(19) DANMARK

(10) DK/EP 1996028 T4

(12) Oversættelse af ændret europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *A 23 K 1/165 (2006.01)* *C 12 N 9/96 (2006.01)* *C 12 N 9/98 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2016-03-07**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om opretholdelse af patentet i ændret form: **2015-12-02**

(86) Europæisk ansøgning nr.: **07726771.4**

(86) Europæisk indleveringsdag: **2007-03-09**

(87) Den europæiske ansøgnings publiceringsdag: **2008-12-03**

(86) International ansøgning nr.: **EP2007052256**

(87) Internationalt publikationsnr.: **WO2007104725**

(30) Prioritet: **2006-03-10 EP 06004998**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC MT NL PL PT RO SE SI SK TR**

(73) Patenthaver: **BASF SE, , 67056 Ludwigshafen, Tyskland**

(72) Opfinder: **LOHSCHEIDT, Markus, Burgstrasse 24, 69121 Heidelberg, Tyskland**
Betz, Roland, Im Brühl 5, 67150 Niederkirchen, Tyskland
BRAUN, Jörg, Osterbergstr. 5a, 76879 Essingen, Tyskland
PELLETIER, Wolf, Ludwigstrasse 51, 76879 Ottersheim, Tyskland

(74) Fuldmægtig i Danmark: **RWS Group, Europa House, Chiltern Park, Chiltern Hill, Chalfont St Peter, Bucks SL9 9FG, Storbritannien**

(54) Benævnelse: **FASTE ENYMFORMULERINGER OG FREMGANGSMÅDE TIL DERES FREMSTILLING**

(56) Fremdragne publikationer:
EP-A1- 0 758 018
EP-A2- 0 257 996
US-A- 5 972 669
US-B1- 6 610 519

Description

The present invention relates to novel solid enzyme formulations comprising mixtures of at least one salt-stabilized enzyme composition, at least one particulate support and at least one hydrophobic liquid. In addition, the invention relates to methods for producing such solid enzyme formulations and also methods for producing animal feed, food and food supplement which comprise such enzyme formulations.

Background of the invention

From the prior art, numerous solid enzyme compositions are known which are produced, for example, by spray-drying liquid enzyme solutions. It is further known that the enzyme stability in such spray-drying processes can be significantly increased by adding stabilizing salts, such as, for example, magnesium sulfate. This therefore produces in this manner solid enzyme compositions which also, even after spray drying, have a high enzyme activity percentage. For example, in EP-A-0 758 018, storage-stable and processing-stable solid enzyme compositions are described which are obtained by drying a solution comprising at least one enzyme and a water-soluble inorganic salt. The enzyme compositions described there are preferably used as additive for solid animal feed compositions.

US 5,972,669 discloses solid, salt-stabilized enzyme solutions that can be used in feedstuffs, wherein said solid, salt-stabilized enzyme solutions can be in a mixture with other feedstuff components, such as meals and soybean oil.

US 6,610,519 discloses solid phytase granules which are stabilized by means of lactic acid (from Corn Steep Liquor), and optionally comprise a fat coating.

For the production of such enzyme-additized animal feed compositions, it is desirable that the enzyme is distributed

as uniformly as possible in the finished feed preparation. Since the dry enzyme preparations comprise the enzyme in high concentration, addition of significantly less than 1% by weight, based on the total weight of the feed composition, is generally completely sufficient to provide the desired enzyme activity for the feed composition. The lower the required amount of enzyme to be added, the more difficult it is, however, to achieve uniform distribution of the enzyme activity in the finished feed preparation. The same difficulty is of course also observed in the production of foods and food supplements to which highly concentrated solid enzyme compositions are to be added distributed as uniformly as possible.

The object of the invention is therefore to find a way which makes it possible to bring highly concentrated solid enzyme compositions which essentially comprise only enzyme and stabilizing support into a form which ensures uniform and reproducible dosage to foods and feeds. At the same time the invention should also ensure that the formulations used therefor have good processing properties, such as reduced dusting tendency, good rheological behavior and narrow particle size distribution.

Summary of the invention

Said object has surprisingly been achieved by providing a solid enzyme formulation according to claim 1, which is obtained by mixing a particulate, salt-stabilized enzyme composition, a particulate support, and also a hydrophobic liquid. In particular, it was surprising that the inventively produced solid formulations are particularly easy to handle, since they exhibit high separation stability, extremely low dusting tendency and, despite addition of hydrophobic liquid, an excellent rheological behavior.

Description of figures

Figure 1 shows on the basis of a flow diagram a preferred embodiment of the present invention, in particular the production of a solid xylanase formulation. For this, a xylanase-comprising liquid concentrate is mixed with magnesium sulfate, dried in a spraying apparatus to give a xylanase-comprising stabilized powder and simultaneously agglomerated, particles, for example having a size in the range from 50 to 250 µm, being able to be obtained. In the next step, the xylanase-comprising dry powder is mixed with a solid organic support and simultaneously or subsequently sprayed with soybean oil. This produces in this manner a xylanase-comprising formulation having low dusting tendency and high separation stability.

Figure 2 illustrates the production of further inventively preferred solid enzyme formulations which comprise, in different embodiments, a mixture of xylanase and glucanase. According to method variant (a), the method proceeds from a liquid mixed concentrate of glucanase and xylanase, whereas in method variant (b), the method first proceeds from a liquid glucanase concentrate. According to method variant (a), the mixed concentrate of glucanase and xylanase as described above for Figure 1 is dried and mixed with an organic support and sprayed with soybean oil. According to method variant (b), in contrast, first a liquid glucanase concentrate is processed to give a glucanase-comprising powder in a similar manner to that described above for the xylanase concentrate. This powder is mixed with a xylanase powder prepared according to Figure 1. At the same time it is mixed with the organic support and sprayed with soybean oil, likewise a xylanase- and glucanase-comprising enzyme formulation being produced. The resultant glucanase- and xylanase-comprising solid enzyme formulations produced are also distinguished by very low dusting tendency and high separation stability.

Detailed description of the invention

a) Preferred embodiments of the invention

The invention relates to solid enzyme formulations according to claim 1, comprising a mixture of a) at least one particulate, granular enzyme composition of at least one enzyme and at least one organic or inorganic salt of a monovalent or divalent metal cation with b) at least one particulate inorganic or organic physiologically compatible support and c) at least one hydrophobic liquid having adhesive properties, and in particular a hydrophobic liquid having a melting point in the range from -60°C to 30°C, in particular from -50 to 0°C, such as, for example, from -40 to -5°C, or from –30 to -10°C; the ratio of the median particle diameter of support to enzyme composition being in the range from about 1 to 8 and the mixing ratio of enzyme composition and support being in the range from about 1:1000 to 1:5 parts by weight.

The invention relates in particular to enzyme formulations of the above-mentioned type comprising

a) a particulate enzyme composition comprising an enzyme in a mixture with at least one organic or inorganic salt of a monovalent or divalent cation; or

b) a particulate enzyme composition comprising at least two enzymes which are different from one another in a mixture with at least one organic or inorganic salt of a monovalent or divalent cation; or

c) at least two particulate enzyme compositions which are different from one another, the two compositions differing in that they comprise at least one different enzyme, the enzymes in each composition being present in a mixture with at least one organic or inorganic salt of a monovalent or divalent cation.

In particular, the invention relates to enzyme formulations, the ratio of the median particle diameters of support to enzyme composition being in the range of from about 1 to 4, or

1 to 2 or 1 to 1.5. The median particle size of enzyme composition used and support used is in the range from 50 to 500 µm, or 150 to 350 µm. Expediently, the mixing ratio of enzyme composition and support is set in the range from about 1:500 to 1:10, or 1:100 to 1:20 parts by weight.

The fraction of the hydrophobic liquid is 0.1 to 5, 0.2 to 2, 0.3 to 1.5, or 0.3 to 0.7% by weight, based on the total weight of the enzyme formulation.

In the enzyme compositions used according to the invention, the salt fraction is in the range from 1 to 30% by weight, 5 to 25% by weight, or 10 to 20% by weight, based on the total weight of the enzyme composition.

The percentage fraction of enzyme protein in the enzyme composition is about 0.01 to 99% by weight, such as, for example, 0.01 to 80% by weight, 10 to 80% by weight, 20 to 75% by weight, or 30 to 60% by weight.

In addition to at least one enzyme and at least one salt, the enzyme composition can, moreover, comprise further components. These can serve as binder (e.g. polymers or sugars), as filler (e.g. lime, loam, carbohydrates, sugars, starch), as dye or further stabilizer. Such further components are known per se from the prior art and are familiar to those skilled in the art.

The residual moisture of the enzyme mixture is according to the invention in a range from 5 to 30% by weight, such as, for example, from 5 to 20% by weight, or from 7 to 16% by weight.

The invention is not limited to any defined enzymes. In particular, however, the usable enzymes are selected from hydrolases (EC 3.), in particular glycosidases (EC 3.2.1), peptidases (EC 3.4) and especially xylanases, glucanases (hemicellulases), cellulases, proteases, keratinases, amylases, peptidases and mixtures thereof.

In preferred enzyme formulations, the enzyme is selected from endo-1,4-ß-xylanases (EC 3.2.1.8), endo-1,4-ß-glucanases (EC 3.2.1.4) and mixtures thereof.

The invention also relates to enzyme formulations which have at least one further of the following properties:

a) gravimetric dusting value (determined according to a method described in the examples) in the range from 0 to 0.5, or 0.001 to 0.3, or 0.001 to 0.2, or 0.01 to 0.2% by weight;

b) bulk density in the range from 200 to 700, 300 to 500, or 350 to 450 g/l (defined as specified in DIN EN ISO 60)

c) flowability (determined by Schulze ring shear test) having an ff_c value in the range from 3 to 30, 5 to 15, or 6 to 10.

The invention relates in particular to enzyme formulations, the formulation comprising a mixture of

a)at least one enzyme composition, the enzyme component of which is selected from xylanases, glucanases and mixtures thereof according to aforesaid definition in a mixture with magnesium sulfate, the magnesium sulfate fraction being about 5 to 25% by weight, or 15 to 20% by weight, based on the total weight of the dry enzyme composition;

b)at least one wheat semolina bran support, the mixing ratio of enzyme composition to support being in the range from 1:5 to 1:500, or 1:10 to 1:100;

c)vegetable oil in a fraction of about 0.1 to1% by weight, or 0.3 to 0.6% by weight based on the final weight of the enzyme formulation,

the median particle size of enzyme composition and support being in the range from about 100 to 500, or 150 to 350 µm,

and the xylanase fraction being about 3000-30 000, or 5200 to 18 000, or 5400 to 9000 TXU/g of formulation and the glucanase fraction being about 2000 to 20 000, or 2200 to 10 000 TGU/g of formulation. The percentage fraction of the xylanase is about 1-20% by weight, preferably 2-10% by weight, and in particular 2.5-5% by weight, and of glucanase about 0.01-10% by weight, preferably 0.1-6% by weight, and in particular 0.2-2% by weight.

Particular preference is given to formulations comprising an enzyme composition of the above-described type, the enzyme component of which is a xylanase.

Particular preference is given to formulations comprising an enzyme composition of the above-described type, the enzyme component of which is a glucanase.

Particular preference is given to formulations comprising an enzyme composition of the above-described type, the enzyme component of which is a mixture of xylanase and glucanase.

Particular preference is given to formulations comprising two enzyme compositions of different enzymes, the one enzyme component being a glucanase and the other a xylanase.

The invention also relates to methods for producing solid enzyme formulations according to aforesaid definition, at least one particulate enzyme composition comprising at least one enzyme and at least one organic or inorganic salt of a monovalent or divalent metal cation being mixed with a particulate inorganic or organic physiologically compatible support and the mixture being wetted with a hydrophobic liquid (having a melting point in the range from -60 to 30°C according to aforesaid definition).

In particular the invention relates to methods in which

a) a particulate enzyme composition comprising an enzyme, in

particular xylanase or glucanase, is in a mixture with at least one organic or inorganic salt of a monovalent or divalent cation is provided; or

b) a particulate enzyme composition comprising at least two enzymes which are different from one another, selected from xylanase and glucanase, is provided in a mixture with at least one organic or inorganic salt of a monovalent or divalent cation; or

c) at least two particulate enzyme compositions which are different from one another are provided, the two compositions differing in that they comprise at least one different enzyme, the enzymes in each composition being present in a mixture with at least one organic or inorganic salt of a monovalent or divalent cation.

Preferred methods are those in which the enzyme composition is obtained by spray drying or by spray drying and agglomeration of an enzyme-comprising liquid in which at least one organic or inorganic salt of a monovalent or divalent cation is taken up.

In addition, preferred methods are those in which at least two enzyme compositions of enzymes which are different from one another are obtained by spray drying or by spray drying and agglomeration of at least two different enzyme-comprising liquids in which at least one organic or inorganic salt of a monovalent or divalent cation is taken up, and

a) each of the at least two enzyme compositions is mixed with a particulate inorganic or organic support, or

b) a particulate inorganic or organic support is mixed with the at least two enzyme compositions; and

the mixture produced according to variant a) or variant b) is wetted with a hydrophobic liquid.

The enzyme-comprising liquid used comprises at least one xylanase, at least one glucanase or a mixture thereof.

In particular, the salt fraction in the enzyme composition used is in the range from 1 to 30% by weight, or at about 10 to 25% by weight or 15 to 20% by weight, based on the total weight of the enzyme composition.

In addition, use is made, in particular, of a support and an enzyme composition the ratio of median particle diameters of which is in the range from about 1 to 8, preferably 1 to 4, or 1 to 2, or 1 to 1.5.

The median particle size of enzyme composition used and support used is in the range from 50 to 500 µm, or 150 to 350 µm. The mixing ratio of enzyme composition and support is in the range from about 1:1000 to 1:5, or 1:500 to 1:10, or 1:100 to 1:20.

The median particle size can, according to the size of the particles, be determined either by means of sieving analysis (e.g. using a shaking sieve machine type Vibro VS 1000 from Retsch), or else by laser diffraction (e.g. using a Mastersizer from Malvern).

The fraction of hydrophobic liquid is 0.1 to 5% by weight, or 0.2 to 2, 0.3 to 1.5, or 0.3 to 0.7% by weight, based on the total weight of the enzyme formulation.

The invention relates in particular to a method for producing a solid enzyme formulation comprising at least one enzyme selected from xylanases, glucanases and mixtures thereof,

a) at least one enzyme-comprising liquid being spray dried or spray dried and agglomerated to give at least one enzyme composition, the enzyme component thereof being selected from xylanases, glucanases and mixtures thereof, and this enzyme

component being present in the liquid in a mixture with magnesium sulfate, and the magnesium sulfate fraction being about 10 to 25% by weight, based on the total weight of the dry enzyme composition;

b) the resultant enzyme composition being mixed with a particulate inorganic or organic support; and

c) the enzyme/support mixture being wetted with a hydrophobic liquid having a melting point between -60 and 30°C.

Preferred method variants comprise

a) a particulate enzyme composition comprising at least one xylanase in a mixture with magnesium sulfate being provided; or

b) a particulate enzyme composition comprising at least one glucanase in a mixture with magnesium sulfate being provided; or

c) a particulate enzyme composition comprising at least one xylanase and at least one glucanase in a mixture with magnesium sulfate being provided; or

d) at least two particulate enzyme compositions which are different from one another being provided, one of the compositions comprising at least one xylanase and the other of the compositions comprising at least one glucanase, the enzymes in each composition being present in a mixture with magnesium sulfate.

In a particular embodiment of the method, an enzyme composition is mixed with at least one wheat semolina bran support, the mixing ratio of enzyme composition to support being in the range from 1:5 to 1:500, or 1:10 to 1:100.

During mixing, in particular vegetable oil is added in a

fraction of from about 0.1 to 1% by weight, or 0.3 to 0.6% by weight, based on the final weight of the enzyme formulation. The median particle size of enzyme composition used and support used is in particular in the range from about 100 to 500 µm, or 150 to 40 µm, and the xylanase fraction is about 5000-30 000, or 5200 to 10 000, or 5400 to 9000 TXU/g of formulation and/or the glucanase fraction is about 2000 to 10 000, or 2200 to 6000 TGU/g of formulation.

The invention also relates to the use of a dry enzyme formulation according to aforesaid definition for producing a food, food supplement or an animal feed.

The invention also relates to methods for producing animal feed, food or food supplement comprising a dry enzyme formulation according to aforesaid definition; in particular animal feed comprising the production of an enzyme formulation according to the invention and subsequent incorporation thereof in animal feed, food or food supplement, in particular in a fraction of from about 0.001 to 1% by weight.

b) Enzymes

The enzymes used according to the invention are not subject to any limitations and can be either of natural or recombinant origin. The enzymes can be enzymes from plants, from fungi, from bacteria or yeasts. Preference is given to enzymes from microbiological sources such as bacteria, yeasts or fungi. The enzyme can be obtained from the respective microorganism by known techniques which typically comprise fermentation of the enzyme-producing microorganism in a suitable nutrient medium and subsequent isolation of the enzyme or enzyme concentrate from the fermentation medium by standard techniques.

If required, to set the pH of the enzyme solution or of the enzyme concentrate, conventional substances such as buffers, bases, acids, can be added to the formulations; preferred pHs are 3.5 to 7, particularly preferably 3.5 to 5, and in

particular 4 to 4.5.

In addition, use can be made of enzyme mutants or enzymes which exhibit an elevated heat stability, such as, for example, proposed in the WO's 95/2997, 97/00020, 97/20920, 97/22691, 98/28410 or 03/062409.

Preferably, however, use is made according to the invention as enzymes of polypeptides having xylanase activity, polypeptides having glucanase activity and mixtures thereof.

b1) Polypeptides having xylanase activity

These are enzymes of class EC 3.2. 1.8 having the official name endo-1, 4-beta-xylanase. The systematic name is 1,4-beta-D-xylanxylanohydrolase. Other names likewise in use are: endo-(1-4)-beta-xylanase; (1-4)-beta-xylan 4-xylanohydrolase; endo-1, 4-xylanase; xylanase; beta-1, 4-xylanase; endo-1, 4-xylanase; endo beta-1, 4-xylanase; endo-1, 4-beta-D-xylanase; 1, 4-beta-xylan xylanohydrolase; beta-xylanase; beta-1, 4-xylan xylanohydrolase; endo-1,4-beta-xylanase; beta-D-xylanase. The enzyme catalyzes the endohydrolysis of 1,4-beta-D-xylosidic bonds in xylans.

The xylanase can be derived, for example, from bacteria, such as, for example, those of the genera Clostridium, Streptomyces, Paenibacillus, Pseudomonas, Thermoascus, Thermotoga, Bacillus, and, for example, xylanases from the following strains Bacillus halodurans, Bacillus pumilus, Bacillus agaradhaerens, Bacillus circulans, Bacillus polymyxa, Bacillus sp., Bacillus stearothermophilus, or Bacillus subtilis.

Fungal xylanases are derived, for example, from yeasts and filamentous fungi, such as, for example, from the following genera: Aspergillus, Aureobasidium, Emericella, Fusarium, Gaeumannomyces, Humicola, Lentinula, Magnaporthe, Neocallimastix, Nocardiopsis, Orpinomyces, Paecilomyces,

Penicillium, Pichia, Saccharomyces, Schizophyllum, Talaromyces, Thermomyces, Trichoderma, such as, for example, Talaromyces emersonii.

The xylanase activity is determined in a manner known per se and is described, for example, in Engelen et al., Journal of AOAC International Vol. 79, No. 5, 1019 (1996). In contrast to the method described there, instead of the xylan substrate from oat spelts (Serva Feinbiochemia GmbH u. Co., Heidelberg), use is made of arabinoxylan from wheat (Megazyme, article P-WAXY, Ireland). The substrate solution is prepared fresh in each case by dissolving 1000 g of arabinoxylan lump-free in 100.00 ml of water over a period of at least 12 hours.

b2) Polypeptides having glucanase activity

Endoglucanases are classified as EC 3.2.1.4 and are frequently called cellulases. Other names are endo-glucanase, endo-1, 4-beta-glucanase, cellulase A or carboxymethylcellulase. The enzymes catalyze the endohydrolysis of 1, 4-beta-D-glucosidic bonds in cellulose and also the 1, 4-links in beta-D-glucans which in addition comprise 1, 3-links.

The glucanase can be derived, for example, from bacteria, such as, for example, from those of the genera Bacillus, Clostridium, Paenibacillus, Pseudomonas, Streptomyces, Thermoascus, Thermotoga. Fungal glucanases are derived, for example, from yeasts and filamentous fungi, such as, for example, from the following genera: Aspergillus, Aureobasidium, Emericella, Fusarium, Gaeumannomyces, Humicola, Lentinula, Magnaporthe, Neocallimastix, Nocardiopsis, Orpinomyces, Paecilomyces, Penicillium, Pichia, Saccharomyces, Schizophyllum, Talaromyces, Thermomyces, Trichoderma, such as, for example, Talaromyces emersonii.

The glucanase activity is determined in a manner known per se and is described, for example, in Engelen et al., Journal of AOAC International Vol. 79, No. 5, 1019 (1996). In contrast to

the method described there, instead of the beta-glucan substrate from barley (Sigma Chemical Co., St. Louis, MO: No. G-6513), use is made of beta-glucan from barley (Megazyme, article P-BGBM, Ireland). The substrate solution is prepared freshly in each case firstly by suspension of 0.750 g of glucan in 20 ml of water and subsequently dissolution by adding 20 ml of sodium hydroxide solution (2 mol/l) with stirring for 15 minutes. 42.5 ml of citric acid solution (1 mol/l) are added, the pH is adjusted to 3.50 +/- 0.03 at 40.0°C +/- 0.1°C using sodium hydroxide solution (2 mol/l) or citric acid solution (1 mol/l). After cooling to room temperature, the mixture is made up to 100.00 ml with water.

c) Stabilizing salts

Examples of suitable stabilizing additives which may be mentioned are inorganic or organic salts.

In particular these are metal salts, in particular alkali metal and alkaline earth metal salts of organic acids, such as, for example, Mg, Ca, Zn, Na, K salts of monovalent or divalent carboxylic acids having 1 to 8 carbon atoms, such as, for example, citrates, acetates, formates and hydrogenformates, in addition inorganic salts, such as, for example, Mg, Ca, Zn, Na, K sulfates, carbonates, silicates or phosphates; alkaline earth metal oxides, such as CaO and MgO; inorganic buffering agents, such as alkali metal hydrogenphosphates, in particular sodium and potassium hydrogenphosphates, such as, for example, K_2HPO_4, KH_2PO_4 and Na_2HPO_4. Particularly preferably, use is made of the following salts in the weight fractions given based on the enzyme composition:

zinc sulfate(0.5 to 10, or 3 to 8% by weight)

calcium sulfate (1 to 30, or 10 to 25% by weight)

magnesium sulfate (5 to 30, or 10 to 25% by weight)

sodium sulfate (1 to 30, or 10 to 20% by weight)

d) Suitable supports

Examples of support materials are carbohydrates, in particular sugars and also starches, for example from corn, rice, potatoes, wheat and cassava; modified starches, for example octenyl succinate anhydride; cellulose and microcrystalline cellulose; inorganic minerals or loam, for example clay, coal, kieselguhr, silicic acid, talc and kaolin; semolina, for example wheat semolina, brans, for example wheat bran or wheat semolina, flours; salts such as metal salts, in particular alkali metal and alkaline earth metal salts of organic acids, for example Mg, Ca, Zn, Na, K citrate, acetate, formate and hydrogenformates, inorganic salts, for example Mg, Ca, Zn, Na, K sulfates, carbonates, silicates or phosphates; alkaline earth metal oxides such as CaO and MgO; inorganic buffering agents such as alkali metal hydrogenphosphates, in particular sodium and potassium hydrogenphosphates, for example K_2HPO_4, KH_2PO_4 and Na_2HPO_4.

e) Suitable hydrophobic liquids

Examples of suitable hydrophobic liquids which may be mentioned are:

In principle all hydrophobic liquids (having a melting point in the range from -60 to 30°C which have a hydrophobic molecule moiety) are usable provided that they are suitable as food or feed additive. Preference is given to naturally occurring plant or animal liquids such as phospholipids and mono-, di- and triacylglycerides and mixtures thereof.

Nonlimiting examples which may be mentioned are soybean lecithin, vegetable oils, such as, for example, sunflower oil, corn germ oil, soybean oil, palm oil, rapeseed oil, palm kernel oil, cottonseed oil, peanut oil, babassu oil, thistle

oil and also animal oils, such as, for example, fish oil.

f) Production of the formulation

The inventive enzyme formulations are produced making use of methods known per se of the prior art, such as, for example, described in Mollet et al., Formulierungstechnik [Formulation technique], 2000, Verlag Wiley-VCH, Weinheim, or Heinze, Handbuch der Agglomerationstechnik [Handbook of agglomeration technique], 2000, Verlag Wiley-VCH, Weinheim.

f1) Drying

For producing the salt-stabilized, preferably agglomerated, enzyme compositions by drying, various technologies come into consideration, such as, in particular

- spray drying
- fluidized-bed granulation
- fluidized-bed agglomeration
- fluidized spray dryer (FSD) technology
- Procell technology from Glatt (WO 2004/108911)

Drying can be performed continuously or batchwise. The dried product, after drying, may still need to be sieved, ground or agglomerated. Combinations of said steps are also possible.

The enzyme solution used according to the invention for spray drying or agglomeration comprises at least one enzyme usable as food additive or feed additive, dissolved or suspended in an aqueous phase such as, for example, the enzyme concentrate which can be obtained from the production process comprising fermentation and workup. The solution has a protein fraction in the range from about 1 to 50% by weight, preferably about

10 to 35% by weight, based on the total weight of the solution. The pH is generally in the range from about 3 to 9. In addition to the abovementioned salt-form enzyme stabilizers such as, for example, alkali metal or alkaline earth metal salts, such as sodium sulfate or magnesium sulfate, the solution can optionally comprise other conventional additives. Examples which may be mentioned are: buffers, such as, for example, phosphate buffers; solubilizers, such as, for example, ethanol or surface-active agents and the like.

In the event that the adhesive properties of the enzyme solution do not suffice to ensure stable sticking-together of the particles after spraying, the use in addition of a binder is advantageous. This avoids the agglomerates from disintegrating again on drying. In such cases it is preferred to spray into the fluidized bed a binder which is soluble or dispersible in aqueous medium. The binder can be sprayed in either dissolved in the enzyme solution to be sprayed in, or separately therefrom, simultaneously or offset in time. Examples of suitable binders which may be mentioned are: solutions of carbohydrates, such as, for example, glucose, sucrose, dextrins, inter alia, sugar alcohols, such as, for example, mannitol, or polymer solutions such as, for example, solutions of hydroxypropylmethylcellulose (HPMC), polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), ethoxylated cellulose (EC), ethylcellulose or propylcellulose. Via targeted selection of amount and adhesive properties of the binder sprayed in, agglomerates of different size and strength can be produced.

If the binder is sprayed on in a mixture with the enzyme, the binder fraction is usually in the range from about 0.5 to 20% by weight, preferably about 1 to 10% by weight, based on the total weight of the solution.

If the binder is sprayed on as a separate solution, the binder fraction of the solution is in the range from about 1 to 30% by weight, based on the total weight of the solution. The

binder in this case is likewise dissolved in an aqueous medium, preferably sterile demineralized water. Conventional additives such as, for example, buffers or solubilizers can likewise be present.

According to the invention, the fraction of the binder in the end product (that is the enzyme composition) is 0 to about 20% by weight, for example about 1 to 6% by weight. The optimum amount is also dependent on the type of binder selected.

The spray drying of liquid enzyme preparations can be carried out in a conventional manner. For this the enzyme solution is pumped to the atomizer in the spraying tower. The atomization is performed, for example, by means of a pressure nozzle (single-fluid nozzle), a twin-fluid nozzle or a centrifugal atomizer. The droplets are dried by a hot air stream passed into the spray dryer. When centrifugal atomizers are used, the drying is preferably performed in co-current flow. With nozzles, the drying can also be performed in counter-current flow or mixed-current flow. The powder can be discharged at the tower or it is entrained by the air stream and separated off in a cyclone and/or filter. Depending on product and procedure, post-drying may be required which can proceed in an internal fluidized bed flanged onto the spray dryer, or an external fluidized bed.

The spray-dried product can be subsequently agglomerated in a fluidized bed. For this purpose pulverulent material, for example enzyme powder obtained by aforesaid spray drying, is charged into a fluidized-bed dryer. The swirling is performed, for example, by feeding preheated air. An enzyme-comprising solution, for example, or a binder solution is sprayed onto the fluidized bed, as a result of which the charged powder is wetted with this solution and increasingly agglomerated owing to its adhesive properties. Spraying into the fluidized bed can proceed from the top (top spray method) or the bottom (bottom spray method). At the same time, simultaneously or semicontinuously, that is timed at intervals, a subquantity of

agglomerate is discharged from the fluidized bed. The discharge is classified, using, for example, a sieve. Coarse material produced can be ground and continuously recirculated to the fluidized bed. Fine fractions, such as, for example, from the exhaust air filter system, can likewise be continuously recirculated.

According to a further method variant, the production of the inventive enzyme agglomerate can proceed continuously, more precisely with continuous feed of a dry pulverulent charge, such as, for example, a dry enzyme powder, into the fluidized-bed dryer. Particularly suitable dryers for this are fluidized-bed dryers having a plurality of spray zones and optionally drying zones. In the first zone, dry enzyme powder is charged, swirled, and enzyme solution and/or binder sprayed in. The agglomerate formed in this zone is transferred to the next zone. Into this, and optionally into one or more further zones, likewise enzyme solution and/or binder solution of identical or different composition can be sprayed in. The water of the enzyme solution or binder solution sprayed on is removed by a feed air stream which is common for all zones, or separate feed air streams which are appropriately heated. In one or more of the last zones, post-drying can further be carried out. Here is also situated the product discharge. The workup of the product is performed as described above.

A further preferred method variant comprises a spray drying of enzyme solution, coupled with subsequent agglomeration of the spray-dried enzyme powder. This can be carried out batchwise or continuously. The continuous procedure is preferred.

Such methods can be carried out using conventional spray-drying plants. However, they can advantageously be carried out in apparatuses which are known as FSD (Fluidized Spray Dryer), SBD (Spray Bed Dryer) or MSD (Multi Stage Dryer).

The resultant fine fraction of the powder can in this case be reincorporated into the process as early as in the spray

dryer, if it is recirculated, for example after precipitation in a cyclone or filter, back to the moist zone of the dryer. The actual agglomeration then takes place in a further stage in a fluidized bed. This stage can be integrated into the spray dryer (internal fluidized bed) or it can be carried out in a separate apparatus (additional fluidized bed). Into the fluidized bed there can be injected, if required, with simultaneous drying, further enzyme solution, an enzyme solution which in addition comprises binder, or only binder in dissolved or dispersed form, in order to support the agglomeration. Examples of suitable binders for the agglomeration are hydroxypropylmethylcellulose, polyvinylpyrrolidone, polyethylene glycols and block polymers of polyoxyethylene and polyoxypropylene. Preferably, the process parameters are set, however, in such a manner that no further addition for agglomerate production is required. The composition and amount of the liquids injected depend on the adhesive properties of the sprayed solution, the agglomerate size to be achieved and the process conditions. Depending on the amount sprayed on, post-drying in a further stage can be required. The product is then worked up in the aforesaid manner.

In the case of a high heat lability of the spray-dried enzymes, during the inventive methods the control of the product temperature is of particular importance. It should be selected to be as low as possible, since with increasing temperature and/or duration of the spray drying and agglomeration method, the losses in activity increase. Typically, the product temperature in spray drying, that is the temperature of the solid spray-dried powder, is at about 40 to 75°C, in particular lower than about 70°C, frequently lower than 60°C. The longer the residence time in the fluidized bed, the lower the temperature should be selected.

The product temperature during agglomeration and drying in the fluidized bed, that is the temperature of the agglomerate in the fluidized bed, is to be selected low during the relatively

long residence time in the apparatus, and is at values of about 30 as 70°C, in particular below 60°C, and preferably below 50°C.

To reduce the residual moisture content further, it can be necessary to carry out a post-drying step. During the post-drying also, the product temperature should be in the abovementioned range, and in particular at 50°C or below. The post-drying reduces the residual moisture content in the inventive preparations to values of less than about 20% by weight, preferably about 5 to 17% by weight.

Drying during agglomeration and the post-drying is achieved by using preheated feed air. The feed air temperature which can be varied depending on the selected preset product temperature, air rate and spray rate, is generally in a range between 30 and 180°C. The post-drying proceeds at a lower temperature, that is to say in the range from about 35 to 55°C.

The duration of agglomeration is likewise dependent on the size of the batch selected, but is in the range from 30 minutes to a plurality of hours.

f2) Production of the enzyme formulation

Using mixing techniques known per se, the spray-dried, if appropriate agglomerated, preproduct (dry enzyme composition) is mixed with the above-described support material. For this the enzyme preparation is added to the support, for example a little at a timer, and this is mixed, if necessary for some time, for example 1 to 5 minutes, until a uniform distribution is achieved. Then the hydrophobic liquid is added. This can be sprayed, added dropwise or poured onto or into the mixture during the mixing operation. After addition is complete, the mixing operation is continued, for example for 5 to 45 minutes, until the oil is uniformly distributed. The resultant product has a very low dust fraction. Further handling steps

are usually not required.

Various mixer types are suitable for the mixing, such as, for example, cone and screw mixers (for example from Nauter), plowshare mixers (for example from Lödige), twin-shaft mixers. The mixing times depend on the mixer type selected and can differ.

g) Food and feed compositions

The enzyme formulations produced according to the invention are suitable, in particular, for additizing foods and feeds.

The formulations are particularly suitable as additives to animal feed in a mixture with single-component feeds of plant or animal origin in accordance with the FMV (German feed regulation), such as, for example, secondary cereal products, wheat feed meal, wheat bran; extraction meals, spent grains, melasses-dried beet pulp, fish meal, meat and bone meals; and/or mineral single-component feeds according to FMV, such as for example, carbonates, phosphates, sulfates, propionates. Those which are likewise suitable are cereals, such as wheat, rye, barley, oats, corn, millet or triticale; secondary cereal products (by-products of milling), such as brans, semolina brans, wheat semolina brans, feed meals or middlings; by-products from oil production (extraction meals, expeller meals, cakes); by-products from sugar production (melasses, dried cossettes, feed sugars, pulps, potato starch, corn gluten, wheat gluten); by-products from the fermentation industry, brewers spent grains, yeast, malt germ, brewers spent wash; and also animal and other feeds, such as blood meal, fish meal, pressing juice, potato protein.

Experimental part

Production Example V1: Xylanase formulation

a) In an aqueous xylanase concentrate having a dry mass

content of about 20 to 35% by weight, a pH in the range of from 3.5 to 5.0 and an activity of 60 000 to 100 000 TXU/g, 10-20% by weight of magnesium sulfate heptahydrate were dissolved, based on the concentrate, at 4-10°C.

b) For spray drying and agglomeration, the enzyme composition produced under a) was sprayed in a laboratory fluidized bed Aeromat type MP-1 from Niro-Aeromatic via a 2-fluid nozzle by the top-spray method. The plastic cone of the fluidized bed had a gas distribution plate diameter of 110 mm and a perforated plate having 12% open surface area. The fluidized bed was charged with an air rate of 50 m^3/h and feed air temperatures of 40 to 100°C. The feed air temperature was regulated, so that the product in the fluidized bed maintained a temperature of approximately 45°C. The spraying time was 240 min. The product was subsequently cooled with swirling at 50 m^3/h feed air at 30°C.

c) The enzyme composition produced under b) was sieved. Fine material and coarse material were sieved out, so that a usable fraction was obtained having a particle size distribution from 100 µm to 400 µm.

This produced a product having the following characteristic data:

Composition:

Xylanase (dry mass)	65% by weight
Magnesium sulfate ($MgSO_4$)	20% by weight
Residual moisture	15% by weight
Activity	from 200 000 to 300 000 TXU/g
Appearance (microscope)	Agglomerates comprising a plurality of primary particles
Median particle diameter	171 µm

d) To produce the enzyme formulation, wheat semolina bran (675.5 g) was charged into a laboratory mixer (Lödige) and homogenized at room temperature and 170 rotations per minute. Under these conditions, 21 g of the enzyme composition produced under c) were added to the mixer and mixed for 5 min. Thereafter, 3.5 g of soybean oil were slowly added dropwise via a pipette and thereafter post-mixed for 30 min.

This produced a product having the following characteristic data:

Composition:

Wheat semolina bran (dry mass)	90% by weight
Enzyme composition (from c))	3% by weight
Soybean oil	0.5% by weight
Residual moisture	6.5% by weight
Activity	from 5000 to 7000 TXU/g
Median particle diameter:	337 µm

Production Example V2: Glucanase formulation

a) In an aqueous ß-glucanase concentrate having a dry mass content of about 20 to 35% by weight, a pH in the range of from 3.5-5.0 and an activity of 150 000 to 400 000 TGU/g, 10 to 20% by weight of magnesium sulfate heptahydrate, based on the concentrate, were dissolved at 4-10°C.

b) For spray drying and agglomeration, the enzyme composition produced under a) was sprayed in by the top-spray method via a 2-fluid nozzle in a laboratory fluidized bed Aeromat type MP-1 from Niro-Aeromatic. The plastic cone of the fluidized bed had a gas distribution plate diameter of 110 mm and a perforated plate having 12% open surface area. The fluidized bed was impinged with an air rate of 50 m^3/h and feed air temperatures of 40 to 100°C. The feed air temperature was controlled in such a way that the product in the fluidized bed maintained a

temperature of approximately 45°C. The spray time was 240 min. The product was then cooled to 30°C with swirling at 50 m^3/h feed air.

c) The enzyme composition produced under b) was sieved. Fine material and coarse material were sieved out, so that a usable fraction having a particle size distribution of 100 µm to 400 µm was obtained.

This produced a product having the following characteristic data:

Composition:

Glucanase (dry mass)	65% by weight
Magnesium sulfate ($MgSO_4$)	20% by weight
Residual moisture	15% by weight
Activity	from 500 000 to 120 000 TGU/g
Appearance (microscope)	Agglomerate comprising a plurality of primary particles
Median particle diameter	167 µm

d) For production of the enzyme formulation, wheat semolina bran (693 g) was charged into a laboratory mixer (Lödige) and homogenized at room temperature and 170 revolutions per minute. Under these conditions, 3.5 g of the enzyme composition produced under c) were placed in the mixer and mixed for 5 min. Thereafter, 3.5 g of soybean oil were slowly added dropwise via a pipette and thereafter post-mixing is performed for 30 min.

This produced a product having the following characteristic data:

Composition:

Wheat semolina bran (dry mass)	92.5% by weight

Enzyme composition (from c))	0.5% by weight
Soybean oil	0.5% by weight
Residual moisture	6.5% by weight
Activity	from 1000 to 7000 TGU/g
Median particle diameter	321 µm

Production Example V3: Xylanase/glucanase formulation

For production of an enzyme formulation, wheat semolina bran (672 g) was charged into a laboratory mixer (Lödige) and homogenized at room temperature and 170 revolutions per minute. Under these conditions, 21 g of the enzyme composition produced under Production Example V1 c) and 3.5 g of the enzyme composition produced under Production Example V2 c) were added to the mixer and mixed for 5 min. Thereafter, 3.5 g soybean oil was slowly added dropwise via a pipette, and subsequently post-mixed for 30 min.

This produced a product having the following characteristic data:

Composition:

Wheat semolina bran (dry mass)	89.5% by weight
Enzyme composition (from V1 c))	3% by weight
Enzyme composition (from V2 c))	0.5% by weight
Soybean oil	0.5% by weight
Residual moisture	6.5% by weight
Xylanase activity	from 5000 to 7000 TXU/g
Glucanase activity	from 1000 to 7000 TGU/g
Median particle diameter	328 µm

Production Example V4: Xylanase/glucanase formulation

a) An aqueous ß-glucanase concentrate having a dry mass content of from about 20 to 35% by weight, a pH in the range of from 3.5-5.0 and an activity of from 150 000 to 400 000 TGU/g was mixed with an aqueous xylanase concentrate having a

dry mass content of from about 20 to 35% by weight, a pH in range of from 3.5 to 5.0 and an activity of from 60 000 to 100 000 TXU/g in the ratio 1:8. In the mixture, 10 to 30% by weight of magnesium sulfate heptahydrate, based on the concentrate, were dissolved at 4-10°C.

Subsequently, the enzyme concentrate obtained under a) was further processed as in Production Example V1 in the steps b) to d).

This produced a product having the following characteristic data:

Composition:

Wheat semolina bran (dry mass)	90% by weight
Enzyme composition (from c))	3% by weight
Soybean oil	0.5% by weight
Residual moisture	6.5% by weight
Xylanase activity	from 5000 to 7000 TXU/g
Glucanase activity	from 1000 to 7000 TGU/g
Median particle diameter	343 µm

Test Example 1: Determination of the dust value

The dust value (% based on total amount of product) of inventive mixtures is determined with and without addition of oil.

The determination proceeded according to the following method:

Three samples each, each of 10 ± 0.03 g of the solid under test are poured slowly (approximately 2 to 3 seconds) through a falling tube (length = 60 cm; diameter = 3 cm) into a container (20.2 cm in height, 19.5 cm in width, 19.5 cm in length; a suction tube is situated on a side wall at a height of approximately 13 cm and is mounted at a right angle (90°) to the falling tube). Using an oil pump connected via the

suction tube to the container, the resultant dust is sucked out of the container and collected on a filter at a constant rate (15 ± 0.5 l/min) for 1 minute. For this, use is made of a glass vacuum filter (diameter 35 mm, D2, 50 ml) provided with a suitable filter (for example Sartorius glass fiber prefilter, 13 400-37-S; diameter 35 mm). The amount of dust removed by suction is determined using an analytical balance, related to the amount of sample used and expressed as a percentage mean. According to the percentage dust values determined, the dust behavior of the samples is described as follows:

Dust value [%]	Description
0–0.05	virtually dust free
0.05–0.25	slightly dust-forming
0.25–1.00	dust-forming
> 1.00	strongly dust-forming

Materials used:

Xylanase powder (XEA): activity: 229 300 TXU/g; median particle diameter = 171; (20% by weight magnesium sulfate heptahydrate); dried in a similar manner to Production Example V1

Wheat semolina bran (WGK) (Hildebrandmühlen); median particle diameter = 370
Soybean oil

Mixing the samples:

The WGK is charged into the Lödige mixer, the SD powder is added thereto and is premixed at room temperature and 5 min at 170 rpm. The soybean oil is heated to approximately 80°C, slowly added dropwise via a fine pipette and post-mixed for 30 min. In each case 1000 g of mixture are prepared. The dust values determined for various mixtures and also for pure XEA and pure WGK are summarized in the following table:

Sample E5/051	Wheat semolina bran (g)	SD powder (g)	Soybean oil (g)	Theoretical activity (TXU/g)	Dust value (%)	Notes	Soybean oil (%)
Batch 1	967.3	32.7	**0.0**	7500	0.081	slightly dust-forming	**0.0**
Batch 2	962.3	32.7	**5.0**	7500	0.025	virtually dust free	**0.5**
Batch 3	957.3	32.7	**10.0**	7500	0.015	virtually dust free	**1.0**
WGK	**pure**	-	**0.0**	0	0.120	slightly dust-forming	**0.0**
XEA	-	**pure**	**0.0**	229300	0.049	virtually dust free	**0.0**

A surprisingly significant reduction in dust forming tendency of inventive oil-comprising mixtures is observed.

In addition, after visual examination and also light-microscopy study of the batches studied no differences in separation behavior could be found (results not shown).

Test Example 2: Determination of flowability

The flow behavior of inventive enzyme formulations is determined by known methods. In the prior art, various methods suitable in principle are described (see Schmitt et al., Part. Part. Syst. Charact. 21 (2004) 403-410).

According to the invention, the determination is performed using the Schulze ring shear tester RST.01-pc. The experiment is performed by the method ASTM D6773 (Schulze Ring Shear Tester 2002).

The following test parameters were used:

Storage time of the sample in the measurement cell: 0 h

Temperature: 22°C

Relative air humidity: 70%

Consolidation force (load): σ_1= 11.18 kPa

Using the ASTM D6773 method, a flowability of ff_C= 8.8 was achieved. Thus the product has high flowability.

Patentkrav

1. Fast enzymformulering til iblanding i dyrefoder, næringseller kosttilskudmidler, omfattende en blanding af
a) mindst en partikelformet enzymsammensætning på mindst et enzym og mindst et organisk eller uorganisk salt af en mono- eller divalent kation med
b) mindst en partikelformet uorganisk eller organisk bærer
og
c) mindst en hydrofob væske;
hvor forholdet mellem den gennemsnitlige partikeldiameter af en bærer og enzymsammensætningen ligger inden for området fra 0,125 til 8, og hvor blandingsforholdet mellem enzymsammensætningen og bæreren ligger inden for området fra 1:1000 til 1:5,
hvor de gennemsnitlige partikelstørrelser for enzymformulering og bærer uafhængigt af hinanden hver især ligger inden for området fra 50 til 500 µm; og hvor andelen af den hydrofobe væske udgør 0,1 til 5 vægt-%, baseret på den samlede vægt af enzymformuleringen.

2. Enzymformulering ifølge krav 1, omfattende en partikelformet enzymsammensætning, indeholdende et enzym i en blanding med mindst et organisk eller uorganisk salt af en mono- eller divalent kation.

3. Enzymformulering ifølge krav 1, omfattende en partikelformet enzymsammensætning, indeholdende mindst to enzymer, som er forskellige fra hinanden, i en blanding med mindst et organisk eller uorganisk salt af en mono- eller divalent kation.

4. Enzymformulering ifølge krav 1, omfattende mindst to partikelformede enzymsammensætninger, som er forskellige fra hinanden, hvor begge sammensætninger adskiller sig ved, at de indeholder mindst et forskelligt enzym, hvor enzymerne i hver sammensætning foreligger i en blanding med mindst et organisk eller uorganisk salt af en mono- eller divalent kation.

5. Enzymformulering ifølge et af kravene 2 til 4, hvor de gennemsnitlige partikelstørrelser af enzymsammensætningen og bæreren uafhængigt af hinanden ligger hver især inden for området fra 150 til 350 µm.

6. Enzymformulering ifølge et af de foregående krav, hvor andelen af den hydrofobe væske er 0,1 til 1,5 vægtprocent, baseret på den samlede vægt af enzymformuleringen.

7. Enzymformulering ifølge et af de foregående krav, hvor saltandelen i enzymsammensætningen ligger inden for området fra 1 til 30 vægtprocent, baseret på den samlede vægt af enzymsammensætningen.

8. Enzymformulering ifølge et af de foregående krav, hvor enzymet/enzymerne er udvalgt blandt xylanaser, glucanaser, cellulaser, proteaser, keratinaser, amylaser og blandinger deraf.

9. Enzymformulering ifølge et af de foregående krav, hvor formuleringen omfatter en blanding af

a) mindst en enzymsammensætning, hvis enzymkomponent er udvalgt blandt xylanaser, glucanaser og blandinger deraf i en blanding med magnesiumsulfat, hvor magnesiumsulfatandelen er omtrent 5 til 25 vægtprocent, baseret på den samlede vægt af den tørre enzymsammensætning;

b) mindst en hvedesemuljeklid-bærer, hvor blandingsforholdet mellem enzymsammensætningen og bæreren ligger inden for området fra 1:5 til 1:500; og

c) planteolie i en andel fra 0,1 til 1 vægtprocent, baseret på enzymformuleringens slutvægt,

hvor den gennemsnitlige partikelstørrelse af enzymsammensætningen og bæreren ligger inden for området fra omtrent 150 til 500 µm, og xylanase-andelen ligger ved 3.000-30.000 TXU/g formulering, og glucanase-andelen ligger ved 2.000 til 20.000 TGU/g formulering.

10. Enzymformulering ifølge krav 9, omfattende en enzymsammensætning, hvis enzymkomponent er en xylanase, en glucanase eller en blanding af xylanase og glucanase; eller omfattende to enzymsammensætninger af forskellige enzymer, hvor den ene enzymkomponent er en glucanase og den anden en xylanase.

11. Fremgangsmåde til fremstilling af en fast enzymformulering ifølge et af de foregående krav, hvor mindst en partikelformet enzymsammensætning, omfattende mindst et enzym og mindst et organisk eller uorganisk salt af en mono- eller divalent kation, blandes med mindst en partikelformet uorganisk eller organisk bærer, og blandingen fugtes med en hydrofob væske.

12. Fremgangsmåde ifølge krav 11, hvor der tilvejebringes en partikelformet enzymsammensætning, indeholdende et enzym i en blanding med mindst et organisk eller uorganisk salt af en mono- eller divalent kation.

13. Fremgangsmåde ifølge krav 11, hvor der tilvejebringes en partikelformet enzym- sammensætning, indeholdende mindst to enzymer, som er forskellige fra hinanden, i en blanding med mindst et organisk eller uorganisk salt af en mono- eller divalent kation.

14. Fremgangsmåde ifølge krav 11, hvor der tilvejebringes mindst to partikelformede enzymsammensætninger, der er forskellige fra hinanden, hvor begge sammensætninger adskiller sig ved, at de indeholder mindst et forskelligt enzym, hvor enzymerne i hver sammensætning foreligger i en blanding med mindst et organisk eller uorganisk salt af en mono- eller divalent kation.

15. Fremgangsmåde ifølge et af kravene 11, 12 eller 13, hvor enzymsammensætningen opnås ved spraytørring eller ved spraytørring og agglomerering af en enzymholdig væske, i hvilken der er optaget mindst et organisk eller uorganisk salt

af en mono- eller divalent kation.

16. Fremgangsmåde ifølge krav 11 eller 14, hvor der opnås mindst to enzymsammensætninger af enzymer, der er forskellige fra hinanden, ved spraytørring eller ved spraytørring og agglomerering af mindst to forskellige enzymholdige væsker, i hvilke der er optaget mindst et organisk eller uorganisk salt af en mono- eller divalent kation, og hvor
a) hver af de i det mindste to enzymsammensætninger blandes med en partikelformet uorganisk eller organisk bærer, eller
b) en partikelformet uorganisk eller organisk bærer blandes med de i det mindste to enzymsammensætninger; og
blandingen, som opstår i henhold til variant a) eller variant b), fugtes med en hydrofob væske.

17. Fremgangsmåde til fremstilling af en fast enzymformulering, omfattende mindst et enzym, som er udvalgt blandt xylanaser, glucanaser og blandinger deraf, hvor
a) mindst en enzymholdig væske spraytørres eller spraytørres og agglomereres til mindst en enzymsammensætning, hvor dens enzymkomponent er udvalgt blandt xylanaser, glucanaser og blandinger deraf, og denne enzymkomponent i en blanding med magnesiumsulfatet er indeholdt i væsken, og hvor magnesiumsulfatandelen er 5 til 25 vægtprocent baseret på den samlede vægt af den tørre enzymsammensætning;
b) den således opnåede enzymsammensætning blandes med en partikelformet uorganisk eller organisk bærer; og
c) enzym/bærer-blandingen blandes med en hydrofob væske.

18. Fremgangsmåde ifølge krav 17, hvor
a) der tilvejebringes en partikelformet enzymsammensætning, indeholdende mindst en xylanase i en blanding med magnesiumsulfat; eller
b) der tilvejebringes en partikelformet enzymsammensætning, indeholdende mindst en glucanase i en blanding med magnesiumsulfat; eller
c) der tilvejebringes en partikelformet enzymsammensætning, indeholdende mindst en xylanase og mindst en glucanase i en

blanding med magnesiumsulfat; eller

d) der tilvejebringes mindst to partikelformede enzymsammensætninger, der er forskellige fra hinanden, hvor en af sammensætningerne indeholder mindst en xylanase, og den anden af sammensætningerne indeholder mindst en glucanase, hvor enzymerne i hver sammensætning foreligger i en blanding med magnesiumsulfat.

19. Fremgangsmåde ifølge krav 17 eller 18, hvor en eller to forskellige enzymsammensætninger blandes med mindst en hvedesemuljeklid-bærer, hvor blandingsforholdet mellem enzymsammensætningen og bæreren ligger inden for området fra 1:5 til 1:1000.

20. Anvendelse af en tør enzymformulering ifølge et af kravene 1 til 10 til fremstilling af et næringsmiddel, kosttilskudmiddel eller et dyrefoder.

21. Fremgangsmåde til fremstilling af dyrefoder, næringsmidler eller kosttilskudmidler, indeholdende en tør enzymformulering ifølge et af kravene 1 til 10, hvilken fremgangsmåde omfatter fremstillingen af en sådan tør enzymformulering ved hjælp af en fremgangsmåde ifølge et af kravene 11 til 19, og dens efterfølgende iblanding i dyrefoder, næringsmidler eller kosttilskudmidler.

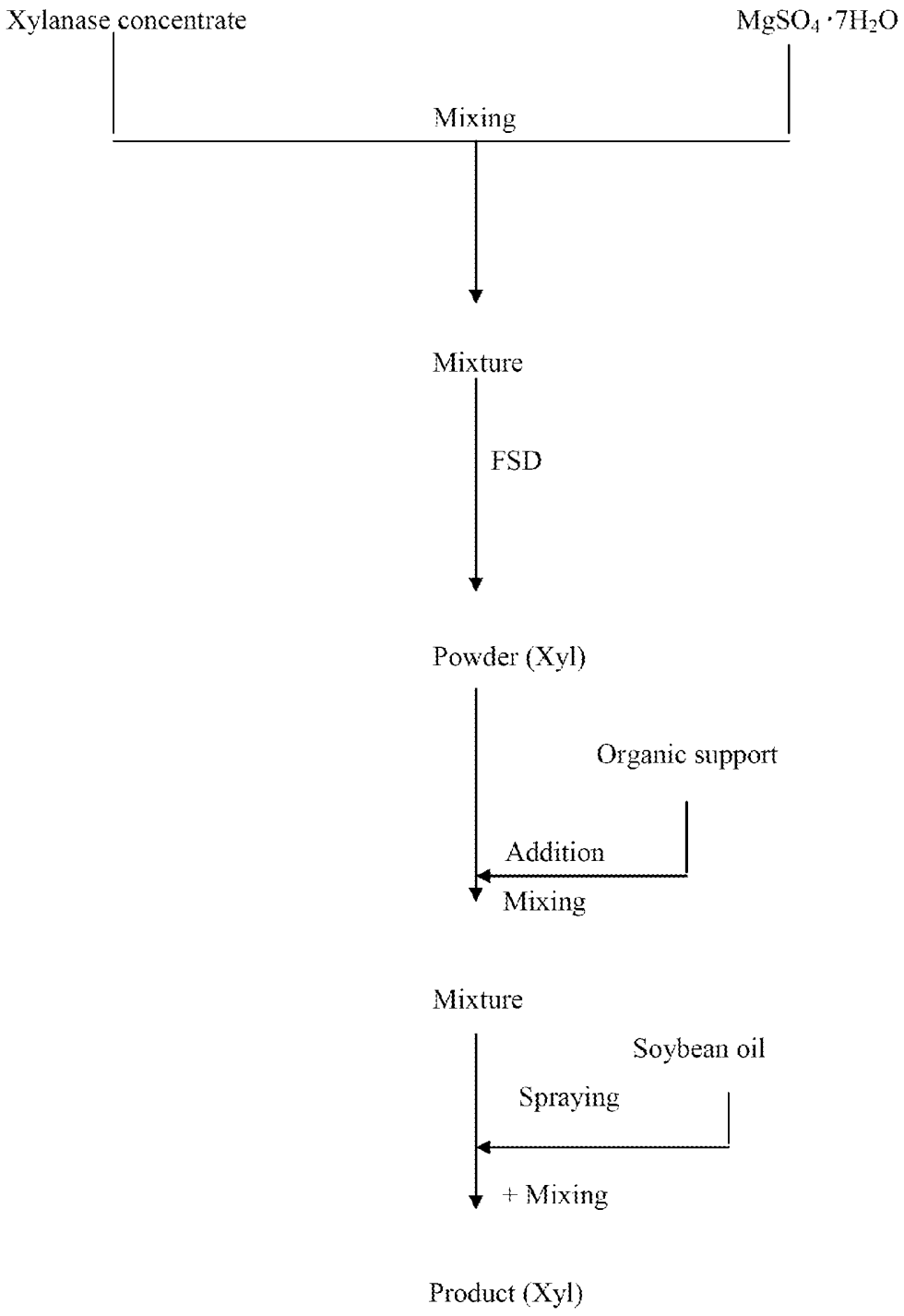

Xylanase concentrate
$MgSO_4 \cdot 7H_2O$
Mixing
Mixture
FSD
Powder (Xyl)
Organic support
Addition
Mixing
Mixture
Soybean oil
Spraying
+ Mixing
Product (Xyl)

Fig. 1

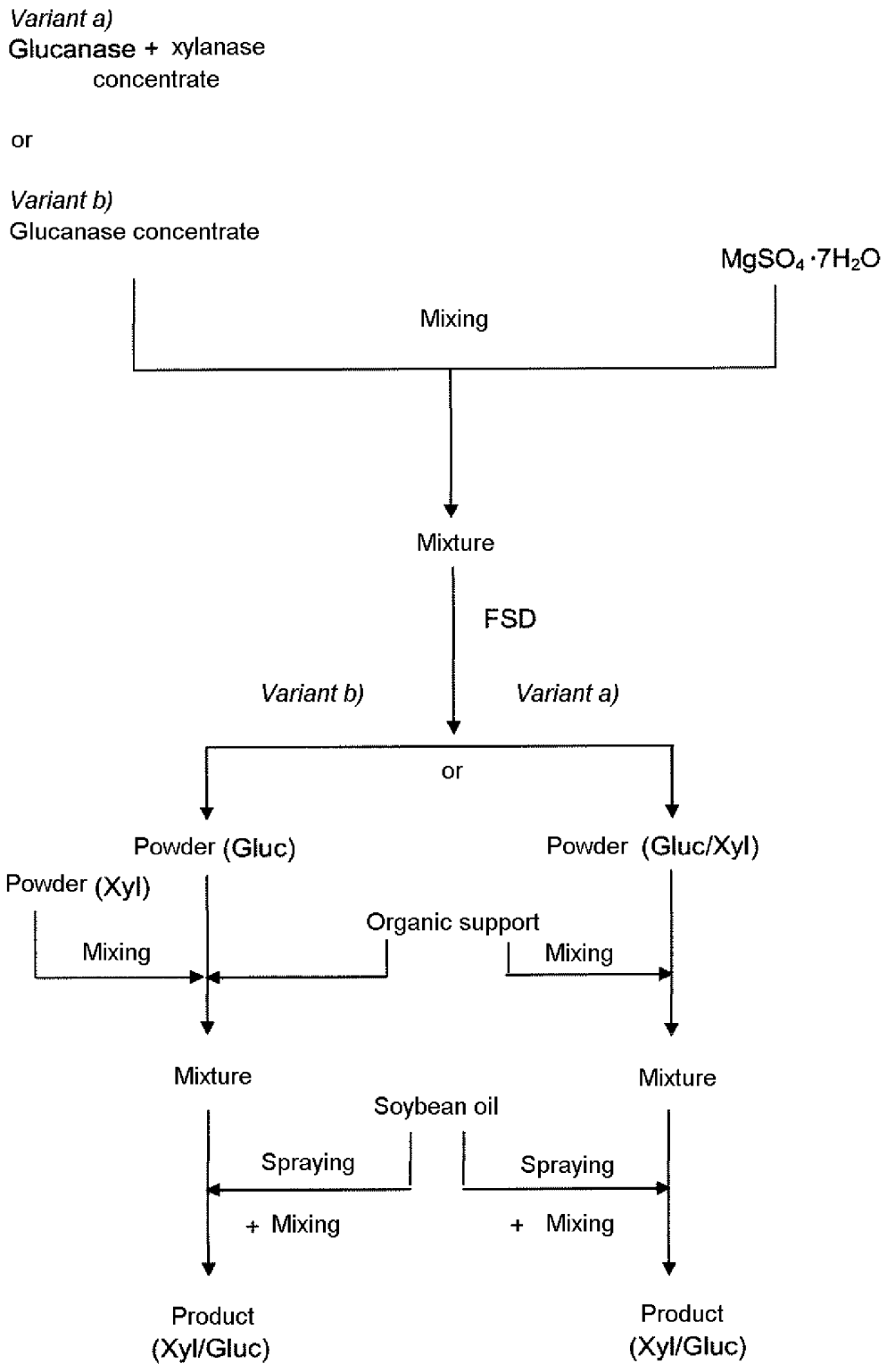

Variant a)
Glucanase + xylanase concentrate
or
Variant b)
Glucanase concentrate
MgSO4 ·7H2O
Mixing
Mixture
FSD
Variant b)
Variant a)
or
Powder (Gluc)
Powder (Gluc/Xyl)
Powder (Xyl)
Organic support
Mixing
Mixing
Mixture
Mixture
Soybean oil
Spraying
Spraying
+ Mixing
+ Mixing
Product (Xyl/Gluc)
Product (Xyl/Gluc)

Fig. 2