United States Patent [19]

Ziffer

[11] **3,717,550**

[45] **Feb. 20, 1973**

[54] **LIQUID COMPOSITIONS OF BACTERIAL PROTEASE AND/OR AMYLASE AND PREPARATION THEREOF**

[75] Inventor: **Jack Ziffer,** Milwaukee, Wis.

[73] Assignee: **Pabst Brewing Company,** Milwaukee, Wis.

[22] Filed: **Sept. 25, 1970**

[21] Appl. No.: **75,764**

[52] **U.S. Cl.**.....**195/63,** 195/68, 252/DIG. 12, 8/138

[51] **Int. Cl.** **C07g 7/02**

[58] **Field of Search**.....195/68, 63, 62, 65, 66; 252/DIG. 12

[56] **References Cited**

UNITED STATES PATENTS

3,033,691	5/1962	Smythe et al.	99/222
3,557,002	1/1971	McCarty	252/89
3,472,783	10/1969	Smillie	195/68 X
3,296,094	1/1967	Cayle	195/63
3,095,358	6/1963	Meister	195/63

OTHER PUBLICATIONS

Yasumatsu et al. Agricultural and Biological Chemistry vol. 29, No. 7 pp. 665–671 (1965) Vol.

Primary Examiner—Lionel M. Shapiro

Attorney—Johnston, Root, O'Keeffe, Keil, Thompson & Shurtleff

[57] **ABSTRACT**

Liquid concentrates of bacterial protease and/or amylase of enhanced stability are prepared. Bacterial protease and/or amylase liquid compositions free of turbidity and a process of producing them are provided. These compositions have advantages over powdered enzymes in avoiding atmospheric contamination and are especially useful in detergents and in textile treatment.

20 Claims, No Drawings

LIQUID COMPOSITIONS OF BACTERIAL PROTEASE AND/OR AMYLASE AND PREPARATION THEREOF

BACKGROUND OF THE INVENTION

In recent years the use of enzymes, especially bacterial protease and bacterial amylase, for presoak and detergent washing products has greatly increased. These enzymes are now being produced in the form of powders in very high potency concentrations and handling problems have developed as a result of these high potency levels. Many individuals have found these enzymes to be a source of irritation. The problem is particularly acute in the manufacturing and handling of the enzymes themselves and also in the handling of the enzymes in plants where they are formulated into various forms of detergent products. In order to alleviate the problem it has heretofore been proposed to produce a dust-free powdered enzyme product.

Another possible solution of the problem would be the provision of the enzymes in the form of a liquid composition. When attempts are made to do this, however, other problems arise. One of these problems is the instability of the enzymes which causes the enzyme activity of a liquid product to be reduced in storage. Another problem arises due to the fact that the enzymes as normally produced form turbid solutions in water containing suspended particles. It would, therefore, be desirable to solve the dusting problem which is a source of irritation to individuals by providing a liquid composition containing enzymes which retain a relatively high activity when the composition is stored prior to use. In addition, It would be desirable to provide high potency liquid concentrates of bacterial protease and/or amylase which are free of turbidity. These liquid concentrates of bacterial protease and/or amylase of enhanced stability would avoid the source of irritation caused by the dusting of powdered enzymes and they could be used in the manufacture of various detergent products. In addition, liquid enzyme compositions tree from turbidity would be acceptable for use as such in household detergent formulations and also as enzyme compositions for the treatment of textiles, for example, in the de-sizing of textile fibers.

OBJECTS

One of the objects of the present invention is to provide new and improved liquid concentrates of bacterial protease and/or bacterial amylase of enhanced stability.

Another object of the invention is to provide liquid compositions containing bacterial protease and/or bacterial amylase which are free of turbidity.

Still a further object of the invention is to provide a new and improved process for producing liquid compositions containing bacterial protease and/or bacterial amylase.

Another object of the invention is to provide new and improved liquid compositions containing bacterial protease and/or bacterial amylase in combination with surfactants. Other objects will appear hereinafter.

BRIEF SUMMARY OF THE INVENTION

In accordance with the invention liquid compositions are prepared consisting essentially of: Component (A), fermentation soluble solids consisting essentially of an enzyme from the group consisting of bacterial protease and bacterial amylase and mixtures thereof, and Component (B), a liquid from the group consisting of water, a normally liquid water miscible polyhydric alcohol, and both water and a normally liquid water miscible polyhydric alcohol, in which said enzyme is soluble to the extent of at least 32 percent by weight fermentation soluble solids at 4.4°C. (40°F), said composition containing a concentration of said components sufficient to maintain an enzyme activity of at least 50 percent when said composition is stored in a container under atmospheric pressure for 6 days at 42°C.

The compositions of the invention will normally have a protease activity of at least 5,000 PV units per gram at 4.4°C., preferably within the range of 5,000 to 2,000,000 PV units per gram at 4.4°C., and generally within the range of 30,000 to 600,000 PV units per gram at 4.4°C. The amylase activity can be up to 1,000,000 DV units per gram at 4.4°C., is preferably at least 10,000 DV units per gram at 4.4°C., and is generally within the range of 30,000 to 400,000 DV units per gram at 4.4°C. The enzymes in the liquid compositions can consist essentially of bacterial protease or bacterial amylase or both bacterial protease and bacterial amylase in the above described proportions.

The liquid compositions can be used as such for washing or they can be mixed with other ingredients for the preparation of detergent compositions. Thus, they can be mixed with various types of compatible surfactants to form liquid concentrates which can be added to wash water in predetermined amounts.

It has been found that where Component (B) is water, the stability of the enzymes is enhanced by maintaining a relatively high enzyme concentration, namely, a concentration of at least 32 percent by weight at 4.4°C. (40°F.). Where a polyhydric alcohol such as glycerol, either alone or mixed with water, constitutes the liquid phase, the retained activity of the enzymes over a period of time is enhanced even at enzyme concentrations below 32 percent by weight. Furthermore, polyhydric alcohols, for example, glycerol, propylene glycol, ethylene glycol, diethylene glycol and higher liquid homologues, have the property of removing the turbidity which is normally present in concentrated bacterial protease and bacterial amylase solutions in water. Hence, the use of polyhydric alcohols in the preparation of these liquid compositions makes it possible to produce clear liquid compositions which do not leave deposits when employed in washing procedures and in textile treatment and are therefore more acceptable for such uses.

DETAILED DESCRIPTION OF THE INVENTION

In preparing compositions in accordance with the invention several different methods may be used. The usual procedure involves first preparing an aqueous concentrate by adding bacterial protease and bacterial amylase to water and agitating the resultant composition at room temperature (20°–32°C.) so as to dissolve the enzyme. The composition is then filtered and the filter cake washed with water. The combined filtrate and wash water after concentration is a turbid solution, probably containing colloidal protein solid in suspension. In accordance with the invention it has been

found that the retained enzyme activity of this solution on standing is enhanced by increasing the proportion of enzyme solids in the solution to the point where they are at least 32 percent by weight, based on the weight of the dry solids, and preferably within the range of 32 percent to saturation. In most cases this range will be from 32 to 45 percent by weight.

Surprisingly, when a liquid polyhydric alcohol is added to the previously described turbid enzyme solution it clarifies the solution. Furthermore, when the polyhydric alcohol is glycerol, the enzyme solution is not only clarified but the retained enzyme activity on storage is greatly enhanced, even when the concentration of enzyme solids is less than 32 percent by weight. Thus, the concentration of enzyme solids can be within the range of 2 to 60 percent by weight and is usually within the range of 32 to 43.5 percent by weight. Where glycerol is employed the composition may contain 40 to 98 percent by weight glycerol. Various types of compositions in which the liquid phase consists essentially of both water and a polyhydric alcohol can be prepared as hereinafter described.

As previously indicated, these liquid enzyme compositions can be employed as such in washing by adding them to the wash water in the proper proportions. In most cases the proportions should be sufficient to give an enzyme concentration of 200–2,000 PV units/liter and 0–500 DV units/liter in a water having a pH within the range of 8 to 12. Compatible surfactants can also be added to the liquid enzyme concentrates. For most purposes the surfactant is either a nonionic or an anionic surfactant. It may also be an amphoteric surfactant. Any of the compatible surfactants described in McCutcheon's Detergents and Emulsifiers, 1969 Annual, can be employed. Examples of such surfactants are Alrosperse DC, Triton QS–15, QS–30, BG–5, and X–102. It is usually preferable to carry out routine experiments in order to determine the compatibility of a particular surfactant. Some of these surfactants are compatible with some polyhydric alcohols but not with others.

The invention will be further illustrated but is not limited by the following examples in which the quantities are stated in parts by weight unless otherwise specified.

EXAMPLE 1

A 20 percent (w/v) solution of $Al_2(SO_4)_3 \cdot 18H_2O$ was slowly added to an agitated bacterial protease fermentation whole culture (500 gal., 10,720 PV units per ml., 20.3×10^9 PV units) while maintaining the whole culture pH at 6.4 by the simultaneous addition of Na_2SO_3 (42.5 lb.). The amount of $Al_2(SO_4)_3 \cdot 18H_2O$ added was 1 percent (w/v) based on the whole culture. Coagulants Nalco 674 (120 grams, as a 0.25 percent (w/v) solution) and Purifloc C–31(5,025 grams, as a 27 percent (w/v) solution were then added, together with filteraid Chem-Flo 325 CK (290 lbs.). The mixture (pH 5.3) was adjusted to pH 6.4 with NaOH and filtered on a plate and frame press. The resultant filtered cake was washed with water and the combined clear solution of filtrate plus wash evaporated in vacuo to 83.6 gal. (pH 6.3, 18.1×10^9 PV units) for a filtration and concentration recovery of 89.2 percent. Sodium sulfate (7,000 grams), sodium sulfite (1,600 grams) and sodium bisulfite (200 grams) were added to a portion of the concentrate warmed to 45°C. (40,000 ml., pH6.4, 25 percent dry solids, 22.40×10^8 PV units) and the mixture filtered on a small Dorr-Oliver rotary filter using a Na_2SO_4 wash. The total PV units recovered in the filtered cake (4,794 grams) and heel totaled 21.94×10^8 PV units for a filtration recovery of 97.9 percent. The overall recovery from the whole culture was 86.7 percent.

EXAMPLE 2

The bacterial protease filtered cake obtained in Example 1 after precipitation with Na_2SO_4, Na_2SO_3 and $NaHSO_3$ was used to prepare a series of aqueous concentrates. Distilled water (7,200 ml.) was added to a portion of the filtered cake (1,765 grams, 680×10^6 PV units) and the mixture agitated at room temperature for 1.5 hours. The mixture was then filtered on a Buchner funnel using a Filteraid FW–20 pre-coat and the filtered cake washed with water (3,000 ml.). The combined filtrate and washed totaled 11,840 ml. and contained 620×10^6 PV units, for a protease enzyme recovery of 91.2 percent. Portions of the combined filtrate and wash were evaporated in vacuo to varying concentrations of dry solids. The resultant concentrates were visually tan turbid solutions, appearing to contain colloidal protein solid in suspension. Aliquots of these concentrates were tested at 4.4°C. (40°F.) and 42°C. (107.6°F.) for storage stability in containers at atmospheric pressure. The results obtained are shown below.

% Dry Solids in Concentrate	Protease Activity (PV/Gm) After 36 Days at Indicated Temperature 4.4°C.	42°C.	% Protease activity remaining
31.4	308,000	180,000	58.4
33.8	334,000	217,000	65.0
37.8	392,000	270,000	68.9
43.5	425,000	362,000	85.2

EXAMPLE 3

Using the procedures described in Examples 1 and 2, an aqueous concentrate was prepared having a per cent dry solids of 32 percent and a protease enzyme activity of 360,000 PV units per gram. The resultant concentrate was a tan turbid solution containing suspended solid.

A portion of the aqueous concentrate (100 grams) was added to distilled water (20 grams). The resultant mixture was still a tan turbid solution containing suspended solid. Aliquots of this solution (without any further evaporation) were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (100 grams) was added to propylene glycol (20 grams, reagent grade) resulting in an almost complete clearing of the turbidity of the aqueous concentrate. Aliquots of this solution (without any further evaporation) were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (100 grams) was added to propylene glycol (50 grams, reagent grade) and the mixture evaporated in vacuo to 100 grams final weight. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (100 grams) was added to propylene glycol (50 grams, reagent grade) and the mixture evaporated in vacuo to 100 grams. An additional 20 grams of propylene glycol was then added to the clear solution resulting in a final total weight of 120 grams, of which 70 grams constituted propylene glycol. Aliquots of this clear solution were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (200 grams) was added to ethylene glycol (100 grams, reagent grade) and the mixture evaporated in vacuo to 200 grams final weight. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

Additional ethylene glycol (20 grams) was added to a portion of the above ethylene glycol-aqueous solution (100 grams). Aliquots of this clear mixture (without any further concentration) were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (200 grams) was added to diethylene glycol (100 grams) and the mixture evaporated in vacuo to 200 grams final weight. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

Additional diethylene glycol (20 grams) was added to a portion of the above diethylene glycol-aqueous solution (100 grams). Aliquots of this clear mixture (without any further concentration were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (200 grams) was added to glycerol (100 grams, U.S.P. grade) and the mixture evaporated in vacuo to 200 grams final weight. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

Additional glycerol (20 grams) was added to a portion of the above glycerol-aqueous solution (100 grams). Aliquots of this clear mixture (without any further concentration) were tested at 4.4°C. and 42°C. for storage stability.

The data obtained for these products are as follows:

Polyhydride alcohol used	% Polyhydric Alcohol in Product	% Non-Alcohol Dry Solids	Storage, Days	Protease activity PV/Gm. remaining after storage at indicated temperatures 4.4°C.	42°C.	% Protease Activity Remaining
None	None	32	14	350 K	192 K	54.9
None	None	26.7	14	304 K	96 K	31.6
propylene glycol	16.7	26.7	7	320 K	200 K	62.5
propylene glycol	50	32	14	380 K	140.8 K	37.1
propylene glycol	58	26.7	7	282 K	125 K	44.3
ethylene glycol	50	32	14	375 K	125.4 k	33.4
ethylene glycol	58	26.7	7	275 k	95 k	34.5
diethylene glycol	50	32	13	375 k	98.4 k	26.2
diethylene glycol	58	26.7	6	282.5 k	207.5 k	73.5
glycerol	50	32	6	340 k	325 k	95.6
			21	340 k	315 k	92.6
			43	355 k	286.4 k	80.7
glycerol	58	26.7	6	302.5 k	275 k	90.9
			21	300 k	252	84.0
			43	310 k	280 .3 k	90.4

EXAMPLE 4

Using the procedures described in Examples 1 and 2, an aqueous protease concentrate was prepared having a per cent dry solids of 38.3 percent and a protease enzyme activity of 355,000 PV units per gram. The resultant concentrate was a tan turbid solution containing suspended solid. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (200 grams) was added to glycerol (100 grams, U.S.P. grade) and the mixture evaporated in vacuo to 200 grams final weight. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

The data obtained for these products are shown below:

% glycerol in product	% Non-glycerol dry solids	storage, days	Protease Activity PV/Gm. Remaining after Storage at Indicated Temperatures 4.4°C.	42°C.	% protease activity remaining
None	38.3	21	367,500	328,000	89.3
50	38.3	20	400,000	400,000	100.0

EXAMPLE 5

Using the procedures described in Examples 1 and 2, an aqueous concentrate was prepared having a per cent dry solids of 32 percent and a protease enzyme activity of 350,000 PV units per gram. The resultant concentrate was a tan turbid solution containing suspended solid.

A portion of the aqueous concentrate (400 grams) was added to glycerol (128 grams, U.S.P. grade) and the mixture evaporated in vacuo to 294 grams. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (100 grams) was added to glycerol (55 grams, U.S.P. grade) and the mixture evaporated in vacuo to 100 grams. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (100 grams) was added to glycerol (60 grams, U.S.P. grade) and the mixture evaporated in vacuo to 100 grams. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (120 grams) was added to glycerol (53 grams, U.S.P. grade) and the mixture evaporated in vacuo to 107 grams. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (120 grams) was added to glycerol (48 grams, U.S.P. grade) and the mixture evaporated in vacuo to 98 grams. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

The data obtained for these products are shown below:

% glycerol in product	% non-glycerol Dry solids	storage, days	Protease Activity PV/Gm. Remaining After Storage At Indicated Temperatures 4.4°C.	42°C.	% Protease activity remaining
43.5	43.5	34	425,000	312,500	73.5
55	32	34	328,000	280,000	85.4
60	32	34	322,000	316,000	98.1
50	36	34	372,500	368,000	98.8
50	38	34	402,500	360,000	89.4

EXAMPLE 6

Using the procedures described in Examples 1 and 2, an aqueous concentrate was prepared having a percent dry solids of 38.9 percent and a protease enzyme activity of 381,000 PV units per gram. The resultant product was a tan turbid solution containing suspended solids. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

A portion of the aqueous concentrate (1,000 grams) was added to glycerol (500 grams, U.S.P. grade) and the mixture evaporated in vacuo to 1,000 grams. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 42°C. for storage stability.

Portions of the glycerol-aqueous concentrate were then further diluted with additional glycerol in the glycerol concentration range of 50–99 percent of the total solution. The resultant products were clear solutions. Aliquots of these solutions were tested at 4.4°C. and 42°C. for storage stability.

The data obtained for these products are shown below:

% Glycerol in Product	% Non-Glycerol Dry Solids	% Water	Protease Activity PV/Gm. Remaining after Storage at Indicated Temperatures Storage, days	4.4°C.	42°C.	% Protease Activity Remaining
None	38.9	61.1	15	367,500	354,000	96.3
50.0	38.9	11.1	15	367,500	360,000	98.0
83.3	13.0	3.7	13	128,000	126,700	98.9
90.0	7.8	2.2	13	74,800	70,400	94.1
95.0	3.9	1.1	13	39,000	32,200	82.6
97.4	2.0	0.6	13	19,000	15,500	81.6
98.7	1.0	0.3	13	9,750	4,750	48.7

EXAMPLE 7

Using the salt treatment and precipitation procedure described in U.S. Ser. No. 849,148, filed Aug. 11, 1969, a filtered precipitated bacterial amylase wet cake was obtained assaying 128,000 DV units per gram. Tap water (4,000 ml.) was added to a portion of the filtered cake (1,000 grams, 128.0×10^6 DV units) and the mixture agitated at room temperature for one hour.

$Al_2(SO_4)_3 \cdot 18H_2O$ (25 grams, as a 20 percent (w/v) solution) was then added and the pH maintained at 6.4 by the simultaneous addition of Na_2SO_3 (30 grams). After the addition of the salts, Chem-Flo 325 CK filteraid (350 grams) was added and the mixture filtered on a Buchner funnel, followed by a tap water wash. The combined filtrate and wash (7,850 ml., pH 6.65) was evaporated in vacuo to a concentrate (33.6 percent dry solids, pH 6.3) containing 121.1×10^6 DV units for an amylase enzyme recovery of 94.6 percent. The resultant concentrate was a visually tan turbid solution, appearing to contain colloidal protein solid in suspension. Aliquots of this concentrate were tested at 4.4°C., 28°C. and 42°C. for storage stability. The results obtained are shown below:

% Dry Solids In Concentrate	Amylase Activity (DV/Gm.) After 36 Days at Indicated Temperature 4.4°C.	28°C.	42°C.
33.6	87,200	86,300	83,700

EXAMPLE 8

a. Using the procedures described in Example 3, a portion of the aqueous concentrate (200 grams) from Example 7 was added to glycerol (100 grams, U.S.P. grade) and the mixture evaporated in vacuo to 200 grams final weight. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C., 28°C., 42°C. and 55°C. for storage stability. The results obtained are shown below:

% glycerol in concentrate	% non-glycerol dry solids	Amylase Activity (DV/Gm.) After 30 Days at Indicated Temperature 4.4°C.	28°C.	42°C.	55°C.
50	33.6	87,600	90,000	92,900	89,200

b. Using the procedures described in Example 3, a portion of the aqueous concentrate (450 grams) from Example 7 was added to glycerol (225 grams, U.S.P. grade) and the mixture evaporated in vacuo to 450 grams final weight. The resultant product was a clear solution. Aliquots of this solution were tested at 4.4°C. and 55°C. for storage stability. The results obtained are shown below:

% Glycerol in Concentrate	% Non-Glycerol Dry Solids	Amylase activity (DV/Gm.) After 12 Days at Indicated Temperature 4.4°C.	55°C.	% Amylase Activity remaining
None	33.6	84,990	44,410	52.3
50	33.6	90,070	85,720	95.2

EXAMPLE 9

An enzyme concentrate was prepared by mixing equal parts by weight of a bacterial protease-glycerol concentrate containing 50 percent glycerol by weight and having an activity of 464,000 PV units per gram with a bacterial amylase concentrate containing 50 percent glycerol by weight and having an activity of 90,680 DV units per gram and 20,000 PV units per gram.

The final product contained a protease concentration of 242,000 PV units per gram and 45,340 DV units per gram.

EXAMPLE 10

The procedure was the same as in Example 9 except that additional glycerol was added and the relative proportions of bacterial protease-glycerol concentrate, bacterial amylase-glycerol concentrate and glycerol were, respectively, 12.42 percent, 65.70 percent and 21.88 percent by weight. The final product had a protease concentration of 70,760 PV units per gram and 59,580 DV units per gram.

EXAMPLE 11

The procedure was the same as in Example 9 except that additional glycerol was added and the relative proportions of bacterial protease-glycerol concentrate, bacterial amylase-glycerol concentrate and glycerol were 46.25 percent, 37.05 percent and 16.70 percent by weight, respectively. The final product contained a protease concentration of 222,200 PV units per gram and 33,600 DV units per gram.

The following examples illustrate the preparation of enzyme compositions of the type described containing surfactants.

EXAMPLE 12

The bacterial protease-glycerol concentrate of Example 9 containing 50 percent by weight glycerol and having a protease concentration of 464,000 PV units per gram was mixed in a weight ratio of 4:1 with Alrospere DC, to produce a composition having a protease concentration of 371,200 PV units per gram.

EXAMPLE 13

The procedure was the same as in Example 12 except that 80 parts of the protease-glycerol concentrate was mixed with 5 parts Alrosperse DC and 15 parts of additional glycerol. The protease concentration was the same as in Example 12.

EXAMPLE 14

The procedure was the same as in Example 12 except that the surfactant Triton QS–15 was used.

EXAMPLE 15

The procedure was the same as in Example 13 except that the surfactant Triton QS–15 was used.

EXAMPLE 16

The procedure was the same as in Example 12 except that the surfactant Triton BG–5 was used.

EXAMPLE 17

The procedure was the same as in Example 13 except that the surfactant Triton BG–5 was used.

EXAMPLE 18

The procedure was the same as in Example 12 except that a bacterial amylase-glycerol concentrate containing 50 percent by weight glycerol was used instead of the bacterial protease-glycerol concentrate. Since the bacterial amylase contained some protease, the final product had an amylase concentration of 72,540 DV units per gram and a protease concentration of 16,000 PV units per gram.

EXAMPLE 19

The procedure was the same as in Example 13 except that a bacterial amylase-glycerol concentrate of the type employed in Example 18 was used. The final product had an amylase concentration of 72,540 DV units per gram and a protease concentration of 16,000 PV units per gram.

EXAMPLE 20

The procedure was the same as in Example 18 except that Triton QS–15 was used instead of Alrosperse DC.

EXAMPLE 21

The procedure was the same as in Example 19 except that Triton QS–15 was used instead of Alrosperse DC.

EXAMPLE 22

The procedure was the same as in Example 18 except that Triton BG–5 was used instead of Alrosperse DC.

EXAMPLE 23

The procedure was the same as in Example 19 except that Triton BG–5 was used instead of Alrosperse DC.

EXAMPLE 24

An enzyme-surfactant concentrate was prepared by mixing 40 parts of bacterial protease-glycerol concentrate containing 50 percent glycerol and having a protease concentration of 464,000 PV units per gram, 40 parts of a bacterial amylase-glycerol concentrate containing 50 percent glycerol and having an amylase activity of 90,680 DV units per gram, and a protease activity of 20,000 PV units per gram, 5 parts of Alrosperse DC and 15 parts glycerol. The final product had a protease concentration of 193,600 PV units per gram and an amylase concentration of 36,270 DV units per gram.

EXAMPLE 25

The procedure was the same as in Example 24 except that Triton BG–5 was substituted for the Alrosperse DC.

EXAMPLE 26

The procedure was the same as in Example 24 except that Triton QS–15 was substituted for the Alrosperse DC.

It should be noted that the bacterial amylase enzyme normally contains some bacterial protease enzyme. In Example 7 the protease enzyme activity is not given.

In Examples 12 to 26 all of the surfaces were found to be compatible with the enzyme concentrates. The Alrosperse DC is an oil soluble surfactant which is non-ionic plus a cationic mixture of fatty alkoamides. Triton BG–5 is a non-ionic surfactant. Triton QS–15 is a liquid anhydrous amphoteric surfactant in the form of an oxyethylated sodium salt containing both anionic and cationic centers. All of these surfactants are identified in McCutcheon's 1969 Annual, supra.

In the foregoing examples the recovery procedures used in preparing bacterial protease and/or the bacterial amylase, as illustrated specifically in Example 1, do not constitute a part of the present invention but are

disclosed and claimed in U.S. application Ser. No. 849,148 filed Aug. 11, 1969.

It may be mentioned that the clarity of the enzyme solution depends upon the particular enzyme and the concentration thereof. The addition of the polyhydric alcohol serves to reduce the turbidity and thereby increase the clarity of the solution. This does not necessarily mean, however, that one can see through a given sample of solution after it has been clarified. Thus, in the case of enzyme compositions which are concentrated to the point that they are of a syrupy consistency, the composition prior to treatment with the polyhydric alcohol will contain suspended particles which cause turbidity and will usually have a tan appearance whereas after the treatment with a polyhydric alcohol the turbidity will be reduced due to the dissolution of the particles and the resultant clarified composition will have a darker brown appearance.

The invention is hereby claimed as follows:

1. A liquid composition consisting essentially of:

Component (A), fermentation soluble solids consisting essentially of an enzyme from the group consisting of bacterial protease and bacterial amylase and mixtures thereof, and

Component (B), a liquid from the group consisting of water, a normally liquid, water miscible polyhydric alcohol, and both water and a normally liquid, water miscible polyhydric alcohol, in which said enzyme is soluble to the extent of at least 32% fermentation soluble solids by weight at 4.4°C., said composition containing a concentration of said components sufficient to maintain an enzyme activity of at least 50% when said composition is stored in a container under atmospheric pressure for six days at 42°C., Component (A) constituting at least 32% by weight of the solids content of said composition, on a dry basis.

2. A composition as claimed in claim 1 in which the protease activity is at least 5,000 PV units per gram at 4.4°C.

3. A composition as claimed in claim 1 in which the protease activity is 5,000 to 2,000,000 PV units per gram at 4.4°C.

4. A composition as claimed in claim 1 in which the protease activity is 30,000 to 600,000 PV units per gram at 4.4°C.

5. A composition as claimed in claim 1 in which the amylase activity is at least 10,000 DV units per gram at 4.4°C.

6. A composition as claimed in claim 1 in which the amylase activity is 10,000 to 1,000,000 DV units per gram at 4.4°C.

7. A composition as claimed in claim 1 in which the amylase activity is 30,000 to 400,000 DV units per gram at 4.4°C.

8. A composition as claimed in claim 1 in which the solids content, on a dry basis, of the enzyme is within the range of 32% by weight to saturation.

9. A composition as claimed in claim 1 in which the solids content, on a dry basis, of the enzyme is within the range of 32 to 43.5 percent by weight.

10. A composition as claimed in claim 1 in which component (A) consists essentially of at least 32 percent by weight, on a dry basis, of the composition of bacterial protease, and component (B) consists essentially of glycerol.

11. A composition as claimed in claim 1 in which component (A) consists essentially of at least 32 percent by weight, on a dry basis, of the composition of bacterial amylase, and component (B) consists essentially of glycerol.

12. A composition as claimed in claim 1 in which component (A) consists essentially of at least 32 percent by weight, on a dry basis, of the composition of both bacterial protease and bacterial amylase, and component (B) consists essentially of glycerol.

13. A composition as claimed in claim 1 in which said enzyme is present in a concentration of at least 32 percent by weight, on a dry basis, and said liquid is from the group consisting of glycerol and water.

14. A process of clarifying an aqueous solution of an enzyme from the group consisting of bacterial protease, bacterial amylase and mixtures thereof which comprises adding to a turbid aqueous solution in which the concentration of said enzyme is at least 32 percent by weight, on a dry basis, a sufficient amount of a liquid, water miscible polyhydric alcohol to reduce the turbidity.

15. A process as claimed in claim 14 in which said polyhydric alcohol is glycerol.

16. A process as claimed in claim 14 in which said enzyme is bacterial protease.

17. A process as claimed in claim 14 in which said aqueous solution containing said polyhydric alcohol is concentrated under conditions facilitating the elimination of at least a part of the water without removing said polyhydric alcohol.

18. A process as claimed in claim 17 in which said concentration is carried out under subatmospheric pressure conditions.

19. A process as claimed in claim 14 in which said enzyme is bacterial amylase.

20. A process as claimed in claim 14 in which said enzyme is a mixture of bacterial protease and bacterial amylase.

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