared of the composition as given in Table II.

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TABLE II

Pentasodium triphosphate	58	66	70	66	70	74
Nonyl phenol condensed with 10 moles of ethylene oxide	10	10	10	10	10	10
Citric Acid		8	4			
Boric Acid				8	4	
KH ₂ PO ₄	16					
Alcalase	16	16	16	16	16	16
Number	1	2	3	4	5	6 (blank)

The granules were prepared from granular anhydrous pentasodium triphosphate having a particle size of 50–250 μ .

The powdered proteolytic enzyme Alcalase was mixed with the acidic substance and subsequently attached to the carrier material by means of dropwise addition of liquid nonylphenol condensed with 10 moles of ethylene oxide. The granules thus formed were coated by means of a second layer of the nonionic. These granules were sieved with a sieve with apertures of 0.5–1 mm and added in an amount of 5 percent to the detergent composition of Example I. Storage tests were carried out for several weeks at 30°C — 80 percent R.H. in laminated and unlaminated carton containers. The residual enzymatic activity was determined, the results of which are shown in Table III.

TABLE III

N° of granule	residual activity (in %)							
	1 week not	3 weeks not	4 weeks not	6 weeks not	8 weeks not	1 week lam.	3 weeks lam.	4 weeks lam.
1	37	88	14	59	6	47	0	27
2	57	109	12	74	6	79	0	66
3	50	96	10	77	5	72	0	36
4	37	10	54	5	44	0	25	0
5	35	97	12	72	4	51	4	40
6	26	78	9	56	1	44	0	24

EXAMPLE III

Several enzyme-containing granules were prepared from granular pentasodium triphosphate which had a particle size of 50–250 μ and a powdered proteolytic enzyme from *Bacillus subtilis*, sold under the trade name Maxatase. The composition of the granules is given in Table IV.

Granules nos. 1 and 4 were prepared in the following way. Citric acid was dispersed in tallow fatty alcohol condensed with 25 moles of ethylene oxide, under

heating to 60°C in a homogenisator for half an hour. The dispersion was a clear liquid, which became viscous at lower temperatures. This dispersion was heated up and sprayed on the granular pentasodium

triphosphate by means of a two-fluid nozzle. Subsequently the powdered enzymes were added, and on the so-obtained granules again the dispersion of citric acid in the nonionic was sprayed.

Granules nos. 2 and 6 were prepared in an identical way, with the exception that first solely the tallow fatty alcohol condensed with 25 moles of ethylene oxide was sprayed onto the granular pentasodium triphosphate, and after addition of the proteolytic enzyme powders the citric acid nonionic dispersion was sprayed on the granules so obtained.

Granules nos. 3 and 5 were prepared as follows. On the granular pentasodium triphosphate first the tallow fatty alcohol condensed with 25 moles of ethylene oxide was sprayed, followed by the addition of the dry mixture of the proteolytic enzyme powder and citric acid, and subsequently again the nonionic was sprayed upon the granules.

Granule no. 7 was prepared by spraying the nonionic on the granular pentasodium triphosphate, adding the enzyme powder and again spraying the nonionic thereupon. Granule no. 7 was made for comparison purposes. All the granules contained 5 percent nonionic in the inner and 5 percent nonionic in the outer layer. From each of these granules, 7.5 percent by weight was added to the detergent composition as given in Example I, and the residual proteolytic activity was determined after 7 days' storage of the composition at 30°C and 80 percent relative humidity. The results are also given in Table IV.

TABLE IV

Granule number	Granular carrier material, percent pentasodium triphosphate	Percent citric acid in inner layer (5%) nonionic	Percent citric acid dry mixed with 16% enzyme	Percent citric acid in outer layer (5%)	Percent residual proteolytic activity
1	70	2		2	33
2	70			4	28
3	70		4		30
4	66	4		4	31
5	66		8		37
6	66			8	26
7	74				23

What is claimed is:

1. A granular proteolytic enzyme composition, adapted to be formulated with a detergent composition, the proteolytic enzyme composition consisting essentially of

i. a core of a non-acidic, non-hygroscopic, nonfriable carrier material selected from the group consisting of alkalimetaltripolyphosphates, -pyrophosphates, -orthophosphates, alkalimetalsulphates, -borates, -silicates, -carbonates, gelatinized starch and starch derivatives,

ii. a coating layer enrobing said core, consisting of a nonionic detergent surfactant selected from the group of alkyl phenols with eight to 18 carbon atoms in the alkyl chain, and condensed with 8 to 15 moles of ethylene oxide, C₈-C₂₀ monohydric aliphatic alcohols condensed with 6 to 25 moles of ethylene oxide, secondary C₁₁₋₁₅ alcohols condensed with 9 moles of ethylene oxide, fatty acid mono and dialkylol amides with 10 to 20 carbon atoms in the fatty acid residue, and ethoxylated derivatives thereof,

iii. an acidic substance, dissolved or dispersed in said coating layer, said acidic substance having a pH of 4 to 6 in a 1%-aqueous solution, selected from the group consisting of citric acid, and adipic acid, said acidic substance being present in an amount of 0.5 to 10 percent by weight of the granule, the weight ratio between the nonionic detergent surfactant coating layer and the acidic substance being from 10:1 to 1:1,

iiii. proteolytic enzymes, attached to the coating layer of the core, in such an amount that the weight ratio between the proteolytic activity, calculated to a standard activity of 1.5 Anson unit/g, and the acidic substance is from 10:1 to 1:10.

2. A composition according to claim 1, in which the granular proteolytic enzyme composition comprises furthermore an outer coating layer of the nonionic detergent surfactant, in which the acidic substance has been dispersed or dissolved.

3. A composition according to claim 1, in which the acidic substance is citric acid.

4. A composition according to claim 1, in which the amount of the acidic substance is from 2 to 8 percent by weight of the granule.

5. A detergent composition consisting essentially of

a. from 2 to 20 percent by weight of an anionic detergent surfactant selected from the group consisting of alkylbenzenesulphonates with 12 to 18 carbon atoms in the alkyl group, alkylsulphonates with 10 to 24 carbon atoms in the alkyl group, olefinesulphonates with 10 to 20 carbon atoms, acylisethionates with 12 to 18 carbon atoms in the acyl group and acyltaurates with 12 to 18 carbon atoms in the acyl group.

b. from 1 to 10 percent by weight of a nonionic detergent surfactant selected from the group consisting of the condensation products of ethylene oxide or propylene oxide with alcohols having from 12 to 24 carbon atoms in the alkyl group, with alkylphenols having from eight to 18 carbon atoms in the alkyl group, with fatty acid amides with a C₁₀-C₂₄ fatty acid residue, with polyalkyleneglycols and with mixed alkylene oxide condensation products,

c. from 1 to 10 percent by weight of an alkalimetal soap of C₁₂-C₂₂ fatty acids,

d. from 20 to 50 percent by weight of a water-soluble alkalimetal builder salt,

e. from 0 to 45 percent by weight of a bleaching agent,

f. from 0.5 to 15 percent by weight of a granular proteolytic enzyme composition consisting essentially of

i. a core of a non-acidic, non-hygroscopic, nonfriable carrier material selected from the group consisting of alkalimetaltripolyphosphates, -pyrophosphates, -orthophosphates, alkalimetalsulphates, -borates, -silicates, -carbonates, gelatinized starch and starch derivatives,

ii. a coating layer enrobing said core, consisting of a nonionic detergent surfactant selected from the group of alkyl phenols with eight to 18 carbon atoms in the alkyl chain, and condensed with 8 to 15 moles of ethylene oxide, C₈-C₂₀ monohydric aliphatic alcohols condensed with 6

to 25 moles of ethylene oxide, secondary C₁₁-C₁₅ alcohols condensed with 9 moles of ethylene oxide, fatty acid mono- and dialkylol amides in the fatty acid residue, and ethoxylated derivatives thereof,

iii. an acidic substance, dissolved or dispersed in said coating layer, said acidic substance having a pH of 4 to 6 in a 1%-aqueous solution, selected from the group consisting of citric acid and adipic acid, said acidic substance being present in an amount of 0.5 to 10 percent by weight of

the granule, the weight ratio between the nonionic detergent surfactant coating layer and the acidic substance being from 10:1 to 1:1.

iiii. proteolytic enzymes, attached to the coating layer of the core, in such an amount that the weight ratio between the proteolytic activity, calculated to a standard activity of 1.5 Anson unit/g, and the acidic substance is from 10:1 to 1:10.

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