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(54) **ENZYME CONTAINING PREPARATION AND DETERGENT CONTAINING SUCH PREPARATION.**

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Description

The invention encompasses an enzyme containing preparation containing a protease and at least one other enzyme and a detergent containing such preparation. In this specification with claims the term "preparation" means either a granulate or a liquid slurry. The slurry can be either anhydrous or substantially anhydrous, or it can be aqueous. These enzyme containing preparations can be used in different fields, e.g. in the field comprising digestive aids and in the detergent field, but their main use is to be found in the detergent field.

In this specification with claims the term "granulate" is to be understood in its widest sense comprising the entire scope from small particles with a size of the order of magnitude around 10 μm to tablets with a size of the order of magnitude around 1 cm.

Enzyme containing granulates are widely used in industry, mainly as dust free additives to detergents. Reference can be made to e.g. US 4,106,991, US 4,661,452, DOS 2,060,095, and GB 1,362,365.

One of the big problems in regard to protease containing granulates is the storage stability of other enzymes present in the granulate. Another big problem in regard to enzyme containing granulates is the storage stability of the enzymes when the enzyme containing granulate is mixed with the other detergent components. Even if several methods for stabilization of the enzymatic activity have been devised, the stability of the enzymatic activity in the enzyme granulate containing detergents is still open to improvement. Another problem in regard to enzyme containing granulates is the color, as most enzyme containing granulates will be discolored, if not coated with e.g. TiO_2. A third problem in regard to enzyme containing granulates is the smell thereof. Even after a fairly good purification of the enzyme containing fermentation broth the finished product often exhibits an unagreeable strong smell.

Anhydrous or substantially anhydrous slurries are widely used in industry, mainly as additives to liquid detergents. Aqueous slurries can be used as additives to liquid detergents, in the starch industry and in the food industry. If the slurry is aqueous the water activity or the ionic strenght in the slurry has to be controlled in such manner that the entire amount or substantially the entire amount of the crystalline enzyme is maintained in the crystalline form.

A big problem in relation to liquid detergents is the stability of enzymes added to these. Several stabilization systems have been used in an attempt to overcome this problem. Within the field of low pH formulations both calcium, formate and borate stabilization has been used and are used. Within the higher pH range some of the inhibition methods are also used. But especially within structured liquids a hydrophobic encapsulation has been used, vide Gb 2,186,884A. However, all these stabilization systems are open to improvement. Another big problem in regard to liquid, enzyme containing preparations is the color. Most liquid, enzyme containing preparations are colored due to impurities, usually with a dull, brownish color, and also, the color varies somewhat from batch to batch. This means that the manufacturer of liquid detergents will have to compensate for this color, when he wants to produce for instance a blue liquid detergent, and furthermore, this compensation may vary from batch to batch of the liquid enzyme containing preparation, which obviously is disadvantageous. As with granulates, also with liquid detergents the smell can be a problem.

Also, some preparations contain allergy generating impurities, and thus, purer preparations is a desideratum.

Thus the purpose of the invention is the provision of an enzyme containing preparation, which exhibits an improved stability, and which is colorless or almost colorless and of a high purity, and thus without any smell problems, and a detergent containing such enzyme containing preparation.

The enzyme containing preparation according to the invention is characterized by the fact that it contains a protease and at least one protease sensitive enzyme, wherein substantially 100% of the proteolytic activity and/or substantially 100% of the protease sensitive enzyme or the protease sensitive enzymes are present as crystals, wherein more than 90% of the crystals possess a maximum crystal dimension between 0.01 μm and 500 μm, preferably between 0.1 μm and 100 μm, and that the preparation preferably contains a stabilizing agent for the enzymes.

If the protease and the protease sensitive enzyme(s) originate from the same microorganism, the stability problems in regard to the protease sensitive enzyme(s) are usually minor. However, for various reasons, e.g. for economic reasons, the microorganism from which the protease is produced, usually differs from the microorganism(s), from which the protease sensitive enzyme(s) is (are) produced, and in such cases serious stability problems may occur. However, with the enzyme containing preparation according to the invention it surprisingly has been found that these stability problems are largely eliminated. Thus, surprisingly it has been found that the enzyme containing preparation according to the invention, when present in a detergent, possesses an equally good or better enzyme stability than otherwise similar but less pure enzyme containing preparations. It is normally assumed that some of the impurities from the fermentation broth stabilize the enzyme, and that

consequently it would be a disadvantage in regard to enzyme stability to purify the enzyme too much, and this assumption is correct. However, if a pure enzyme can be maintained as crystals in the preparations according to the invention it has been found that the stability is almost equal to or better than the stability of less pure preparations. Also, it has been found that the enzyme containing preparation according to the invention possesses improved color and smell characteristics, when the crystallization of the enzyme is performed without later introduction of impurities.

If substantially 100% of the proteolytic activity is present as crystals only a small amount of the crystalline protease or nothing thereof will be dissolved in the preparation according to the invention, and thus, the stability of the other enzyme or the other enzymes in the preparation according to the invention will be improved. If the protease is Savinase® and the preparation contains the lipase Lipolase® as the other enzyme, and if the preparation is a slurry it has been found that the lipase exhibits an excellent stability. If substantially 100% of the protease sensitive enzyme or the protease sensitive enzymes are present as crystals only a small amount of the protease sensitive enzyme(s) or nothing thereof will be dissolved in the preparation according to the invention, and thus, the stability of the protease sensitive enzyme(s) in the preparation according to the invention will be improved. If the protease is Savinase® and the preparation contains the lipase Lipolase® as the other enzyme, and if the preparation is a granulate it has been found that the lipase exhibits an excellent stability.

WO 89/08703 describes a method for production of crystalline subtilisin. This crystalline protease could be used as a constituent in the preparation according to the invention. However, WO 89/08703 does not indicate anything about the problems, which arise in relation to the preparations, which besides the crystalline subtilisin contain protease sensitive enzymes.

It is to be understood that the enzyme containing preparations according to the invention can be produced in any conventional manner, if only the conventional enzyme starting material, i.e. enzyme in the form of an amorphous powder, usually with a relatively large amount of impurities, or in the form of an enzyme solution or slurry, is substituted by an enzymatic starting material, in which substantially 100% of the enzymatic activity is present as enzyme crystals. If the preparation is a granulate, a non limiting list of such usable granulation methods is the previously cited US 4,106,991, US 4,661,452, DOS 2,060,095, and Gb 1,362,365.

A preferred form of enzyme crystals is described in co-pending patent applications Nos. 847/90 and 848/90 (our refs. 3411.010-DK and 3411.020), filed on the same date as this application. Other types of enzyme crystals usable in the enzyme containing preparation are known per se. Reference can be made to DK 872/86, US 4,699,882, and Northrop, J.H. et al, Crystalline Enzymes, 2nd edition, New York, 1948. Also, reference can be made to the current catalogues from Sigma Chemical Company, P.O. Box 14508, St. Louis, MO, USA.

A further advantage in relation to the preparation according to the invention is to be found in the washing process in a washing float with chlorine in the tap water. In such washing floats the chlorine will be consumed in the beginning of the washing cycle, and due to the fact that crystalline enzymes are dissolved slower in the washing float than enzymes usually used, the enzymes in the preparation according to the invention will not be present in dissolved form, before part of the chlorine has been consumed, and thus they will be protected from inactivation by the chlorine. Documentation for this will be presented later in this specification.

The stabilizing agent for the enzymes provides a further improvement of the enzyme stability. In case of a granulate PVP (polyvinyl pyrrolidone) can be used; in case of a slurry preservatives against microbial growth, sucrose and Ca^{++} can be used as stabilizing agents.

In the preparation according to the invention more than 90% of the crystals possess a maximum crystal dimension between 0.1 μm and 500 μm, preferably between 1 μm and 100 μm. Such crystals are preferably prepared according to co-pending patent applications Nos. 847/90 and 848/90 (our refs. 3411.010-DK and 3411.020-DK), filed on the same date as this application, but they can also be prepared by means of other crystallization methods.

In a preferred embodiment of the preparation according to the invention the protease sensitive enzyme is a lipase, amylase, cellulase, hemicellulase, pectinase, amidase or oxidase, or protein engineered variants of these. These enzymes are common enzymes used as additives in detergents.

The protease in the preparation according to the invention may be a Subtilisin type protease. Examples are Savinase®, Esperase®, Alcalase®, Subtilisin NOVO or protein engineered variants of these. These enzymes are preferred proteases in detergents.

In a preferred embodiment of the preparation according to the invention the protease sensitive enzyme is a lipase and the lipase is Lipolase®.

The preparation according to the invention may be a granulate. This preparation is well suited as an additive to a detergent in granulate form.

The preparation according to the invention may be a slurry, which may be anhydrous or substantially anhydrous, or aqueous.

The preparation according to the invention may be used as a detergent additive. This is the main use of the preparation according to the invention.

Also the invention comprises a detergent, which contains the preparation according to the invention, whereby the detergent either is solid and contains the preparation as a granulate in a concentration of between 0.001 and 10 mg of enzyme protein/g of detergent, preferably between 0.005 and 5 mg of enzyme protein/g of detergent, most preferably from 0.01 to 1 mg of enzyme protein/g of detergent, or is liquid and contains the preparation as an anhydrous or substantially anhydrous slurry in an amount of between 0.001 to 10 mg of enzyme protein per g of detergent, preferably from 0.005 to 5 mg of enzyme protein per g of detergent, most preferably from 0.01 to 1 mg of enzyme protein per g of detergent. Depending upon the enzymatic strength of the granulate the concentrations indicated above will usually be obtained by addition of between 0.01 and 10% w/w of the granulate to the detergent. The above indicated liquid detergents exhibit a satisfactory stability of the enzyme.

Use of binders in relation to the granulate preparations according to the invention is a necessity, and optionally such binders can be carbohydrate binders, e.g. dextrins or cellulose derivatives, for instance hydroxypropyl cellulose, methyl cellulose or CMC.

EXAMPLES

EXAMPLE 1

13.3 kg of a powder composition with the formulation
0.5 kg crystalline SAVINASE® with an activity of 244 KNPU/g
1.5 kg bentonite ASB 350 (ECCI)
2.2 kg fibrous cellulose, ARBOCEL BC200
9.1 kg finely grounded sodium sulphate
is granulated in a Lödige mixer FM 50 with 4.5 kg of a binder solution consisting of 3.0 kg of water, 0.15 kg of polyvinyl pyrrollidone K30 and 1.35 kg of a carbohydrate binder. The granulation is performed in a manner as described in US patent No. 4,106,991, Example 1.

Savinase® is an alkaline Bacillus protease prepared as indicated in US patent No. 3,723,250. Also, reference can be made to the product sheet for Savinase®, B345 b-GB, March 1988, obtainable from Novo Nordisk A/S on request.

The KNPU proteolytic activity unit is defined in AF 101.10, which on request can be obtained from Novo Nordisk A/S, Denmark.

The granulate is dried in a fluid bed to a water content below 1%, whereafter a light colored granulate is obtained with particle distribution:

11% > 1180 μm
17% > 1000 μm
25% > 850 μm
40% > 710 μm
56% > 600 μm
77% > 500 μm
86% > 420 μm
93% > 355 μm
2.4% < 300 μm

with an activity of 7.3 KNPU/g and with the Hunter color coordinates (L:a:b)=(79.6: -1.3: 11.3).

The granulate is finally sifted to get a product with the particle range 300 μm to 1000 μm and coated with 7% of PEG 4000 and 12% of a 1:1 mixture of TiO_2 and kaolin in a manner as described in US patent No. 4,106,991, Example 22.

EXAMPLE 2

Preparation of a Lipolase® 100 T granulate containing crystalline Lipolase®

The following components are introduced into a Lödige mixer FM 50:

2.0 kg of bentonite ASB 350
3.0 kg of fibrous cellulose, Arbocel BC 200
2.1 kg of carbohydrate binder
0.63 kg of crystalline Lipolase® (Lipolase® produced according to EP 238023)

12.3 kg of ground Na_2SO_4

The mixed dry components are sprayed with 3.6 kg of water. During and after the spraying the moist mixture is exposed to a compacting and granulation influence from the multiple set of knives, as descibed in Example 1 of US patent no. 4,106,991. When the granulation is finished, the granulate is dried in a fluid bed, and the part thereof defined as product fraction (in this cae 300-900 μm) is separated for quality testing. The undersize fraction, which is not treated and the oversize fraction, which is crushed, are recirculated.

The color is measured according to the Hunter Lab Scale (AF 78):

L = 70.1
a = 0.4
b = 12.0

The above-mentioned product fraction is coated according to US 4, 106,991, Example 22 for storage stability testing.

EXAMPLE 3

Preparation of a Lipolase® 100 T granulate containing amorphous Lipolase® as a reference for comparison

Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350
3.0 kg of fibrous cellulose, Arbocel BC 200
2.1 kg of carbohydrate binder
0.63 kg of amorphous Lipolase®
9.7 kg of ground Na_2SO_4

The mixed dry components are sprayed with 3.0 kg of water.

Color coordinates:

L = 68.0
a = 0.7
b = 15.0

It appears that the color of the preparation in Example 2 (crystalline Lipolase®) is brighter than the color of the preparation in Example 3 (amorphous Lipolase®).

The product fraction is coated for storage stability testing.

The storage stability of the coated granulates produced according to Examples 2 and 3 is tested using accelerated conditions:

2% of the granulate is added to a European heavy duty detergent and stored in closed jars at 50°C. The activity is measured after 0, 1, 3 and 7 days and calculated in percent.

	Residual activity (%)		
	1 day	3 days	7 days
Example 2: crystalline Lipolase®	99	85	69
Example 3: amorphous Lipolase®	90	82	59

EXAMPLE 4

Preparation of a Savinase®/Lipolase® 3.0/50 T cogranulate containing amorphous Savinase® and crystalline Lipolase®

Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350
3.0 kg of fibrous cellulose, Arbocel BC 200
2.1 kg of carbohydrate binder
0.32 kg of crystalline Lipolase®
1.84 kg of amorphous Savinase®
10.7 kg of ground Na_2SO_4

The mixed dry components are sprayed with 3.3 kg of water.

The product fraction is coated for storage stability testing.

EXAMPLE 5

Preparation of a Savinase®/Lipolase® 3.0/50 T cogranulate containing amorphous Savinase® and amorphous Lipolase®

Produced according to Example 2 with the following formulation:
2.0 kg of bentonite ASB 350
3.0 kg of fibrous cellulose, Arbocel BC 200
2.1 kg of carbohydrate binder
1.6 kg of amorphous Lipolase®
1.84 kg of amorphous Savinase®
9.7 kg of ground Na_2SO_4
The mixed dry components are sprayed with 3.3 kg of water.
The product fraction is coated for storage stability testing.

EXAMPLE 6

Preparation of a Savinase®/Lipolase® 3.0/50 T cogranulate containing crystalline Savinase® and amorphous Lipolase®

Produced according to Example 2 with the following formulation:
2.0 kg of bentonite ASB 350
3.0 kg of fibrous cellulose, Arbocel BC 200
2.1 kg of carbohydrate binder
1.6 kg of amorphous Lipolase®
11.0 kg of ground Na_2SO_4
The mixed dry components are sprayed with 0.7 kg of crystalline Savinase® filter cake produced according to DK patent application no. 847/90 suspended in 2.9 kg of water.
The product fraction is coated for storage stability testing.

EXAMPLE 7

Preparation of a Savinase®/Lipolase® 3.0/50 T cogranulate containing crystalline Savinase® and crystalline Lipolase®

Produced according to Example 2 with the following formulation:
2.0 kg of bentonite ASB 350
3.0 kg of fibrous cellulose, Arbocel BC 200
2.1 kg of carbohydrate binder
0.32 kg of crystalline Lipolase®
12.2 kg of ground Na_2SO_4
The mixed dry components are sprayed with 0.7 kg of crystalline Savinase® filter cake produced according to DK patent application no. 847/90 suspended in 3.0 kg of water.
The product fraction is coated for storage stability testing.
The storage stability of the coated granulates produced according to Examples 4 through 7, determined in regard to the protease sensitive enzyme Lipolase®, is tested under accelerated conditions:
4% of the granulate is added to European heavy duty detergent and stored in closed jars at 50°C. The activity is measured after 0, 1, 3 and 7 days and calculated in percent.

		Residual activity of Lipolase (%)		
		1 day	3 days	7 days
Example 4:	amorphous Savinase®/- crystalline Lipolase®	99	84	77
Example 5:	amorphous Savinase®/- amorphous Lipolase®	89	61	39
Example 6:	crystalline Savinase®/- amorphous Lipolase®	81	54	45
Example 7:	crystalline Savinase®/- crystalline Lipolase®	95	84	77

In another example the granulates produced according to Examples 4 through 7, determined in regard to the protease sensitive enzyme Lipolase®, is tested under other accelerated conditions:

4% of the granulate is added to European heavy duty detergent and stored in closed jars at 37°C and 70% relative humidity. The activity is measured after 0, 3, 7 and 14 days and calculated in percent.

		Residual activity of Lipolase (%)		
		3 days	7 days	14 days
Example 4:	amorphous Savinase®/- crystalline Lipolase®	87	66	41
Example 5:	amorphous Savinase®/- amorphous Lipolase®	51	15	5
Example 6:	crystalline Savinase®/- amorphous Lipolase®	46	15	4
Example 7:	crystalline Savinase®/- crystalline Lipolase®	81	58	36

It appears that the best results are obtained with crystalline lipase.

EXAMPLE 8

Preparation of a Savinase® 4.0 T granulate containing crystalline Savinase®

Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350

3.0 kg of fibrous cellulose, Arbocel BC 200

2.4 kg of carbohydrate binder
11.7 kg of ground Na_2SO_4

The mixed dry components are sprayed with 1.2 kg of crystalline Savinase® filter cake produced according to DK patent application no. 847/90, suspended in 2.2 kg of water, 0.11 kg of Na_2SO_4 and 0.2 kg of PVP K30.

Color coordinates:

L = 79.9
a = 0.8
b = 7.8

EXAMPLE 9

Preparation of a Savinase® 4.0 T granulate containing amorphous Savinase®

Produced according to Example 2 with the following formulation:

0.8 kg of kaolin, Speswhite
3.0 kg of fibrous cellulose, Arbocel BC 200
3.0 kg of amorphous Savinase® concentrate
11.2 kg of ground Na_2SO_4

The mixed dry components are sprayed with 1.8 kg of carbohydrate binder, 0.2 kg of PVP K30 in 3.0 kg of water.

Color coordinates:

L = 48.2
a = 6.0
b = 17.7

It clearly appears that the color of the preparation in Example 8 (crystalline Savinase®) is much brighter than the color of the preparation in Example 9 (amorphous Savinase®), to the point, that no TiO_2 is needed in the preparation of Example 8.

EXAMPLE 10

Preparation of a Durazym® 6.0 T granulate containing crystalline Durazym®

Produced according to Example 2 with the following formulation:

0.7 kg of bentonite ASB 350
1.0 kg of fibrous cellulose, Arbocel BC 200
0.4 kg of carbohydrate binder
4.3 kg of ground Na_2SO_4

The mixed dry components are sprayed with 0.354 kg of crystalline Durazym® filter cake produced according to DK patent application no. 847/90, suspended in 1.5 kg of water, 0.07 kg of PVP K30 and 0.4 kg of carbohydrate binder.

Color coordinates:

L = 82.5
a = 1.0
b = 10.0

EXAMPLE 11

Preparation of a Durazym® 6.0 T granulate containing amorphous Durazym®

Produced according to Example 2 with the following formulation:

1.5 kg of bentonite ASB 350
3.25 kg of fibrous cellulose, Arbocel BC 200
0.9 kg of carbohydrate binder
7.7 kg of ground Na_2SO_4
2.0 kg of amorphous Durazym®

The mixed dry components are sprayed with 2.5 kg of water, 0. 15 kg of PVP K30 and 0.5 kg of carbohydrate binder.

Color coordinates:

L = 52.8
a = 5.2
b = 17.4

It clearly appears that the color of the preparation in Example 10 (crystalline Durazym®) is much brighter than the color of the preparation in Example 11 (amorphous Durazym®), to the point, that no TiO_2 is needed in the preparation of Example 10.

EXAMPLE 12

Preparation of a Savinase® 4.0 T granulate containing crystalline Savinase®

Produced according to Example 2 with the following formulation:
2.0 kg of bentonite ASB 350
3.0 kg of fibrous cellulose, Arbocel BC 200
1.2 kg of carbohydrate binder
11.9 kg of ground Na_2SO_4
0.46 kg of dry crystalline Savinase®

The mixed dry components are sprayed with 2.3 kg of water containing 0.2 kg of PVP K30 and 1.2 kg of carbohydrate binder.

EXAMPLE 13

Preparation of a Savinase® 4.0 T granulate containing crystalline Savinase®

Produced according to Example 2 with the following formulation:
2.0 kg of bentonite ASB 350
3.0 kg of fibrous cellulose, Arbocel BC 200
2.4 kg of carbohydrate binder
11.6 kg of ground Na_2SO_4

The mixed dry components are sprayed with 1.5 kg of crystalline Savinase® filter cake produced according to DK patent application no. 847/90, suspended in 2.5 kg of water with 0.1 kg of Na_2SO_4 and 0.2 kg of PVP K30.

EXAMPLE 14

Preparation of a Savinase® 6.0 T granulate containing amorphous Savinase®

Produced according to Example 2 with the following formulation:
0.8 kg of kaolin, Speswhite
1.8 kg of fibrous cellulose, Arbocel BC 200
2.8 kg of amorphous Savinase®
12.6 kg of ground Na_2SO_4

The mixed dry components are sprayed with 1.8 kg of carbohydrate binder, 0.2 kg of PVP K30 in 3.6 kg of water.

The storage stability of the coated granulates produced according to Examples 12 through 14, determined in regard to Savinase®, is tested under accelerated conditions:

1% of the granulate is added to a European heavy duty detergent and stored in closed jars at 50°C. The activity is measured after 0, 1, 3 and 7 days and calculated in percent.

	Residual activity of Savinase (%)		
	1 day	3 days	7 days
Example 12: dry crystalline Savinase®	60	58	52
Example 13: crystalline Savinase®	68	57	49
Example 14: amorphous Savinase®	60	51	42

It appears that the stability of crystalline Savinase® is better than of amorphous Savinase®.

EXAMPLE 15

This example illustrates the further advantage in regard to a chlorine containing washing float. The release velocity in seconds, i.e. the time interval needed to dissolve 50, 90 and 95% of the enzyme, appears from the below indicated table.

	Release velocity (s)		
	50%	90%	95%
Example 12: dry crystalline Savinase®	57	85	92
Example 13: crystalline Savinase®	69	116	122
Example 14: amorphous Savinase®	43	62	59

It clearly appears that the crystalline Savinase® dissolves more slowly than the amorphous Savinase®.

EXAMPLE 16

The liquid preparation Lipolase® 100L in an amount of 1%, crystalline Savinase in an amount of 0.2% and dissolved Savinase® in an amount of 0.625% was added to NOVO standard liquid detergent with builder with the following composition:

Berol 160	15%
NANSA 1169/P	33.3%
Coconut fatty acid	9%
Oleic acid	1%
Triethanolamin	9%
Glycerol	12%
Ethanol	1.5%
Trisodium citrate $2H_2O$	8%
$CaCl_2$ $2H_2O$	0.1%
NaOH	1%
Water	10.1%
pH	8.5

The below indicated table shows the residual activity of the dissolved Lipolase® in the liquid detergent after 3 days at 4°C and 35°C.

	Residual activity after 3 days	
Sample	4°C	35°C
Lipolase® + crystalline Savinase®	66%	23%
Lipolase® + liquid Savinase®	16%	0.9%

It clearly appears that the stability of Lipolase® was better in the presence of crystalline Savinase® than in the presence of the dissolved Savinase®.

EXAMPLE 17

This example illustrates the storage stability of aqueous and anhydrous Savinase® slurries.

The aqueous slurry base was 54% $(NH_4)_2SO_4$ in deionized water.

The anhydrous slurry base was 305 g of Surfactant T9, 140 g Na_2SO_4 and 15 g Aerosil 200 which were well mixed and homogenized with an Ultra-Turrax mixer.

8.7 g crystalline Savinase® or 17 g of amorphous Savinase® were mixed with each base. The slurries were homogenized with an Ultra-Turrax mixer.

The slurries were incubated at 60°C for 5 days and the residual activities were measured.

Storage stability

	Crystalline		Amorphous	
	Aqueous KNPU/g (%)	Anhydrous KNPU/g (%)	Aqueous KNPU/g (%)	Anhydrous KNPU/g (%)
0 days	63.0(100)	15.8(100)	9.18(100)	7.63(100)
1 -	65.6(104)	17.5(111)	7.45(81)	7.05(92)
5 -	57.7(92)	14.7(93)	7.61(83)	6.48(85)

It clearly appears from the above table that the stability of the crystalline preparations is better than the stability of the amorphous preparations.

Claims

1. Enzyme containing preparation, characterized by the fact that it contains a protease and at least one protease sensitive enzyme, wherein substantially 100% of the proteolytic activity and/or substantially 100% of the protease sensitive enzyme or the protease sensitive enzymes are present as crystals, wherein more than 90% of the crystals possess a maximum crystal dimension between 0.01 μm and 500 μm, preferably between 0.1 μm and 100 μm, and that the preparation preferably contains a stabilizing agent for the enzymes.

2. Preparation according to Claim 1, characterized by the fact, that the protease sensitive enzyme is a lipase, amylase, cellulase, hemicellulase, pectinase, amidase or oxidase, or protein engineered variants of these.

3. Preparation according to Claims 1 - 2, characterized by the fact that the protease sensitive enzyme is a lipase and that the lipase is Lipolase®.

4. Detergent, characterized by the fact that it contains the preparation according to Claims 1 - 3, and that the detergent either is solid and contains the enzyme containing preparation as a granulate in a concentration of between 0.001 and 10 mg of enzyme protein/g of detergent, preferably between 0.005 and 5 mg of enzyme protein/g of detergent, most preferably from 0.01 to 1 mg of enzyme protein/g of detergent, or that the detergent is liquid and contains the enzyme containing preparation as a slurry in an amount of between 0.001 and 10 mg of enzyme protein per g of detergent, preferably from 0.005 to 5 mg of enzyme protein per g of detergent, most preferably from 0.01 to 1 mg of enzyme protein per g of detergent.

Patentansprüche

1. Enzymhaltige Zubereitung, gekennzeichnet durch die Tatsache, daß sie eine Protease und wenigstens ein protease-sensitives Enzym enthält, wobei im wesentlichen 100% der proteolytischen Aktivität und-/oder im wesentlichen 100% des protease-sensitiven Enzyms oder der protease-sensitiven Enzyme als Kristalle vorliegen, wobei mehr als 90% der Kristalle eine maximale Kristallgröße zwischen 0,01 μm und 500 μm, vorzugsweise zwischen 0,1 μm und 100 μm besitzen, und daß die Zubereitung vorzugsweise ein Stabilisierungsmittel für die Enzyme enthält.

2. Zubereitung nach Anspruch 1, gekennzeichnet durch die Tatsache, daß das protease-sensitive Enzym

eine Lipase, Amylase, Cellulase, Hemicellulase, Pektinase, Amidase oder Oxidase ist, oder proteintechnologische Varianten derselben.

3. Zubereitung nach den Ansprüchen 1-2, gekennzeichnet durch die Tatsache, daß das protease-sensitive Enzym eine Lipase ist und daß die Lipase Lipolase® ist.

4. Detergens, gekennzeichnet durch die Tatsache, daß es die Zubereitung nach den Ansprüchen 1-3 enthält und daß das Detergens entweder fest ist und die enzymhaltige Zubereitung als ein Granulat in einer Konzentration von zwischen 0,001 und 10 mg Enzymprotein/g Detergens, vorzugweise zwischen 0,005 und 5 mg Enzymprotein/g Detergens, am bevorzugtesten von 0,01 bis 1 mg Enzymprotein/g Detergens enthält, oder daß das Detergens flüssig ist und die enzymhaltige Zubereitung als eine Aufschlämmung in einer Menge von zwischen 0,001 und 10 mg Enzymprotein pro g Detergens, vorzugsweise von 0,005 bis 5 mg Enzymprotein pro g Detergens, am bevorzugtesten von 0,01 bis 1 mg Enzymprotein pro g Detergens enthält.

Revendications

1. Préparation contenant des enzymes, caractérisée par le fait qu'elle contient une protéase et au moins une enzyme sensible aux protéases, dans laquelle pratiquement 100 % de l'activité protéolytique et/ou pratiquement 100 % de l'enzyme sensible aux protéases ou des enzymes sensibles aux protéases sont présentes sous forme de cristaux, plus de 90 % des cristaux ayant une dimension maximale comprise entre 0,01 µm et 500 µm, de préférence entre 0,1 µm et 100 µm, et par le fait que la préparation contient de préférence un agent stabilisant pour les enzymes.

2. Préparation selon la revendication 1, caractérisée par le fait que l'enzyme sensible aux protéases est une lipase, une amylase, une cellulase, une hémicellulase, une pectinase, une amidase ou une oxydase, ou des dérivés de ces enzymes obtenus par génie protéique.

3. Préparation selon les revendications 1-2, caractérisée par le fait que l'enzyme sensible aux protéases est une lipase et que la lipase est la Lipolase®.

4. Détergent caractérisé par le fait qu'il contient la préparation selon les revendications 1-3, et que, soit le détergent est solide et contient la préparation contenant les enzymes sous forme d'un granulé à une concentration comprise entre 0,001 et 10 mg de protéine enzymatique/g de détergent, de préférence entre 0,005 et 5 mg de protéine enzymatique/g de détergent, de façon la plus préférable entre 0,01 et 1 mg de protéine enzymatique/g de détergent, soit le détergent est liquide et contient la préparation contenant les enzymes sous forme d'une suspension épaisse en une quantité comprise entre 0,001 et 10 mg de protéine enzymatique par g de détergent, de préférence entre 0,005 et 5 mg de protéine enzymatique par g de détergent, de façon la plus préférable entre 0,01 et 1 mg de protéine enzymatique par g de détergent.