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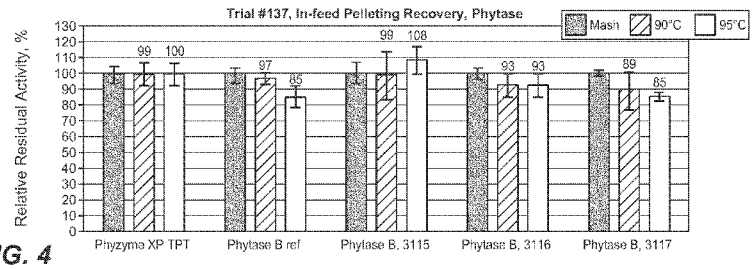


FIG. 4

(57) Abstract: The present teachings provide an improved population of granules comprising small smooth core particles. Methods of making and using are also provided.

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# GRANULES WITH SMALL SMOOTH CORES

## CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority from US provisional application USSN 61/837,122, filed 19 June 2013 and is incorporated herein by reference in their entirety.

## FIELD OF THE INVENTION

This disclosure is directed towards improved populations of granules containing active agents and methods of making and using.

## BACKGROUND OF THE INVENTION

The use of active agents, such as enzymes, in dry product formulations in industrial and consumer applications, such as detergents, textile compositions, baking, foods and animal feed has become a common practice. Enzymes are known to break down stains, modify fabric colors and textures, modify the viscosity of dough and foods, and improve digestibility of food and animal feed, by improving the availability of nutrients such as soluble phosphate and reducing anti-nutritional factors such as phytic acid in food and animal feed, thereby improving animal productivity.

Inactivation of enzymes can occur during storage in dry product formulations such as powdered laundry and dish detergents, textile processing blends, baking flours, and animal feeds composed of ingredients such as grains (such as corn or soy), vitamins, minerals and nutrients, such choline chloride, and additionally during industrial processing, (such as steam pelleting of animal feed) by exposure to high temperatures, steam or high humidity, compression or shear stress, and chemicals (such as acids, bases, surfactants, bleaches, or organic solvents). The inactivation is at least partially reversible if the enzyme reactivates after processing, for example, upon cooling after steam treatment and pelleting; however, the inactivation is frequently irreversible, such that the catalytic activity of the enzyme does not fully recover after processing, for example, upon cooling after steam treatment and pelleting. The irreversible

inactivation and reduced activity of an enzyme is generally not desirable in processes such as steam pelleting.

When compared with dry feed mixes, feed pellets have properties that are favored by the industry, such as improved feed quality, decreased pathogens, lower dust levels during manufacture, ease of handling, and more uniform ingredient dosing. Preferred industry pelleting processes utilize steam injection, in a process known as conditioning, which adds moisture and elevates the temperature prior to the pelleting step which forces the steam heated feed ingredients, or conditioned mash, through a die. The pelleting process temperatures may be from about 70°C to 95°C, or higher.

Because of the steam, temperatures, compression forces and chemicals used in pelleting processes, the activity or potency of enzymes are often significantly reduced during processing and subsequent storage. In fact, feed enzymes are often provided to the industry as stabilized liquid products that are sprayed onto feed pellets after the pelleting process to avoid enzyme inactivation. However, homogeneous dosing is difficult to achieve when the enzyme is applied post pelleting, for instance, by spraying the enzyme onto the pellets, and the cost of the equipment to add enzyme post-pelleting is high. Alternatively, liquid enzyme formulations, or dry mix enzyme formulations, may be added to the mixer prior to pelleting. In certain instances, higher levels of enzymes than otherwise needed may be added in order to compensate for losses during pelleting.

There is a need in the detergent, textile, baking, food and feed industries for stable, durable enzyme granules to serve as components in product and premix formulations that are stored for up to several months or years, or subjected to industrial processing operations, such as mixing or steam treatment pelleting processes, without appreciable loss of enzyme activity.

Approaches to avoid the problem of irreversibly inactivating enzymes or reducing the activity of the enzyme in industrial processes include identifying new sources of an enzyme (e.g. the identification of a known enzyme in an extreme thermophile microorganism) or identifying means to stabilize known enzymes. Klibanov, 1983, (*Stabilization of Enzymes against Thermal Inactivation*, Advances in Applied Microbiology, volume 29, page 1-28) discloses that there are three basic means for stabilizing enzymes: (1) immobilization, (2) chemical modification and (3) inclusion of additives. However, Klibanov (1983) further discloses that any one of these methods could lead to stabilization or destabilization, or have no effect at all. While previous formulation approaches have made some progress in this area (see for example WO97/23606, WO9854980, WO9739116, WO2007044968, EP1224273B1, WO2009/102770, US Patent

6,602,841, EP1804592B1, EP0098631B1, and EP1996028) the present teachings make an additional advance in overcoming some of these problems by use of an improved granule structure.

It is often desirable to provide a solid formulation with a high concentration of active enzyme in a granular or particulate form. The particulate granule is formulated with coatings, barrier coatings, and additives or excipients designed to provide protection of the enzyme against inactivation. To minimize the cost of these protective coatings, additives and excipients, and to minimize the impact of product transportation costs, it is desirable to formulate the granular enzyme with the highest feasible ratio of active enzyme to inactive ingredients. However, if the particle size of granules is held constant as the enzyme concentration or payload is increased, the number of particles for a given amount of enzyme dosed into the final product or application decreases. Too great a reduction in the number of particles per dose, however, can lead to high variability between individual doses of product. For example, a low average number of enzyme granules in a scoop of detergent, textile treatment blend, baking dough, or serving of animal feed, may lead to inhomogeneous distribution of enzyme concentration between individual doses or servings, and thus variability in efficacy of the enzyme treatment from dose to dose.

To achieve the economic benefits of increased enzyme payload, while avoiding the problems of variability resulting from a dearth of particles per dose, one can attempt to reduce the average particle diameter of the enzyme granule in order to maintain a sufficient number of particles per dose. However, it is difficult to apply enzyme coatings and protective barrier coatings to smaller core particles in coating processes such as fluid bed spray-coating, pan coating, drum coating and the like. As the diameter of core particles becomes smaller, particularly less than about 250, less than 200, less than 175, less than 150 microns, the tendency of particles to agglomerate increases. In addition, the application of a given weight percentage of coating material to a smaller core results in a thinner coating, hence a less protective coating. The problem of applying uniform coatings to small cores is challenging particularly when the particles are irregular in shape. Applying a coating to cores with pointed or angular corners and edges on the surface can result in regions of thin or discontinuous coating. Coatings applied to irregular coatings also may not adhere as well, or may result in agglomerates where asperities or surface protrusions on neighboring core particles come into contact with each other. Compensation for this by applying thicker coatings, if it can be done without agglomeration, will tend to add cost.

Thus, there is a need for improved methods of applying enzymes coatings to cores without incurring significant agglomeration while ensuring effective, continuous protective coatings. Achieving such high quality coatings on smaller particles will provide enhanced stability of the enzymes during processing and storage, while providing higher payloads, and while maintaining adequate homogeneity of the mixture in order not to increase dose-to-dose variability.

## BRIEF SUMMARY OF THE INVENTION

An object of the invention is to ensure or improve stability of an enzyme during storage alone, industrial processing, or in mixtures with other ingredients, while at the same time improving the ability to apply a continuous coating of consistent minimum thickness to the particle, that is, a coating with a reduced number of defects or thin spots in relationship to the weight percentage of coating material applied to the core or enzyme-containing core.

We have surprisingly found that providing cores that have a smoother, more regular surface, and a narrow particle size distribution allows for the application of improved coatings, with consistent minimum thickness, to smaller particles. In some embodiments, the improved coated enzyme granules are achieved by starting with a population of cores with a smoothness index less than 2.5, a weight average mean particle diameter of less than 300 microns, and a particle size dispersity index (PSDI) of less than 2.0, and coating the enzyme-containing cores with a further coating that comprises a polymer comprising at least 7% w/w of the final granule.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts illustrative small smooth core particles according to the present teachings, compared to other core particles that are small but irregular.

Figure 2 depicts some illustrative data according to the present teachings.

Figure 3 depicts some illustrative data according to the present teachings.

Figure 4 is a graph depicting the pelleting results of three representative granule batches. The black bars represent the mash activities, while dark gray and light grey bars represent recovery yields in 90°C and 95°C respectively.

Figure 5 is a graph depicting the pelleting results of three representative phytase standard TPT batches. The black bars represent the mash activities, while dark gray and light grey bars represent recovery yields in 90°C and 95°C respectively.

## DETAILED DESCRIPTION

The practice of the present teachings will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, granulation, and animal feed pelleting, which are within the skill of the art. Such techniques are explained fully in the literature, for example, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989); Oligonucleotide Synthesis (M. J. Gait, ed., 1984; Current Protocols in Molecular Biology (F. M. Ausubel et al., eds., 1994); PCR: The Polymerase Chain Reaction (Mullis et al., eds., 1994); Gene Transfer and Expression: A Laboratory Manual (Kriegler, 1990), Granulation Technology for Bioproducts (Kadam, 1990), and Fairfield, D. 1994. Chapter 10, Pelleting Cost Center. In Feed Manufacturing Technology IV. (McEllhiney, editor), American Feed Industry Association, Arlington, Va., pp. 110-139.

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present teachings belong. Singleton, et al., Dictionary of Microbiology and Molecular Biology, second ed., John Wiley and Sons, New York (1994), and Hale & Markham, The Harper Collins Dictionary of Biology, Harper Perennial, NY (1991) provide one of skill with a general dictionary of many of the terms used in this invention. Any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present teachings.

For ease of reference we have described elements of the present teachings under one or more headings. It is to be noted that the teachings under each of the headings also apply to the teachings under the other headings. For example, each of the stated embodiments and aspects concerning the use of the present teachings is equally an embodiment or aspect concerning the

method of the present teachings or the composition of the present teachings. Likewise, each of the stated embodiments and aspects concerning the method or use of the present teachings is equally an embodiment or aspect concerning the composition of the present teachings. In this application, the use of the singular includes the plural unless specifically stated otherwise.

All patents, patent applications, publications, documents, and articles cited herein are all incorporated herein by reference in their entireties.

Numeric ranges provided herein are inclusive of the numbers defining the range.

**Definitions**

As used herein, the term "granule" refers to a particle which contains a core (typically a small smooth core particle), an active agent (typically an enzyme), and optionally at least one additional coating.

As used herein, the term "core" refers to the inner nucleus of a granule, and typically comprises a "small smooth core particle". The cores of the present teachings may be produced by a variety of fabrication techniques including: rotary atomization, wet granulation, dry granulation, spray drying, disc granulation, extrusion, pan coating, spheronization, drum granulation, fluid-bed agglomeration, high-shear granulation, fluid-bed spray coating, crystallization, precipitation, emulsion gelation, spinning disc atomization and other casting approaches, and prill processes. Such processes are known in the art and are described in US Pat. No. 4689297 and US Pat. No. 5324649 (fluid bed processing); EP656058B1 and US Pat. No. 454332 (extrusion process); US Pat. No. 6248706 (granulation, high-shear); and EP804532B1 and US Pat. No. 6534466 (combination processes utilizing a fluid bed core and mixer coating).

The active agent is typically coated around the core. Suitable cores for use in the present teachings are can be any material meeting the smoothness index, mass median diameter, and particle size disparity definitions as provided herein. In one embodiment of the present teachings the core comprises a sodium chloride or sodium sulfate crystal. In another embodiment of the present teachings, the core comprises a sucrose crystal seed. Particles composed of inorganic salts and/or sugars and/or small organic molecules may be used as the cores of the present teachings. Suitable water soluble ingredients for incorporation into cores include: inorganic salts such as sodium chloride, ammonium sulfate, sodium sulfate, magnesium sulfate, zinc sulfate; or urea, citric acid, sugars such as sucrose, lactose and the like. In some embodiments, the core

particles are crystalline inorganic salts. In some embodiments, the core particles are crystalline sodium sulfate.

The term "small smooth core particle" as used herein refers to a particle, typically the inner particle of a granule, that exhibits certain smoothness index, mass median diameter, and particle size dispersity index measures, as provided herein.

The term "smoothness index" as used herein refers to the ratio of the empirical specific surface area to the envelope specific surface area calculated for a representative sample of cores.

As used herein, "representative sample of cores" refers to a random sample of at least 15 core particles.

The term "empirical specific surface area" as used herein refers to the empirically measured surface area per gram (measured in square meters per gram) of a representative sample of cores as determined, using the BET (Brunauer-Emmett-Teller) gas adsorption method (S. Brunauer, P. H. Emmett and E. Teller, *J. Am. Chem. Soc.*, 1938, **60**, 309. doi:10.1021/ja01269a023). The specific details of the BET method are described in the Methods section below.

The term "envelope specific surface area" as used herein refers to the calculated specific surface area (in units of square meters per gram) based upon the measured particle size distribution and average true density of a representative sample of cores, using the idealized assumption that all core particles are perfect spheres. An algorithm for calculating the envelope specific surface area is described in the Methods section below.

The term "particle size distribution" (abbreviated PSD) as used herein, refers to relative amount by mass percent of small smooth core particles present in each of several diameter size intervals. The PSD of the core sample is measured by laser light scattering (see for example T. Allen, Particle Size Measurement, Vol. 1 (Chapman and Hall, 1997). In the present application, the PSD is characterized by three mass percentile diameters, which can be determined from a log-normal plot of the PSD.

$D_{50}$ or MMD (mass median diameter) The log-normal distribution mass median diameter. The MMD is considered to be the mass average particle diameter, below which 50% w/w of the particles a representative sample have a smaller diameter.

$D_{10}$: Tenth percentile diameter (TPD), the diameter at which 10% w/w of the particles in a representative sample have a smaller diameter.

$D_{90}$: Ninetieth percentile diameter (NPD) the diameter at which 90% w/w of the particles in a representative sample have a smaller diameter.

Typically, the $D_{10}$, $D_{50}$ and $D_{90}$ are expressed in microns.

The "particle size dispersity index" (abbreviated PSDI) of a core sample is the $D_{90}$ / $D_{10}$ ratio of a representative sample.

The term "coating" as used herein refers to a layer of material surrounding an underlying material. The first coating layer generally encapsulates the core in order to form a substantially continuous layer so that the core surface has few or no uncoated areas, and is typically an active agent coating. Subsequent additional coating layers can encapsulate the growing granule to form one or more additional substantially continuous layer(s) ("additional coatings"). The materials (e.g. the active agents and components detailed herein) used in the granule can, but need not be, suitable for the use in foods and/or animal feeds, and accordingly can be food grade or feed grade.

The coatings of the present teachings may further comprise one or more of the following: inorganic salts, salts of organic acids, sugars, sugar alcohols, starches (native, pre-gelatinized, hydrolyzed, or chemically modified) and other polysaccharides, gums, additional active agents, feed or food grade polymers, pigments, clays, plasticizers, surfactants, fibrous materials, anti-tack agents, fillers, extenders and other compounds known to be used in coatings. Suitable polymers include polyvinyl alcohol (PVA), including partially and fully hydrolyzed PVA, polyethylene glycol, polyethylene oxide, polyvinyl pyrrolidine, and carbohydrate polymers (such as starch, amylose, amylopectin, alpha and beta-glucans, pectin, glycogen), including mixtures and derivatives thereof. Suitable fillers useful in the coatings include inert materials used to add bulk and reduce cost, or used for the purpose of adjusting the intended enzyme activity in the finished granule. Examples of such fillers include, but are not limited to, water soluble agents such as salts, sugars and water dispersible agents such as clays, talc, silicates, cellulose and starches, and cellulose and starch derivatives. Suitable plasticizers useful in the coatings of the present teachings are low molecular weight organic compounds and are highly specific to the polymer being plasticized. Examples include, but are not limited to, sugars (such as, glucose, fructose and sucrose), sugar alcohols (such as, sorbitol, xylitol and maltitol and other glycols), polar low molecular weight organic compounds, such as urea, or other known plasticizers such as water or feed grade plasticizers. Suitable fibrous materials useful in the coatings of the present teachings include, but are not limited to: cellulose, and cellulose

derivatives such as HPMC (hydroxy-propyl-methyl cellulose), CMC (carboxy-methyl cellulose), HEC (hydroxy-ethyl cellulose). In one embodiment, particularly for feed applications, of the present teachings, a coating comprises a water-soluble or dispersible corn cob material or sugar or salt crystal. In another embodiment particularly suitable for household cleaning applications, the coating comprises a water-soluble or dispersible sugar or salt crystal or a non pareil. Those skilled in the art will recognize that, for feed and food applications, the coatings (and any polymers, fillers, plasticizers, fibrous materials, and extenders), are acceptable for food and/or feed applications. For household cleaning applications, such a restriction need not apply.

The term "active agent coating" as used herein refers to the coating that contains the active agent (typically an enzyme). Generally, the active agent coating will be applied to the small smooth core particle, and thereafter additional coatings may optionally be applied.

The term "additional coating" as used herein refers to one or more coatings that are optionally applied to a small smooth core particle. Typically, the additional coating will be applied to a nascent granule that contains a small smooth core particle and an active agent coating. Non-limiting examples of additional coatings include moisture barrier coatings and moisture hydrating coatings.

The term "moisture barrier coating" as used herein refers to a coating that comprises a moisture barrier material.

The term "moisture hydrating coating" as used herein refers to a coating that comprises a moisture hydrating material.

The term "moisture barrier material" refers to materials that exclude, prevent or substantially retard water uptake. These materials typically are hydrophobic or amphiphilic, provide insulation against water and do not inherently absorb and/or bind water and include, but are not limited to, film-forming materials. Examples of moisture barrier materials include barrier polymers, proteins, lipids, fats and oils, fatty acids and gums. Examples of film forming moisture barrier materials are natural and modified barrier polymers, such as gum arabic, whey, whey protein concentrate, PVA, including modified PVA and fully hydrolyzed PVA, and synthetic polymers such as latex, HPMC, and acid-thinned hydroxypropyl starch, for example, PureCote™, oxidized starch, and modified starch. Non-film forming moisture barrier materials include, for instance, waxes, fats, oils and lipids, and lecithin. Selected moisture barrier materials that do not readily oxidize are, for example, latex polymer and barrier polymers such as gum arabic.

The term "moisture hydrating material" refers to materials that take up aqueous liquids, such as water, by one several mechanisms. In a first non-limiting mechanism, the materials

absorb free water. In a second non-limiting mechanism, the materials take up bound water that generally is present as crystalline waters of hydration. Accordingly, the materials may be provided as partially or fully hydrated materials or as non-hydrated materials that will absorb or bind aqueous liquids and retard or reduce the rate or extent of migration of such liquids to the active agent. In a third non-limiting mechanism, moisture hydrating materials thermally insulate the active agent by retarding heat transfer to the active agent within the granule and by maintaining the active agent at a lower temperature than the temperature at the exterior surface of the granule. Moisture hydrating materials include carbohydrates and inorganic salts, including hydrated salts, such as magnesium sulfate, sodium sulfate, and ammonium sulfate; maltodextrin; sugars, for example, sucrose; starch, including cornstarch.

As used herein, the terms "pellets" and "pelleting" refer to solid, rounded, spherical and cylindrical tablets or pellets and the processes for forming such solid shapes, particularly feed pellets and solid, extruded animal feed. Known food and animal feed pelleting manufacturing processes generally include admixing together food or feed ingredients for about 1 to about 5 minutes at room temperature, transferring the resulting admixture to a surge bin, conveying the admixture to a steam conditioner, optionally transferring the steam conditioned admixture to an expander, transferring the admixture to the pellet mill or extruder, and finally transferring the pellets into a pellet cooler. Fairfield, D. 1994. Chapter 10, Pelleting Cost Center. In Feed Manufacturing Technology IV. (McEllhiney, editor), American Feed Industry Association, Arlington, Va., pp. 110-139.

As used herein, the term "unpelleted mixtures" refers to premixes or precursors, base mixes, mash, and diluents. Premixes typically contain vitamins and trace minerals. Base mixes typically contain food and feed ingredients such as dicalcium phosphate, limestone, salt and a vitamin and mineral premix, but not grains and protein ingredients. Diluents include, but are not limited to grains (for example wheat middlings and rice bran) and clays, such as phyllosilicates (the magnesium silicate sepiolite, bentonite, kaolin, montmorillonite, hectorite, saponite, beidellite, attapulgite, and stevensite). Clays also function as carriers and fluidizing agent, or diluents, for feed premixes. Mash typically comprises a complete animal diet.

As used herein, the term "recovered activity" refers to the ratio of (i) the activity of an active agent after a treatment involving one or more of the following stressors: heating, increased pressure, increased pH, decreased pH, storage, drying, exposure to surfactant(s), exposure to solvent(s) (including water/moisture), and mechanical stress) to (ii) the activity of the phytase before the treatment. The recovered activity may be expressed as a percentage.

The percent recovered activity is calculated as follows:

$$\% \text{ recovered activity} = \left( \frac{\text{activity after treatment}}{\text{activity before treatment}} \right) \times 100\ \%$$

In the context of pelleting experiments, the "activity before treatment" can be approximated by measuring the active agent activity present in the mash that does not undergo treatment in a manner that is otherwise matched to the active agent that does undergo treatment. For example, the active agent in the untreated mash is handled and stored for a similar time and under similar conditions as the active agent in the treated (e.g, pelleted) mash, to control for possible interactions or other effects outside of the specified treatment per se.

As used herein, the term "active agent" may be any material that is to be added to a granule to provide the intended functionality for a given use. The active agent may be a biologically viable material, a food or feed ingredient, an antimicrobial agent, an antibiotic replacement agent, a prebiotic, a probiotic, an agrochemical ingredient, such as a pesticide, fertilizer or herbicide; a pharmaceutical ingredient or a household care active ingredient, or combinations thereof. In a preferred embodiment, the active ingredient is a protein, enzyme, peptide, polypeptide, amino acid, carbohydrate, lipid or oil, vitamin, co-vitamin, hormone, or combinations thereof. In another embodiment, the active ingredient is an enzyme, bleach, bleach activator, perfume, or other biologically active ingredient. Inherently thermostable active agents are encompassed by the present teachings and can exhibit enhanced thermostability in the granules. Most preferred active ingredients for food and feed applications are enzymes, peptides and polypeptides, amino acids, antimicrobials, gut health promoting agents, vitamins, and combinations thereof. Any enzyme may be used, and a nonlimiting list of enzymes include phytases, xylanases, β-glucanases, phosphatases, proteases, amylases (alpha or beta or glucoamylases) cellulases, lipases, cutinases, oxidases, transferases, reductases, hemicellulases, mannanases, esterases, isomerases, pectinases, lactases, peroxidases, laccases, other redox enzymes and mixtures thereof. Particularly preferred enzymes include a xylanase from *Trichoderma reesei* and a variant xylanase from *Trichoderma reesei*, both available from DuPont Industrial Biosciences or the inherently thermostable xylanase described in EP1222256B1, as well as other xylanases from *Aspergillus niger, Aspergillus kawachii, Aspergillus tubigensis, Aspergillus clavatus, Bacillus circulans, Bacillus pumilus, Bacillus subtilis, Fusarium verticilloides, Fusarium oxysporum, Neocallimastix patriciarum, Penicillium*

*species, Streptomyces lividans, Streptomyces thermoviolaceus, Thermomonospora fusca, Trichoderma harzianum, Trichoderma reesei, Trichoderma viride.* Additional particularly preferred enzymes include phytases, such as for example Finase L®, a phytase from Aspergillus sp., available from AB Enzymes, Darmstadt, Germany; Phyzyme™ XP, a phytase from *E. Coli*, and Axtra Phy, a phytase from *Buttiauxella,* both available from Danisco Animal Nutrition, DuPont, and other phytases from, for example, the following organisms: Trichoderma, Penicillium, Fusarium, Buttiauxella, Citrobacter, Enterobacter, Penicillium, Humicola, Hafnia, Bacillus, and Peniophora, as well as those phytases described in US patent applications 61/595,923 and 61/595,941, both filed February 12, 2012 . An example of a cellulase is Multifect® BGL, a cellulase (beta glucanase), available from Danisco Animal Nutrition, DuPont and other cellulases from species such as Aspergillus, Trichoderma, Penicillium, Humicola, Bacillus, Cellulomonas, Penicillium, Thermomonospore, Clostridium, and Hypocrea. The cellulases and endoglucanases described in US20060193897A1 also may be used. Amylases may be, for example, from species such as Aspergillus, Trichoderma, Penicillium, Bacillus, for instance, *B. subtilis*, *B. stearothermophilus*, *B. lentus*, *B. licheniformis*, *B. coagulans*, and *B. amyloliquefaciens.* Suitable fungal amylases are derived from *Aspergillus*, such as *A. oryzae* and *A. niger.* Proteases may be from *Bacillus amyloliquefaciens*, *Bacillus lentus* , *Bacillus subtilis, Bacillus licheniformis, and Aspergillus and Trichoderma species*. Phytases, xylanases, phosphatases, proteases, amylases, esterases, redox enzymes, lipases, transferases, cellulases, and β-glucanases are enzymes frequently used for inclusion in animal feed. Enzymes suitable for inclusion into tablets for household care applications are similar, particularly proteases, amylases, lipases, pectate lyases, mannanases, hemicellulases, redox enzymes, peroxidases, transferases, and cellulases. In particularly preferred aspects of the present teachings, the enzymes are selected from phytases, xylanases, beta glucanases, amylases, proteases, lipases, esterases, and mixtures thereof. In one embodiment of the present invention, two enzymes are provided in the granule, a xylanase and a beta-glucanase. In another embodiment of the present invention, two enzymes provided in the granule are a protease and amylase. The enzymes may be mixed together or applied to the granule separately. In another embodiment, three enzymes are provided in the granule, namely beta-glucanase, xylanase and phytase. The above enzyme lists are examples only and are not meant to be exclusive. Any enzyme may be used in the granules of the present teachings, including wild type, recombinant and variant enzymes of bacterial, fungal, yeast, plant, insect and animal sources, and acid, neutral or alkaline enzymes. It will be recognized by those skilled in the art that the amount of enzyme used will depend, at least in part, upon the type and property of the selected enzyme and the intended use.

## Methods

### BET specific surface area measurement.

To measure the specific surface area of core particles, the BET method was used to evaluate pore distribution of the cores by krypton gas adsorption / desorption, using a Micromeritics model ASAP 2420 Accelerated Porosimetry and Surface Area System. The BJH method (Elliott P. Barrett , Leslie G. Joyner , Paul P. Halenda, *J. Am. Chem. Soc.*, 1951, *73* (1), pp 373–380, **DOI:** 10.1021/ja01145a126) was used to determine pore volume distribution in the range 20 – 1000 Å. Samples, approximately 4-6 grams in mass, were outgassed in vacuo at 150°C temperature overnight to remove adsorbed water and / or volatile contaminants. They were re-weighed and transferred to the porosimeter, placed under vacuum and cooled to 77K at which point gas ( Kr) adsorption isotherms were recorded via pressure measurements. Surface area coverage is automatically computed by the ASAP 2420 V2.07J software package provided by Micromeritics.

### Envelope specific surface area calculation.

The envelope specific surface area represents the specific surface area of an equivalent idealized sample of perfectly smooth, spherical particles whose PSD matches that of the actual sample of cores. The particle size distribution (PSD) of the cores is determined via laser diffraction (reference ISO 13320-1:1999) using a Malvern Mastersizer 2000 with hexane as the background fluid in the recirculation loop. An amount of the sample of cores is added of sufficient mass to reach a laser obscuration of approximately 10%. The PSD is automatically calculated from the diffraction pattern using Mie theory. In the case of sodium sulfate, the complex refractive index of the particle is taken as 1.468 + 0.01i, and the refractive index of the fluid as 1.375. The generated PSD is a differential mass fraction showing the percentage of particles in each of 81 logarithmically-spaced size bins spanning 0.01 microns to 10,000 microns. If necessary, the PSD should be corrected and renormalized to exclude artifacts such as dust fines or agglomerates, specifically any particles finer than 60 microns or larger than 500 microns. The geometric mean diameter of the upper and lower size demarcations of each size bin is calculated by taking the square root of the product of the upper and lower size demarcation. The envelope specific surface area in square meters per gram for a given bin is given by $6/(d*\rho)$, where d is particle diameter (in cm), $\rho$ is the true density (density excluding voids) of the

particulate solid (in g/cm$^3$). In the case of sodium sulfate, $\rho = 2.664$ g/cm$^3$. Since the PSD is a mass-weighted distribution, the total envelope surface area is calculated by multiplying the differential mass fraction in each size bin by the specific surface area for that size bin and summing over all size bins.

## Exemplary Embodiments

In some embodiments, the present teachings provide a granule composition which surprisingly possesses equivalent stability compared to granules containing non-small smooth cores, thus allowing for savings in material cost. For example, the granule composition of the present teachings can possess surprisingly equivalent storage stability. The desirable storage stability can reside in any of a variety of contexts, including dish detergent, laundry detergent, animal feed, textiles, and human food. The granules of the present teachings can demonstrate improved stability under these various conditions as compared to identically stored granules lacking the small smooth core particle of the present teachings. In some embodiments, the present teachings are believed to provide improved stability compared to granules containing larger and/or non-smooth cores.

In some embodiments, the present teachings provide a granule composition with equivalent resistance to dust generation as compared to granules containing non small smooth cores as measured by the Heubach dust test.

In some embodiments, the present teachings are believed to provide a granule composition with improved resistance to dust generation relative to granules containing a non small smooth core.

In some embodiments, the present teachings provide for a granule composition of equivalent resistance to steam-pelleting compared to granules containing a non small smooth core. For example, the degree of "recovered activity" as defined supra can be determined for the granules of the present teachings, and compared to identically-treated granules containing a different core from the small smooth core particle of the present teachings. In some embodiments, the present teachings are believed to provide for a granule composition of improved resistance to steam-pelleting compared to granules containing a small smooth core.

Additional applications and methods employing the granules of the present teachings are described in the below non-limiting sections.

## Compositions and Methods for Baking and Food Preparation

The present teachings also relate to a "food composition," including but not limited to a food product, animal feed and/or food/feed additives, comprising the present granule composition, and methods for preparing such a food composition comprising mixing the present granule composition with one or more food ingredients, or uses thereof.

The present granule composition can be used in the preparation of a food composition, wherein the food composition is baked subsequent to the addition of the present granule composition. As used herein the term "baking composition" means any composition and/or additive prepared in the process of providing a baked food product, including but not limited to bakers flour, a dough, a baking additive and/or a baked product. The food composition or additive may be liquid or solid.

As used herein, the term "flour" means milled or ground cereal grain. The term "flour" also may mean Sago or tuber products that have been ground or mashed. In some embodiments, flour may also contain components in addition to the milled or mashed cereal or plant matter. An example of an additional component, although not intended to be limiting, is a leavening agent. Cereal grains include wheat, oat, rye, and barley. Tuber products include tapioca flour, cassava flour, and custard powder. The term "flour" also includes ground corn flour, maize-meal, rice flour, whole-meal flour, self-rising flour, tapioca flour, cassava flour, ground rice, enriched flower, and custard powder.

For the commercial and home use of flour for baking and food production, it is important to maintain an appropriate level of enzyme activity in the flour. A level of activity that is too high may result in a product that is sticky and/or doughy and therefore unmarketable. Flour with insufficient enzyme activity may not contain enough sugar for proper yeast function, resulting in dry, crumbly bread, or baked products. Accordingly, the present granule composition in combination with an α-amylase(s), may be added to the flour to augment the level of endogenous enzyme activity in flour.

An amylase in the present granule composition can be added alone or in a combination with other amylases to prevent or retard staling, i.e., crumb firming of baked products. The amount of anti-staling amylase will typically be in the range of 0.01-10 mg of enzyme protein per kg of flour, e.g., 0.5 mg/kg ds. Additional anti-staling amylases that can be used and include an endo-amylase, e.g., a bacterial endo-amylase from *Bacillus*. The additional amylase can be another maltogenic α-amylase (EC 3.2.1.133), e.g., from *Bacillus*. Novamyl® is an exemplary maltogenic α-amylase from *B. stearothermophilus* strain NCIB 11837 and is

described, for example, in Christophersen et al. (1997) *Starch* 50: 39-45. Other examples of anti-staling endo-amylases include bacterial α-amylases derived from *Bacillus*, such as *B. licheniformis* or *B. amyloliquefaciens*. The anti-staling amylase may be an exo-amylase, such as β-amylase, e.g., from plant sources, such as soybean, or from microbial sources, such as *Bacillus*.

The baking composition comprising the present granule composition further can comprise a phospholipase or enzyme with phospholipase activity. An enzyme with phospholipase activity has an activity that can be measured in Lipase Units (LU). The phospholipase may have $A_1$ or $A_2$ activity to remove fatty acid from the phospholipids, forming a lysophospholipid. It may or may not have lipase activity, i.e., activity on triglyceride substrates. The phospholipase typically has a temperature optimum in the range of 30-90°C., e.g., 30-70°C. The added phospholipases can be of animal origin, for example, from pancreas, e.g., bovine or porcine pancreas, snake venom, or bee venom. Alternatively, the phospholipase may be of microbial origin, e.g., from filamentous fungi, yeast or bacteria, for example.

The phospholipase is added in an amount that improves the softness of the bread during the initial period after baking, particularly the first 24 hours. The amount of phospholipase will typically be in the range of 0.01-10 mg of enzyme protein per kg of flour, e.g., 0.1-5 mg/kg. That is, phospholipase activity generally will be in the range of 20-1000 LU/kg of flour, where a Lipase Unit is defined as the amount of enzyme required to release 1 µmol butyric acid per minute at 30°C, pH 7.0, with gum arabic as emulsifier and tributyrin as substrate.

Compositions of dough generally comprise wheat meal or wheat flour and/or other types of meal, flour or starch such as corn flour, cornstarch, rye meal, rye flour, oat flour, oatmeal, soy flour, sorghum meal, sorghum flour, potato meal, potato flour or potato starch. The dough may be fresh, frozen, or par-baked. The dough can be a leavened dough or a dough to be subjected to leavening. The dough may be leavened in various ways, such as by adding chemical leavening agents, e.g., sodium bicarbonate or by adding a leaven, i.e., fermenting dough. Dough also may be leavened by adding a suitable yeast culture, such as a culture of *Saccharomyces cerevisiae* (baker's yeast), e.g., a commercially available strain of *S. cerevisiae*.

The dough may also comprise other conventional dough ingredients, e.g., proteins, such as milk powder, gluten, and soy; eggs (e.g., whole eggs, egg yolks or egg whites); an oxidant, such as ascorbic acid, potassium bromate, potassium iodate, azodicarbonamide (ADA) or ammonium persulfate; an amino acid such as L-cysteine; a sugar; or a salt, such as sodium

chloride, calcium acetate, sodium sulfate, or calcium sulfate. The dough further may comprise fat, e.g., triglyceride, such as granulated fat or shortening. The dough further may comprise an emulsifier such as mono- or diglycerides, diacetyl tartaric acid esters of mono- or diglycerides, sugar esters of fatty acids, polyglycerol esters of fatty acids, lactic acid esters of monoglycerides, acetic acid esters of monoglycerides, polyoxyethylene stearates, or lysolecithin. For example, the dough can be made without addition of emulsifiers.

The dough product may be any processed dough product, including fried, deep fried, roasted, baked, steamed and boiled doughs, such as steamed bread and rice cakes. In one embodiment, the food product is a bakery product. Typical bakery (baked) products include bread - such as loaves, rolls, buns, bagels, pizza bases etc. pastry, pretzels, tortillas, cakes, cookies, biscuits, crackers etc.

Optionally, an additional enzyme may be used together with the anti-staling amylase and the phospholipase. The additional enzyme may be a second amylase, such as an amyloglucosidase, a β-amylase, a cyclodextrin glucanotransferase, or the additional enzyme may be a peptidase, in particular an exopeptidase, a transglutaminase, a lipase, a cellulase, a xylanase, a protease, a protein disulfide isomerase, e.g., a protein disulfide isomerase as disclosed in WO 95/00636, for example, a glycosyltransferase, a branching enzyme (1,4-α-glucan branching enzyme), a 4-α-glucanotransferase (dextrin glycosyltransferase) or an oxidoreductase, e.g., a peroxidase, a laccase, a glucose oxidase, a pyranose oxidase, a lipooxygenase, an L-amino acid oxidase or a carbohydrate oxidase. The additional enzyme(s) may be of any origin, including mammalian and plant, and particularly of microbial (bacterial, yeast or fungal) origin and may be obtained by techniques conventionally used in the art.

The xylanase is typically of microbial origin, e.g., derived from a bacterium or fungus, such as a strain of *Aspergillus*. Xylanases include Pentopan® and Novozym 384®, for example, which are commercially available xylanase preparations produced from *Trichoderma reesei*. The amyloglucosidase may be an *A. niger* amyloglucosidase (such as AMG®). Other useful amylase products include Grindamyl® A 1000 or A 5000 (Grindsted Products, Denmark) and Amylase® H or Amylase® P (DSM). The glucose oxidase may be a fungal glucose oxidase, in particular an *Aspergillus niger* glucose oxidase (such as Gluzyme®). An exemplary protease is Neutrase®.

The process may be used for any kind of baked product prepared from dough, either of a soft or a crisp character, either of a white, light or dark type. Examples are bread, particularly white, whole-meal or rye bread, typically in the form of loaves or rolls, such as, but

not limited to, French baguette-type bread, pita bread, tortillas, cakes, pancakes, biscuits, cookies, piecrusts, crisp bread, steamed bread, pizza and the like.

The present granule composition may be used in a pre-mix, comprising flour together with an anti-staling amylase, a phospholipase, and/or a phospholipid. The pre-mix may contain other dough-improving and/or bread-improving additives, e.g., any of the additives, including enzymes, mentioned above. The present granule composition can be a component of an enzyme preparation comprising an anti-staling amylase and a phospholipase, for use as a baking additive.

The storing, handling and incorporation of the loaded delivery vehicle can be accomplished by means of a packaged mix. The packaged mix can comprise the present granule composition. However, the packaged mix may further contain additional ingredients as required by the manufacturer or baker. After the present granule composition has been incorporated into the dough, the baker continues through the normal production process for that product.

A food composition is contemplated where the food is an oil, meat, lard, composition comprising the present granule composition. In this context the term "[oil/meat/lard] composition" means any composition, based on, made from and/or containing oil, meat or lard, respectively. A method is contemplated for preparing an oil or meat or lard composition and/or additive comprising the present granule composition, comprising mixing the present granule composition with a oil/meat/lard composition and/or additive ingredients.

The food composition can be an animal feed composition, animal feed additive, and/or pet food comprising the present granule composition. A method is contemplated for preparing such an animal feed composition, animal feed additive composition and/or pet food comprising mixing the present granule composition thereof with one or more animal feed ingredients and/or animal feed additive ingredients and/or pet food ingredients. The present granule composition can be used in the preparation of an animal feed composition and/or animal feed additive composition and/or pet food.

The term "animal" includes all non-ruminant and ruminant animals. In a particular embodiment, the animal is a non-ruminant animal, such as a horse and a mono-gastric animal. Examples of mono-gastric animals include, but are not limited to, pigs and swine, such as piglets, growing pigs, sows; poultry such as turkeys, ducks, chicken, broiler chicks, layers; fish such as salmon, trout, tilapia, catfish and carps; and crustaceans such as shrimps and prawns. In a further embodiment the animal is a ruminant animal including, but not limited to, cattle, young

calves, goats, sheep, giraffes, bison, moose, elk, yaks, water buffalo, deer, camels, alpacas, llamas, antelope, pronghorn and nilgai.

In the present context, it is intended that the term "pet food" is understood to mean a food for a household animal such as, but not limited to dogs, cats, gerbils, hamsters, chinchillas, fancy rats, guinea pigs; avian pets, such as canaries, parakeets, and parrots; reptile pets, such as turtles, lizards and snakes; and aquatic pets, such as tropical fish and frogs.

The terms "animal feed composition," "feedstuff" and "fodder" are used interchangeably and may comprise one or more feed materials selected from the group comprising a) cereals, such as small grains (e.g., wheat, barley, rye, oats and combinations thereof) and/or large grains such as maize or sorghum; b) by products from cereals, such as corn gluten meal, Distillers Dried Grain Solubles (DDGS) (particularly corn based Distillers Dried Grain Solubles (cDDGS), wheat bran, wheat middlings, wheat shorts, rice bran, rice hulls, oat hulls, palm kernel, and citrus pulp; c) protein obtained from sources such as soya, sunflower, peanut, lupin, peas, fava beans, cotton, canola, fish meal, dried plasma protein, meat and bone meal, potato protein, whey, copra, sesame; d) oils and fats obtained from vegetable and animal sources; e) minerals and vitamins.

**Textile Desizing Compositions and Use**

Also contemplated are compositions and methods of treating fabrics (e.g., to desize a textile) using the present granule composition. Fabric-treating methods are well known in the art (*see*, *e.g.*, U.S. Patent No. 6,077,316). For example, the feel and appearance of a fabric can be improved by a method comprising contacting the fabric with the present granule composition in a solution. The fabric can be treated with the solution under pressure.

The present granule composition can be applied during or after the weaving of a textile, or during the desizing stage, or one or more additional fabric processing steps. During the weaving of textiles, the threads are exposed to considerable mechanical strain. Prior to weaving on mechanical looms, warp yarns are often coated with sizing starch or starch derivatives to increase their tensile strength and to prevent breaking. The present granule composition can be applied during or after the weaving to remove these sizing starches or starch derivatives. After weaving, a present granule composition can be used to remove the size coating before further processing the fabric to ensure a homogeneous and wash-proof result.

The present granule composition can be used alone or with other desizing chemical reagents and/or desizing enzymes to desize fabrics, including cotton-containing fabrics, as detergent additives, e.g., in aqueous compositions. The present granule composition also can be used in compositions and methods for producing a stonewashed look on indigo-dyed denim fabric and garments. For the manufacture of clothes, the fabric can be cut and sewn into clothes or garments, which are afterwards finished. In particular, for the manufacture of denim jeans, different enzymatic finishing methods have been developed. The finishing of denim garment normally is initiated with an enzymatic desizing step, during which garments are subjected to the action of amylolytic enzymes to provide softness to the fabric and make the cotton more accessible to the subsequent enzymatic finishing steps. The present granule composition can be used in methods of finishing denim garments (e.g., a "bio-stoning process"), enzymatic desizing and providing softness to fabrics, and/or finishing process.

**Cleaning Compositions**

An aspect of the present compositions and methods is a cleaning composition that includes the present granule composition as a component. A protease polypeptide, or other relevant enzyme, can be used as a component in detergent compositions for hand washing, laundry washing, dishwashing, and other hard-surface cleaning.

Preferably, the present granule composition is incorporated into detergents at or near a concentration conventionally used for amylase in detergents. For example, protease polypeptide may be added in amount corresponding to 0.00001 – 1 mg (calculated as pure enzyme protein) of amylase per liter of wash/dishwash liquor. Exemplary formulations are provided herein, as exemplified by the following:

A protease polypeptide may be a component of a detergent composition, as the only enzyme or with other enzymes including other amylolytic enzymes. As such, it may be included in the detergent composition in the form of the present granule composition. When employed in this context, non-limiting examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1,000 to 20,000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in, for example, GB 1483591.

The detergent composition can comprise one or more surfactants, each of which may be anionic, nonionic, cationic, or zwitterionic. The detergent will usually contain 0% to about 50% of anionic surfactant, such as linear alkylbenzenesulfonate (LAS); α-olefinsulfonate (AOS); alkyl sulfate (fatty alcohol sulfate) (AS); alcohol ethoxysulfate (AEOS or AES); secondary alkanesulfonates (SAS); α-sulfo fatty acid methyl esters; alkyl- or alkenylsuccinic acid; or soap. The composition may also contain 0% to about 40% of nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, or polyhydroxy alkyl fatty acid amide (as described for example in WO 92/06154).

The detergent composition may additionally comprise one or more other enzymes, such as another protease, another amylolytic enzyme, cutinase, lipase, cellulase, pectate lyase, perhydrolase, mannanase, xylanase, peroxidase, and/or laccase in any combination.

The detergent may contain about 1% to about 65% of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (*e.g.,* SKS-6 from Hoechst). The detergent may also be unbuilt, *i.e.* essentially free of detergent builder. Enzymes, and specifically amylases, either with or without starch binding domains, can be used in a variety of compositions including laundry and dishwashing applications, surface cleaners, as well as in compositions for ethanol production from starch or biomass.

The detergent may comprise one or more polymers. Examples include carboxymethylcellulose (CMC), poly(vinylpyrrolidone) (PVP), polyethyleneglycol (PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

The detergent may contain a bleaching system, which may comprise a $H_2O_2$ source such as perborate or percarbonate, which may be combined with a peracid-forming bleach activator such as tetraacetylethylenediamine (TAED) or nonanoyloxybenzenesulfonate (NOBS). Alternatively, the bleaching system may comprise peroxyacids (*e.g.,* the amide, imide, or sulfone type peroxyacids). The bleaching system can also be an enzymatic bleaching system, for example, perhydrolase, such as that described in International PCT Application WO 2005/056783.

The enzymes of the detergent composition may be stabilized using conventional stabilizing agents, *e.g.,* a polyol such as propylene glycol or glycerol; a sugar or sugar alcohol; lactic acid; boric acid or a boric acid derivative such as, *e.g.,* an aromatic borate ester; and the composition may be formulated as described in, *e.g.,* WO 92/19709 and WO 92/19708.

The detergent may also contain other conventional detergent ingredients such as *e.g.*, fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil redeposition agents, dyes, bactericides, tarnish inhibiters, optical brighteners, or perfumes.

The pH (measured in aqueous solution at use concentration) is usually neutral or alkaline, *e.g.*, pH about 7.0 to about 11.0.

Particular forms of detergent compositions for inclusion of the present teachings are described, below.

**Heavy Duty Dry/Solid (HDD) laundry detergent composition**

Exemplary HDD laundry detergent compositions includes a detersive surfactant, including anionic detersive surfactants (e.g., linear or branched or random chain, substituted or unsubstituted alkyl sulphates, alkyl sulphonates, alkyl alkoxylated sulphate, alkyl phosphates, alkyl phosphonates, alkyl carboxylates and/or mixtures thereof), non-ionic detersive surfactant (e.g., linear or branched or random chain, substituted or unsubstituted $C_8$-$C_{18}$ alkyl ethoxylates, and/or $C_6$-$C_{12}$ alkyl phenol alkoxylates), cationic detersive surfactants (e.g., alkyl pyridinium compounds, alkyl quaternary ammonium compounds, alkyl quaternary phosphonium compounds, alkyl ternary sulphonium compounds, and mixtures thereof), zwitterionic and/or amphoteric detersive surfactants (e.g., alkanolamine sulpho-betaines), ampholytic surfactants, semi-polar non-ionic surfactants, and mixtures thereof; builders including phosphate free builders (for example zeolite builders examples which include zeolite A, zeolite X, zeolite P and zeolite MAP in the range of 0wt% to less than 10wt%), phosphate builders (for example sodium tri-polyphosphate in the range of 0wt% to less than 10wt%), citric acid, citrate salts and nitrilotriacetic acid, silicate salt (e.g., sodium or potassium silicate or sodium meta-silicate in the range of 0wt% to less than 10wt%, or layered silicate (SKS-6)); carbonate salt (e.g., sodium carbonate and/or sodium bicarbonate in the range of 0 wt% to less than 80 wt%); and bleaching agents including photobleaches (e.g., sulfonated zinc phthalocyanines, sulfonated aluminum phthalocyanines, xanthenes dyes, and mixtures thereof) hydrophobic or hydrophilic bleach activators (e.g., dodecanoyl oxybenzene sulfonate, decanoyl oxybenzene sulfonate, decanoyl

oxybenzoic acid or salts thereof, 3,5,5-trimethy hexanoyl oxybenzene sulfonate, tetraacetyl ethylene diamine-TAED, nonanoyloxybenzene sulfonate-NOBS, nitrile quats, and mixtures thereof), sources of hydrogen peroxide (e.g., inorganic perhydrate salts examples of which include mono or tetra hydrate sodium salt of perborate, percarbonate, persulfate, perphosphate, or persilicate), preformed hydrophilic and/or hydrophobic peracids (e.g., percarboxylic acids and salts, percarbonic acids and salts, perimidic acids and salts, peroxymonosulfuric acids and salts, and mixtures thereof), and/or bleach catalysts (e.g., imine bleach boosters (examples of which include iminium cations and polyions), iminium zwitterions, modified amines, modified amine oxides, N-sulphonyl imines, N-phosphonyl imines, N-acyl imines, thiadiazole dioxides, perfluoroimines, cyclic sugar ketones, and mixtures thereof, and metal-containing bleach catalysts (e.g., copper, iron, titanium, ruthenium, tungsten, molybdenum, or manganese cations along with an auxiliary metal cations such as zinc or aluminum and a sequestrate such as ethylenediaminetetraacetic acid, ethylenediaminetetra(methylenephosphonic acid), and water-soluble salts thereof).

The composition preferably includes enzymes, e.g., proteases, amylases, lipases, cellulases, choline oxidases, peroxidases/oxidases, pectate lyases, mannanases, cutinases, laccases, phospholipases, lysophospholipases, acyltransferase, perhydrolase, arylesterase, and any mixture thereof.

The composition may optionally include additional detergent ingredients including perfume microcapsules, starch encapsulated perfume accord, hueing agents, additional polymers, including fabric integrity and cationic polymers, dye-lock ingredients, fabric-softening agents, brighteners (for example C.I. Fluorescent brighteners), flocculating agents, chelating agents, alkoxylated polyamines, fabric deposition aids, and/or cyclodextrin.

**Automatic dishwashing (ADW) detergent composition**

Exemplary ADW detergent composition includes non-ionic surfactants, including ethoxylated non-ionic surfactants, alcohol alkoxylated surfactants, epoxy-capped poly(oxyalkylated) alcohols, or amine oxide surfactants present in amounts from 0 to 10% by weight; builders in the range of 5-60% including phosphate builders (e.g., mono-phosphates, di-phosphates, tri-polyphosphates, other oligomeric-poylphosphates, sodium tripolyphosphate-STPP) and phosphate-free builders (e.g., amino acid-based compounds including methyl-glycine-diacetic acid (MGDA) and salts and derivatives thereof, glutamic-N,N-diacetic acid (GLDA) and salts and derivatives thereof, iminodisuccinic acid (IDS) and salts and derivatives

thereof, carboxy methyl inulin and salts and derivatives thereof, nitrilotriacetic acid (NTA), diethylene triamine penta acetic acid (DTPA), B-alaninediacetic acid (B-ADA) and their salts, homopolymers and copolymers of poly-carboxylic acids and their partially or completely neutralized salts, monomeric polycarboxylic acids and hydroxycarboxylic acids and their salts in the range of 0.5% to 50% by weight; sulfonated/carboxylated polymers in the range of about 0.1 % to about 50% by weight to to provide dimensional stability; drying aids in the range of about 0.1 % to about 10% by weight (e.g., polyesters, especially anionic polyesters, optionally together with further monomers with 3 to 6 functionalities - typically acid, alcohol or ester functionalities which are conducive to polycondensation, polycarbonate-, polyurethane- and/or polyurea-polyorganosiloxane compounds or precursor compounds, thereof, particularly of the reactive cyclic carbonate and urea type); silicates in the range from about 1 % to about 20% by weight (including sodium or potassium silicates for example sodium disilicate, sodium meta-silicate and crystalline phyllosilicates); inorganic bleach (e.g., perhydrate salts such as perborate, percarbonate, perphosphate, persulfate and persilicate salts) and organic bleach (e.g., organic peroxyacids, including diacyl and tetraacylperoxides, such as diperoxydodecanedioc acid, diperoxytetradecanedioc acid, and diperoxyhexadecanedioc acid); bleach activators (i.e., organic peracid precursors in the range from about 0.1 % to about 10% by weight); bleach catalysts (e.g., manganese triazacyclononane and related complexes, Co, Cu, Mn, and Fe bispyridylamine and related complexes, and pentamine acetate cobalt(III) and related complexes); metal care agents in the range from about 0.1% to 5% by weight (e.g., benzatriazoles, metal salts and complexes, and/or silicates); enzymes in the range from about 0.01 to 5.0 mg of active enzyme per gram of automatic dishwashing detergent composition (e.g., proteases, amylases, lipases, cellulases, choline oxidases, peroxidases/oxidases, pectate lyases, mannanases, cutinases, laccases, phospholipases, lysophospholipases, acyltransferase, perhydrolase, arylesterase, and mixtures thereof); and enzyme stabilizer components (e.g., oligosaccharides, polysaccharides, and inorganic divalent metal salts).

**Additional detergent compositions**

Additional exemplary detergent formulations to which any of a variety of relevant enzymes in the present granule compositions can be added are described, below, in the numbered paragraphs.

1) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising linear alkylbenzenesulfonate (calculated as acid) about 7% to about 12%; alcohol

ethoxysulfate (*e.g.*, $C_{12-18}$ alcohol, 1-2 ethylene oxide (EO)) or alkyl sulfate (*e.g.*, $C_{16-18}$) about 1% to about 4%; alcohol ethoxylate (*e.g.*, $C_{14-15}$ alcohol, 7 EO) about 5% to about 9%; sodium carbonate (*e.g.*, $Na_2CO_3$) about 14% to about 20%; soluble silicate (*e.g.*, $Na_2O$, $2SiO_2$) about 2 to about 6%; zeolite (*e.g.*, $NaA1SiO_4$) about 15% to about 22%; sodium sulfate (*e.g.*, $Na_2SO_4$) 0% to about 6%; sodium citrate/citric acid (*e.g.*, $C_6H_5Na_3O_7/C_6H_8O_7$) about 0% to about 15%; sodium perborate (*e.g.*, $NaBO_3H_2O$) about 11% to about 18%; TAED about 2% to about 6%; carboxymethylcellulose (CMC) and 0% to about 2%; polymers (*e.g.*, maleic/acrylic acid, copolymer, PVP, PEG) 0-3%; enzymes (calculated as pure enzyme) 0.0001-0.1% protein; and minor ingredients (*e.g.*, suds suppressors, perfumes, optical brightener, photobleach) 0-5%.

2) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising linear alkylbenzenesulfonate (calculated as acid) about 6% to about 11%; alcohol ethoxysulfate (*e.g.*, $C_{12-18}$ alcohol, 1-2 EO) or alkyl sulfate (*e.g.*, $C_{16-18}$) about 1% to about 3%; alcohol ethoxylate (*e.g.*, $C_{14-15}$ alcohol, 7 EO) about 5% to about 9%; sodium carbonate (*e.g.*, $Na_2CO_3$) about 15% to about 21%; soluble silicate (*e.g.*, $Na_2O$, $2SiO_2$) about 1% to about 4%; zeolite (*e.g.*, $NaA1SiO_4$) about 24% to about 34%; sodium sulfate (*e.g.*, $Na_2SO_4$) about 4% to about 10%; sodium citrate/citric acid (*e.g.*, $C_6H_5Na_3O_7/ C_6H_8O_7$) 0% to about 15%; carboxymethylcellulose (CMC) 0% to about 2%; polymers (*e.g.*, maleic/acrylic acid copolymer, PVP, PEG) 1-6%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; minor ingredients (*e.g.*, suds suppressors, perfume) 0-5%.

3) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising linear alkylbenzenesulfonate (calculated as acid) about 5% to about 9%; alcohol ethoxylate (*e.g.*, $C_{12-15}$ alcohol, 7 EO) about 7% to about 14%; Soap as fatty acid (*e.g.*, $C_{16-22}$ fatty acid) about 1 to about 3%; sodium carbonate (as $Na_2CO_3$) about 10% to about 17%; soluble silicate (*e.g.*, $Na_2O$, $2SiO_2$) about 3% to about 9%; zeolite (as $NaA1SiO_4$) about 23% to about 33%; sodium sulfate (*e.g.*, $Na_2SO_4$) 0% to about 4%; sodium perborate (*e.g.*, $NaBO_3H_2O$) about 8% to about 16%; TAED about 2% to about 8%; phosphonate (*e.g.*, EDTMPA) 0% to about 1%; carboxymethylcellulose (CMC) 0% to about 2%; polymers (*e.g.*, maleic/acrylic acid copolymer, PVP, PEG) 0-3%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; minor ingredients (*e.g.*, suds suppressors, perfume, optical brightener) 0-5%.

4) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising linear alkylbenzenesulfonate (calculated as acid) about 8% to about 12%; alcohol ethoxylate (*e.g.*, $C_{12-15}$ alcohol, 7 EO) about 10% to about 25%; sodium carbonate (as $Na_2CO_3$) about 14% to about 22%; soluble silicate (*e.g.*, $Na_2O$, $2SiO_2$) about 1% to about 5%; zeolite (*e.g.*, $NaA1SiO_4$) about 25% to about 35%; sodium sulfate (*e.g.*, $Na_2SO_4$) 0% to about 10%; carboxymethylcellulose (CMC) 0% to about 2%; polymers (*e.g.*, maleic/acrylic acid copolymer, PVP, PEG) 1-3%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, suds suppressors, perfume) 0-5%.

5) An aqueous liquid detergent composition comprising linear alkylbenzenesulfonate (calculated as acid) about 15% to about 21%; alcohol ethoxylate (*e.g.*, $C_{12-15}$ alcohol, 7 EO or $C_{12-15}$ alcohol, 5 EO) about 12% to about 18%; soap as fatty acid (*e.g.*, oleic acid) about 3% to about 13%; alkenylsuccinic acid ($C_{12-14}$) 0% to about 13%; aminoethanol about 8% to about 18%; citric acid about 2% to about 8%; phosphonate 0% to about 3%; polymers (*e.g.*, PVP, PEG) 0% to about 3%; borate (*e.g.*, $B_4O_7$) 0% to about 2%; ethanol 0% to about 3%; propylene glycol about 8% to about 14%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, dispersants, suds suppressors, perfume, optical brightener) 0-5%.

6) An aqueous structured liquid detergent composition comprising linear alkylbenzenesulfonate (calculated as acid) about 15% to about 21%; alcohol ethoxylate (*e.g.*, $C_{12-15}$ alcohol, 7 EO, or $C_{12-15}$ alcohol, 5 EO) 3-9%; soap as fatty acid (*e.g.*, oleic acid) about 3% to about 10%; zeolite (as $NaA1SiO_4$) about 14% to about 22%; potassium citrate about 9% to about 18%; borate (*e.g.*, $B_4O_7$) 0% to about 2%; carboxymethylcellulose (CMC) 0% to about 2%; polymers (*e.g.*, PEG, PVP) 0% to about 3%; anchoring polymers such as, *e.g.*, lauryl methacrylate/acrylic acid copolymer; molar ratio 25:1, MW 3800) 0% to about 3%; glycerol 0% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, dispersants, suds suppressors, perfume, optical brighteners) 0-5%.

7) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising fatty alcohol sulfate about 5% to about 10%; ethoxylated fatty acid monoethanolamide about 3% to about 9%; soap as fatty acid 0-3%; sodium carbonate (*e.g.*, $Na_2CO_3$) about 5% to about 10%; Soluble silicate (*e.g.*, $Na_2O$, $2SiO_2$) about 1% to about 4%; zeolite (*e.g.*, $NaA1SiO_4$) about 20% to about 40%; Sodium sulfate (*e.g.*, $Na_2SO_4$) about 2% to

about 8%; sodium perborate (*e.g.*, $NaBO_3H_2O$) about 12% to about 18%; TAED about 2% to about 7%; polymers (*e.g.*, maleic/acrylic acid copolymer, PEG) about 1% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, optical brightener, suds suppressors, perfume) 0-5%.

8) A detergent composition formulated as a granulate comprising linear alkylbenzenesulfonate (calculated as acid) about 8% to about 14%; ethoxylated fatty acid monoethanolamide about 5% to about 11%; soap as fatty acid 0% to about 3%; sodium carbonate (*e.g.*, $Na_2CO_3$) about 4% to about 10%; soluble silicate ($Na_2O$, $2SiO_2$) about 1% to about 4%; zeolite (*e.g.*, $NaA1SiO_4$) about 30% to about 50%; sodium sulfate (*e.g.*, $Na_2SO_4$) about 3% to about 11%; sodium citrate (*e.g.*, $C_6H_5Na_3O_7$) about 5% to about 12%; polymers (*e.g.*, PVP, maleic/acrylic acid copolymer, PEG) about 1% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, suds suppressors, perfume) 0-5%.

9) A detergent composition formulated as a granulate comprising linear alkylbenzenesulfonate (calculated as acid) about 6% to about 12%; nonionic surfactant about 1% to about 4%; soap as fatty acid about 2% to about 6%; sodium carbonate (*e.g.*, $Na_2CO_3$) about 14% to about 22%; zeolite (*e.g.*, $NaA1SiO_4$) about 18% to about 32%; sodium sulfate (*e.g.*, $Na_2SO_4$) about 5% to about 20%; sodium citrate (*e.g.*, $C_6H_5Na_3O_7$) about 3% to about 8%; sodium perborate (*e.g.*, $NaBO_3H_2O$) about 4% to about 9%; bleach activator (*e.g.*, NOBS or TAED) about 1% to about 5%; carboxymethylcellulose (CMC) 0% to about 2%; polymers (*e.g.*, polycarboxylate or PEG) about 1% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, optical brightener, perfume) 0-5%.

10) An aqueous liquid detergent composition comprising linear alkylbenzenesulfonate (calculated as acid) about 15% to about 23%; alcohol ethoxysulfate (*e.g.*, $C_{12-15}$ alcohol, 2-3 EO) about 8% to about 15%; alcohol ethoxylate (*e.g.*, $C_{12-15}$ alcohol, 7 EO, or $C_{12-15}$ alcohol, 5 EO) about 3% to about 9%; soap as fatty acid (*e.g.*, lauric acid) 0% to about 3%; aminoethanol about 1% to about 5%; sodium citrate about 5% to about 10%; hydrotrope (*e.g.*, sodium toluensulfonate) about 2% to about 6%; borate (*e.g.*, $B_4O_7$) 0% to about 2%; carboxymethylcellulose 0% to about 1%; ethanol about 1% to about 3%; propylene glycol about 2% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, polymers, dispersants, perfume, optical brighteners) 0-5%.

11) An aqueous liquid detergent composition comprising linear alkylbenzenesulfonate (calculated as acid) about 20% to about 32%; alcohol ethoxylate (*e.g.*, $C_{12-15}$ alcohol, 7 EO, or $C_{12-15}$ alcohol, 5 EO) 6-12%; aminoethanol about 2% to about 6%; citric acid about 8% to about 14%; borate (*e.g.*, $B_4O_7$) about 1% to about 3%; polymer (*e.g.*, maleic/acrylic acid copolymer, anchoring polymer such as, *e.g.*, lauryl methacrylate/acrylic acid copolymer) 0% to about 3%; glycerol about 3% to about 8%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, hydrotropes, dispersants, perfume, optical brighteners) 0-5%.

12) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising anionic surfactant (linear alkylbenzenesulfonate, alkyl sulfate, α-olefinsulfonate, α-sulfo fatty acid methyl esters, alkanesulfonates, soap) about 25% to about 40%; nonionic surfactant (*e.g.*, alcohol ethoxylate) about 1% to about 10%; sodium carbonate (*e.g.*, $Na_2CO_3$) about 8% to about 25%; soluble silicates (*e.g.*, $Na_2O$, $2SiO_2$) about 5% to about 15%; sodium sulfate (*e.g.*, $Na_2SO_4$) 0% to about 5%; zeolite ($NaA1SiO_4$) about 15% to about 28%; sodium perborate (*e.g.*, $NaBO_3{\cdot}4H_2O$) 0% to about 20%; bleach activator (TAED or NOBS) about 0% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; minor ingredients (*e.g.*, perfume, optical brighteners) 0-3%.

13) Detergent compositions as described in compositions 1)-12) *supra,* wherein all or part of the linear alkylbenzenesulfonate is replaced by ($C_{12}$-$C_{18}$) alkyl sulfate.

14) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising ($C_{12}$-$C_{18}$) alkyl sulfate about 9% to about 15%; alcohol ethoxylate about 3% to about 6%; polyhydroxy alkyl fatty acid amide about 1% to about 5%; zeolite (*e.g.*, $NaA1SiO_4$) about 10% to about 20%; layered disilicate (*e.g.*, SK56 from Hoechst) about 10% to about 20%; sodium carbonate (*e.g.*, $Na_2CO_3$) about 3% to about 12%; soluble silicate (*e.g.*, $Na_2O$, $2SiO_2$) 0% to about 6%; sodium citrate about 4% to about 8%; sodium percarbonate about 13% to about 22%; TAED about 3% to about 8%; polymers (*e.g.*, polycarboxylates and PVP) 0% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, optical brightener, photobleach, perfume, suds suppressors) 0-5%.

15) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising ($C_{12}$-$C_{18}$) alkyl sulfate about 4% to about 8%; alcohol ethoxylate about 11% to about 15%; soap about 1% to about 4%; zeolite MAP or zeolite A about 35% to about 45%; sodium carbonate (as $Na_2CO_3$) about 2% to about 8%; soluble silicate (*e.g.*, $Na_2O$, $2SiO_2$) 0% to about 4%; sodium percarbonate about 13% to about 22%; TAED 1-8%; carboxymethylcellulose (CMC) 0% to about 3%; polymers (*e.g.*, polycarboxylates and PVP) 0% to about 3%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, optical brightener, phosphonate, perfume) 0-3%.

16) Detergent formulations as described in 1)-15) supra, which contain a stabilized or encapsulated peracid, either as an additional component or as a substitute for already specified bleach systems.

17) Detergent compositions as described supra in 1), 3), 7), 9), and 12), wherein perborate is replaced by percarbonate.

18) Detergent compositions as described supra in 1), 3), 7), 9), 12), 14), and 15), which additionally contain a manganese catalyst. The manganese catalyst for example is one of the compounds described in "Efficient manganese catalysts for low-temperature bleaching," *Nature* 369: 637-639 (1994).

19) Detergent composition formulated as a non-aqueous detergent liquid comprising a liquid nonionic surfactant such as, *e.g.*, linear alkoxylated primary alcohol, a builder system (*e.g.*, phosphate), an enzyme(s), and alkali. The detergent may also comprise anionic surfactant and/or a bleach system.

As above, the present enzyme may be incorporated at a concentration conventionally employed in detergents. It is at present contemplated that, in the detergent composition, the enzyme may be added in an amount corresponding to 0.00001-1.0 mg (calculated as pure enzyme protein) of enzyme polypeptide per liter of wash liquor.

The detergent composition may also contain other conventional detergent ingredients, *e.g.,* deflocculant material, filler material, foam depressors, anti-corrosion agents, soil-suspending agents, sequestering agents, anti-soil redeposition agents, dehydrating agents, dyes, bactericides, fluorescers, thickeners, and perfumes.

The detergent composition may be formulated as a hand (manual) or machine (automatic) laundry detergent composition, including a laundry additive composition suitable for pre-treatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations, or be formulated for manual or automatic dishwashing operations.

Any of the cleaning compositions described, herein, may include any number of additional enzymes. In general the enzyme(s) should be compatible with the selected detergent, (*e.g.*, with respect to pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, and the like), and the enzyme(s) should be present in effective amounts. The following enzymes are provided as examples.

*Proteases*: Suitable proteases include those of animal, vegetable or microbial origin. Chemically modified or protein engineered mutants are included, as well as naturally processed proteins. The protease may be a serine protease or a metalloprotease, an alkaline microbial protease, a trypsin-like protease, or a chymotrypsin-like protease. Examples of alkaline proteases are subtilisins, for example those derived from *Bacillus*, *e.g.*, subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147, and subtilisin 168 (*see, e.g.,* WO 89/06279). Examples of trypsin-like proteases are trypsin (*e.g.,* of porcine or bovine origin), and *Fusarium* proteases (*see, e.g.,* WO 89/06270 and WO 94/25583). Examples of useful proteases also include but are not limited to the variants described in WO 92/19729, WO 98/20115, WO 98/20116, and WO 98/34946. Commercially available protease enzymes include but are not limited to: ALCALASE®, SAVINASE®, PRIMASE™, DURALASE™, ESPERASE®, KANNASE™, and BLAZE™ (Novo Nordisk A/S and Novozymes A/S); MAXATASE®, MAXACAL™, MAXAPEM™, PROPERASE®, PURAFECT®, PURAFECT OXP™, FN2™, and FN3™ (Danisco US Inc.). Other exemplary proteases include NprE from *Bacillus amyloliquifaciens* and ASP from *Cellulomonas* sp. strain 69B4.

*Lipases*: Suitable lipases include those of bacterial or fungal origin. Chemically modified, proteolytically modified, or protein engineered mutants are included. Examples of useful lipases

include but are not limited to lipases from *Humicola* (synonym *Thermomyces*), *e.g.*, from *H. lanuginosa* (*T. lanuginosus*) (*see e.g.,* EP 258068 and EP 305216), from *H. insolens* (*see e.g.,* WO 96/13580); a *Pseudomonas* lipase (*e.g.*, from *P. alcaligenes* or *P. pseudoalcaligenes*; *see, e.g.,* EP 218 272), *P. cepacia* (*see e.g.,* EP 331 376), *P. stutzeri* (*see e.g.,* GB 1,372,034), *P. fluorescens*, *Pseudomonas* sp. strain SD 705 (*see e.g.,* WO 95/06720 and WO 96/27002), *P. wisconsinensis* (*see e.g.,* WO 96/12012); a *Bacillus* lipase (*e.g.,* from *B. subtilis*; *see e.g.,* Dartois *et al. Biochemica et Biophysica Acta*, 1131: 253-360 (1993)), *B. stearothermophilus* (*see e.g.,* JP 64/744992), or *B. pumilus* (*see e.g.,* WO 91/16422). Additional lipase variants contemplated for use in the formulations include those described for example in: WO 92/05249, WO 94/01541, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079, WO 97/07202, EP 407225, and EP 260105. Some commercially available lipase enzymes include LIPOLASE® and LIPOLASE ULTRA™ (Novo Nordisk A/S and Novozymes A/S).

*Polyesterases*: Suitable polyesterases can be included in the composition, such as those described in, for example, WO 01/34899, WO 01/14629, and US6933140.

*Amylases*: The compositions can be combined with amylases, such as non-production enhanced amylase. These can include commercially available amylases, such as but not limited to STAINZYME®, NATALASE®, DURAMYL®, TERMAMYL®, FUNGAMYL® and BAN™ (Novo Nordisk A/S and Novozymes A/S); RAPIDASE®, POWERASE®, and PURASTAR® (from Danisco US Inc.).

*Cellulases*: Cellulases can be added to the compositions. Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, *e.g.*, the fungal cellulases produced from *Humicola insolens*, *Myceliophthora thermophila* and *Fusarium oxysporum* disclosed for example in U.S. Patent Nos. 4,435,307; 5,648,263; 5,691,178; 5,776,757; and WO 89/09259. Exemplary cellulases contemplated for use are those having color care benefit for the textile. Examples of such cellulases are cellulases described in for example EP 0495257, EP 0531372, WO 96/11262, WO 96/29397, and WO 98/08940. Other examples are cellulase variants, such as those described in WO 94/07998; WO 98/12307; WO 95/24471; PCT/DK98/00299; EP 531315; U.S. Patent Nos.

5,457,046; 5,686,593; and 5,763,254. Commercially available cellulases include CELLUZYME® and CAREZYME® (Novo Nordisk A/S and Novozymes A/S); and PURADAX HA® (Danisco US Inc.); and KAC-500(B)™ (Kao Corporation).

*Peroxidases/Oxidases*: Suitable peroxidases/oxidases contemplated for use in the compositions include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus*, *e.g.*, from *C. cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257. Commercially available peroxidases include for example GUARDZYME™ (Novo Nordisk A/S and Novozymes A/S).

The detergent composition can also comprise 2,6-β-D-fructan hydrolase, which is effective for removal/cleaning of biofilm present on household and/or industrial textile/laundry.

The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive, *i.e.* a separate additive or a combined additive, can be formulated *e.g.*, as a granulate, a liquid, a slurry, and the like. Exemplary detergent additive formulations include but are not limited to granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids or slurries.

Reduced-dusting granulates may be produced, and added with the present granule compositions, *e.g.*, as disclosed in U.S. Patent Nos. 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (*e.g.,* polyethyleneglycol, PEG) with mean molar weights of 1,000 to 20,000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in, for example, GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods.

The detergent composition may be in any convenient form, *e.g.*, a bar, a tablet, a powder, a granule, a paste, or a liquid. A liquid detergent may be aqueous, typically containing

up to about 70% water, and 0% to about 30% organic solvent. Compact detergent gels containing about 30% or less water are also contemplated. The detergent composition can optionally comprise one or more surfactants, which may be non-ionic, including semi-polar and/or anionic and/or cationic and/or zwitterionic. The surfactants can be present in a wide range, from about 0.1% to about 60% by weight.

When included therein the detergent will typically contain from about 1% to about 40% of an anionic surfactant, such as linear alkylbenzenesulfonate, α-olefinsulfonate, alkyl sulfate (fatty alcohol sulfate), alcohol ethoxysulfate, secondary alkanesulfonate, α-sulfo fatty acid methyl ester, alkyl- or alkenylsuccinic acid, or soap.

When included therein, the detergent will usually contain from about 0.2% to about 40% of a non-ionic surfactant such as alcohol ethoxylate, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, polyhydroxy alkyl fatty acid amide, or N-acyl-N-alkyl derivatives of glucosamine ("glucamides").

The detergent may contain 0% to about 65% of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, carbonate, citrate, nitrilotriacetic acid, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid, alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (*e.g.*,SKS-6 from Hoechst).

The detergent may comprise one or more polymers. Exemplary polymers include carboxymethylcellulose (CMC), poly(vinylpyrrolidone) (PVP), poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), poly(vinylpyridine-N-oxide), poly(vinylimidazole), polycarboxylates *e.g.,* polyacrylates, maleic/acrylic acid copolymers), and lauryl methacrylate/acrylic acid copolymers.

The enzyme(s) of the detergent composition may be stabilized using conventional stabilizing agents, *e.g.*, as polyol (*e.g.,* propylene glycol or glycerol), a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative (*e.g.*, an aromatic borate ester), or a phenyl boronic acid derivative (*e.g.,* 4-formylphenyl boronic acid). The composition may be formulated as described in WO 92/19709 and WO 92/19708.

It is contemplated that in the enzyme-containing detergent compositions, it may be added in an amount corresponding to about 0.01 to about 100 mg of enzyme protein per liter of wash liquor (*e.g.*, about 0.05 to about 5.0 mg of enzyme protein per liter of wash liquor or 0.1 to about 1.0 mg of enzyme protein per liter of wash liquor).

Although the present compositions and methods have been described with reference to the details below, it would be understood that various modifications can be made.

Numerous enzyme cleaning assays are known in the art, including swatch and micro-swatch assays.

**Brewing Compositions**

The present granule composition may be a component of a brewing composition used in a process of providing a fermented beverage, such as brewing. It is believed that non-fermentable carbohydrates form the majority of the dissolved solids in the final beer. This residue remains because of the inability of malt amylases to hydrolyze the alpha-1,6-linkages of the starch. The non-fermentable carbohydrates contribute about 50 calories per 12 ounces (about 340 grams) of beer. The present granule composition, usually in combination with a glucoamylase and optionally a pullulanase and/or isoamylase, assist in converting the starch into dextrins and fermentable sugars, lowering the residual non-fermentable carbohydrates in the final beer.

The principal raw materials used in making these beverages are water, hops and malt. In addition, but also exclusively, adjuncts such as common corn grits, refined corn grits, brewer's milled yeast, rice, sorghum, refined corn starch, barley, barley starch, dehusked barley, wheat, wheat starch, torrified cereal, cereal flakes, rye, oats, potato, tapioca, and syrups, such as corn syrup, sugar cane syrup, inverted sugar syrup, barley and/or wheat syrups, and the like may be used as a source of starch.

For a number of reasons, the malt, which is produced principally from selected varieties of barley, has an important effect on the overall character and quality of the beer. First, the malt is the primary flavoring agent in beer. Second, the malt provides the major portion of the fermentable sugar. Third, the malt provides the proteins, which will contribute to the body and foam character of the beer. Fourth, the malt provides the necessary enzymatic activity during mashing. Hops also contribute significantly to beer quality, including flavoring. In particular, hops (or hops constituents) add desirable bittering substances to the beer. In addition, the hops can act as protein precipitants, establish preservative agents and aid in foam formation and stabilization.

Cereals (grains), such as barley, oats, wheat, but also corn and rice are often used for brewing, both in industry and for home brewing, but also other plant components, such as hops

are often added. The components used in brewing may be unmalted or may be malted, i.e., partially germinated, resulting in an increase in the levels of enzymes, including α-amylase. For successful brewing, adequate levels of α-amylase enzyme activity are necessary to ensure the appropriate levels of sugars for fermentation. The present granule composition may also be added to the components used for brewing.

As used herein, the term "stock" means grains and plant components that are crushed or broken. For example, barley used in beer production is a grain that has been coarsely ground or crushed to yield a consistency appropriate for producing a mash for fermentation. As used herein, the term "stock" includes any of the aforementioned types of plants and grains in crushed or coarsely ground forms. The methods described herein may be used to determine α-amylase activity levels in both flours and stock.

Processes for making beer are well known in the art. *See, e.g.*, Wolfgang Kunze (2004) "Technology Brewing and Malting," Research and Teaching Institute of Brewing, Berlin (VLB), 3rd edition. Briefly, the process involves: (a) preparing a mash, (b) filtering the mash to prepare a wort, and (c) fermenting the wort to obtain a fermented beverage, such as beer. Typically, milled or crushed malt, malt and adjunct, or adjunct is mixed with water and held for a period of time under controlled temperatures to permit the enzymes present in the malt and/or adjunct to convert the starch present in the malt into fermentable sugars. The mash is then transferred to a mash filter where the liquid is separated from the grain residue. This sweet liquid is called "wort," and the left over grain residue is called "spent grain." The mash is typically subjected to an extraction, which involves adding water to the mash in order to recover the residual soluble extract from the spent grain. The wort is then boiled vigorously to sterilizes the wort and help develop the color, flavor and odor. Hops are added at some point during the boiling. The wort is cooled and transferred to a fermentor.

The wort is then contacted in a fermentor with yeast. The fermentor may be chilled to stop fermentation. The yeast that may flocculate is removed. Finally, the beer is cooled and stored for a period of time, during which the beer clarifies and its flavor develops, and any material that might impair the appearance, flavor, and shelf life of the beer settles out. The beer usually contains from about 2% to about 10% v/v alcohol, although beer with a higher alcohol content, e.g., 18% v/v, may be obtained. Prior to packaging, the beer is carbonated and, optionally, filtered, and pasteurized.

The brewing composition comprising an alpha-amylase, often, but not necessarily in combination with one or more exogenous enzymes, such as glucoamylase(s), pullulanase(s)

and/or isoamylase(s), and any combination thereof, may be added to the mash of step (a) above, i.e., during the preparation of the mash. Alternatively, or in addition, the brewing composition may be added to the mash of step (b) above, such as during the filtration of the mash. Alternatively, or in addition, the brewing composition may be added to the wort of step (c) above, such as during the fermenting of the wort.

Particular embodiments pertains to any of the above uses, methods or fermented beverages, wherein said fermented beverage is a beer, such as full malted beer, beer brewed under the "Reinheitsgebot," ale, IPA, lager, bitter, Happoshu (second beer), third beer, dry beer, near beer, light beer, low alcohol beer, low calorie beer, porter, bock beer, stout, malt liquor, non-alcoholic beer, non-alcoholic malt liquor and the like, but also alternative cereal and malt beverages such as fruit flavoured malt beverages, e.g., citrus flavoured, such as lemon-, orange-, lime-, or berry-flavoured malt beverages, liquor flavoured malt beverages, e.g., vodka-, rum-, or tequila-flavoured malt liquor, or coffee flavoured malt beverages, such as caffeine-flavoured malt liquor, and the like.

Additional Animal Feed Embodiments

In some embodiments, especially in the context of animal feed applications, the granules of the present teachings can be used with any of a variety of at least one of the following enzymes:

**XYLANASES**

| **Commercial Name ®** | **Company** | **Xylanase type** | **Xylanase source** |
|---|---|---|---|
| Allzyme PT | Alltech | endo-1,4-β-xylanase | *Aspergillus Niger* |
| Amylofeed | Andrés Pintaluba S.A | endo-1,4-β-xylanase | *Aspergillus Niger (phoenicis)* |
| Avemix 02 CS | Aveve | endo-1,4-β-xylanase | *Trichoderma reesei* |
| AveMix XG 10 | Aveve, NL | endo-1,4-β-xylanase | *Trichoderma reesei* |
| Avizyme 1100 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Avizyme 1110 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Avizyme 1202 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Avizyme 1210 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Avizyme 1302 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Avizyme 1500 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |

| | | | |
|---|---|---|---|
| Avizyme 1505 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Avizyme SX | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Belfeed MP100 | Beldem | endo-1,4-β-xylanase | *Bacillus subtilis* |
| Biofeed Plus | DSM | endo-1,4-β-xylanase | *Humicola insolens* |
| Bio Feed® Wheat | Novozymes | endo-1,4-β-xylanase | |
| Danisco Glycosidase (TPT/L) | Danisco Animal Nutrition | endo-1,4-β-xylanase | *Trichoderma reesei* |
| Danisco Xylanase | Danisco | endo-1,4-β-xylanase | *Trichoderma reesei* |
| Dyadic® Xylanase PLUS | Dyadic | endo-1,4-β-D-xylanase | *Trichoderma longibrachiatum (formerly Trichoderma reesei)* |
| Econase XT | ABVista | endo-1,4-β-xylanase | *Trichoderma reesei* |
| Endofeed® DC | Andres Pintaluba S.A. | endo-1,4-β-xylanase | *Aspergillus Niger* |
| Feedlyve AXL | Lyven | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Grindazym GP | Danisco | endo-1,4-β-xylanase | *Aspergillus Niger* |
| Grindazym GV | Danisco | endo-1,4-β-xylanase | *Aspergillus Niger* |
| Hostazym X | Huvepharma | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Kemzyme Plus Dry | Kemin | endo-1,4-β-xylanase | *Trichoderma viride* |
| Kemzyme Plus Liquid | Kemin | endo-1,4-β-xylanase | *Trichoderma viride* |
| Kemzyme W dry | Kemin | endo-1,4-β-xylanase | *Trichoderma viride* |
| Kemzyme W liquid | Kemin | endo-1,4-β-xylanase | *Trichoderma viride* |
| Natugrain | BASF | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Natugrain TS Plus | BASF | endo-1,4-β-xylanase | *Aspergillus Niger* |
| Natugrain Wheat | BASF | endo-1,4-β-xylanase | *Aspergillus Niger* |
| Natugrain® TS/L | BASF | endo-1,4-β-xylanase | *Aspergillus Niger* |
| Natuzyme | Bioproton | endo-1,4-β-xylanase | *Trichoderma longibrachiatum /Trichoderma reesei* |
| Porzyme 8100 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Porzyme 8300 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |

| | | | |
|---|---|---|---|
| Porzyme 9102 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Porzyme 9310/Avizyme 1310 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Porzyme tp100 | Danisco | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Ronozyme AX | DSM | endo-1,4-β-xylanase | *Thermomyces lanuginosus gene expressed in Aspergillus oryzae* |
| Ronozyme WX | DSM/Novozymes | endo-1,4-β-xylanase | *Thermomyces lanuginosus gene expressed in Aspergillus oryzae* |
| Rovabio Excel | Adisseo | endo-1,4-β-xylanase | *Penicillium funiculosum* |
| Roxazyme G2 | DSM/Novozymes | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Safizym X | Le Saffre | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |
| Xylanase | Lyven | endo-1,4-β-xylanase | *Trichoderma longibrachiatum* |

**Amylase**

| **Commercial product®** | **Company** | **Amylase type** | **Amylase source** |
|---|---|---|---|
| AlphaStar PLUS | Dyadic | alpha amylase | *Bacillus subtilis* |
| GlucoStar PLUS | Dyadic | gluco-amylase | *Aspergillus niger* |
| Amylofeed | Andrés Pintaluba S.A | alpha amylase | *Aspergillus oryzae* |
| Avizyme 1500 | Danisco | alpha amylase | *Bacillus amyloliquefaciens* |
| Avizyme 1505 | Danisco | alpha amylase | *Bacillus amyloliquefaciens* |
| Kemzyme Plus Dry | Kemin | alpha-amylase | *Bacillus amyloliquefaciens* |
| Kemzyme Plus Liquid | Kemin | alpha-amylase | *Bacillus amyloliquefaciens* |
| Kemzyme W dry | Kemin | alpha-amylase | *Bacillus amyloliquefaciens* |
| Kemzyme W liquid | Kemin | alpha-amylase | *Bacillus amyloliquefaciens* |
| Natuzyme | Bioproton | alpha-amylase | *Trichoderma longibrachiatum /Trichoderma reesei* |
| Porzyme 8100 | Danisco | alpha-amylase | *Bacillus amyloliquefaciens* |
| Porzyme tp100 | Danisco | alpha-amylase | *Bacillus amyloliquefaciens* |
| Ronozyme A | DSM/Novozymes | alpha-amylase | *Bacillus amyloliquefaciens* |
| Ronozyme AX | DSM | alpha-amylase | *Bacillus amyloliquefaciens* |
| Ronozyme® RumiStar (L/CT) | DSM/Novozymes | alpha-amylase | *Bacillus stearothermophilus* expressed in *Bacillus licheniformis* |

**Protease**

| Commercial product® | Company | Protease type | Protease source |
|---|---|---|---|
| Avizyme 1100 | Danisco A/S | Subtilisin | *Bacillus subtilis* |
| Avizyme 1202 | Danisco A/S | Subtilisin | *Bacillus subtilis* |
| Avizyme 1302 | Danisco A/S | Subtilisin | *Bacillus subtilis* |
| Avizyme 1500 | Danisco A/S | Subtilisin | *Bacillus subtilis* |
| Avizyme 1505 | Danisco A/S | Subtilisin | *Bacillus subtilis* |
| Kemzyme Plus Dry | Kemin | Bacillolysin | *Bacillus amyloliquefaciens* |
| Kemzyme W dry | Kemin | Bacillolysin | *Bacillus amyloliquefaciens* |
| Natuzyme | Bioproton | Protease | *Trichoderma longibrachiatum /Trichoderma reesei* |
| Porzyme 8300 | Danisco | Subtilisin | *Bacillus subtilis* |
| Ronozyme ProAct | DSM/Novozymes | Alkaline serine protease | *Neocardiopsis prasina gene* expressed in *Bacillus licheniformis* |
| Cibenza DP100 | Novus | Keratinase | *Bacillus licheniformis* |
| CIBENZA IND900 | Novus | | |
| Protease PLUS | Dyadic | alkaline protease | *Bacillus licheniformis* |
| Protease AP CONC | Dyadic | alkaline protease | *Bacillus licheniformis* |

**Phytase**

| Commercial product® | Company | Phytase type | Phytase source |
|---|---|---|---|
| Axtra Phy | Danisco | 6-phytase | *Buttiauxella sp.* |
| Bio-feed® Phytase | Novozymes. | | |
| CIBENZA® PHOS | Novus | | |
| Finase | ABVista | 3-phytase | *Trichoderma reesei* |
| Finase EC | ABVista | 6-phytase | *E. coli* gene expressed in *Trichoderma reesei* |
| MICROTECH 5000 plus | VTR | | |
| Natuphos | BASF | 3-phytase | *Aspergillus Niger* |
| Natuzyme | Bioproton | phytase (type not specified) | *Trichoderma longibrachiatum /Trichoderma reesei* |
| OPTIPHOS® | Huvepharma AD | 6-phytase | *E. coli* gene expressed in *Pichia pastoris* |
| Phytase sp1002 | DSM | 3-phytase | *Hansenula polymorpha* |
| Phyzyme XP | Danisco | 6-phytase | *E. coli* gene expressed in *Schizosaccahomyces pombe* |

| | | | |
|---|---|---|---|
| Quantum 2500D, 5000L | ABVista | 6-phytase | *E. coli* gene expressed in *Pichia pastoris* |
| Quantum Blue | ABVista | | |
| Quantum® XT | ABVista | | |
| Ronozyme Hi-Phos (M/L) | DSM/Novozymes | 6-phytase | *Citrobacter braakii* |
| Ronozyme NP | DSM/Novozymes | 6-phytase | *Aspergillus oryzae* |
| Ronozyme P | DSM/Novozymes | 6-phytase | *Aspergillus oryzae* |
| Rovabio PHY | Adisseo | 3-phytase | *Penicillium funiculosum* |

**Cellulases**

| **Commercial product®** | **Company** | **Enzyme type** | **Source** |
|---|---|---|---|
| Multifect® BGL | Danisco Animal Nutrition | | |
| Natugrain® TS | BASF | | |
| Econase Barley | ABVista | | |
| Dyadic® Cellulase PLUS | Dyadic | acid cellulase | |
| Dyadic® Beta Glucanase BP CONC | Dyadic | beta-1, 3-1,4-glucanase/cellulase | *Trichoderma longibrachiatum* |
| Dyadic® Cellulase CP CONC | Dyadic | acid cellulase | |
| HOSTAZYM C® | Huvepharma | | |
| Hostazym® suis | Huvepharma | | |

**Other**

| **Commercial product®** | **Company** | **Enzyme type** | **Source** |
|---|---|---|---|
| CIBENZA DE200 | Novus | Mannanase | |
| RONOZYME® A | DSM | amylase and beta-glucanase | |
| ROXAZYME® G2 | DSM | beta-glucanases, cellulases and xylanases | |
| RONOZYME® VP | DSM | hemicellulases and pectinases | |
| CIBENZA® CSM | Novus | xylanase, α-galactosidase and β-glucanase | |

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By way of example only a feedstuff for chickens, e.g. broiler chickens may be comprises of one or more of the ingredients listed in the table below, for example in the % ages given in the table below:

| Ingredients | Starter (%) | Finisher (%) |
|---|---|---|
| Maize | 46.2 | 46.7 |
| Wheat Middlings | 6.7 | 10.0 |
| Maize DDGS | 7.0 | 7.0 |
| Soyabean Meal 48%CP | 32.8 | 26.2 |
| An/Veg Fat blend | 3.0 | 5.8 |
| L-Lysine HCl | 0.3 | 0.3 |
| DL-methionine | 0.3 | 0.3 |
| L-threonine | 0.1 | 0.1 |
| Salt | 0.3 | 0.4 |
| Limestone | 1.1 | 1.1 |
| Dicalcium Phosphate | 1.2 | 1.2 |
| Poultry Vitamins and Micro-minerals | 0.3 | 0.3 |

By way of example only the diet specification for chickens, such as broiler chickens, may be as set out in the Table below:

| Diet specification | | |
|---|---|---|
| Crude Protein (%) | 23.00 | 20.40 |
| Metabolizable Energy Poultry (kcal/kg) | 2950 | 3100 |
| Calcium (%) | 0.85 | 0.85 |
| Available Phosphorus (%) | 0.38 | 0.38 |
| Sodium (%) | 0.18 | 0.19 |
| Dig. Lysine (%) | 1.21 | 1.07 |
| Dig. Methionine (%) | 0.62 | 0.57 |
| Dig. Methionine + Cysteine (%) | 0.86 | 0.78 |
| Dig. Threonine (%) | 0.76 | 0.68 |

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By way of example only a feedstuff laying hens may be comprises of one or more of the ingredients listed in the table below, for example in the %ages given in the table below:

| Ingredient | Laying phase (%) |
|---|---|
| Maize | 10.0 |
| Wheat | 53.6 |
| Maize DDGS | 5.0 |
| Soybean Meal 48%CP | 14.9 |
| Wheat Middlings | 3.0 |
| Soybean Oil | 1.8 |
| L-Lysine HCl | 0.2 |
| DL-methionine | 0.2 |
| L-threonine | 0.1 |
| Salt | 0.3 |
| Dicalcium Phosphate | 1.6 |
| Limestone | 8.9 |
| Poultry Vitamins and Micro-minerals | 0.6 |

By way of example only the diet specification for laying hens may be as set out in the Table below:

| **Diet specification** | |
|---|---|
| Crude Protein (%) | 16.10 |
| Metabolizable Energy Poultry (kcal/kg) | 2700 |
| Lysine (%) | 0.85 |
| Methionine (%) | 0.42 |
| Methionine + Cysteine (%) | 0.71 |
| Threonine (%) | 0.60 |
| Calcium (%) | 3.85 |
| Available Phosphorus (%) | 0.42 |
| Sodium (%) | 0.16 |

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By way of example only a feedstuff for turkeys may be comprises of one or more of the ingredients listed in the table below, for example in the % ages given in the table below:

| **Ingredient** | **Phase 1 (%)** | **Phase 2 (%)** | **Phase 3 (%)** | **Phase 4 (%)** |
|---|---|---|---|---|
| Wheat | 33.6 | 42.3 | 52.4 | 61.6 |
| Maize DDGS | 7.0 | 7.0 | 7.0 | 7.0 |
| Soyabean Meal 48%CP | 44.6 | 36.6 | 27.2 | 19.2 |
| Rapeseed Meal | 4.0 | 4.0 | 4.0 | 4.0 |
| Soyabean Oil | 4.4 | 4.2 | 3.9 | 3.6 |
| L-Lysine HCl | 0.5 | 0.5 | 0.4 | 0.4 |
| DL-methionine | 0.4 | 0.4 | 0.3 | 0.2 |
| L-threonine | 0.2 | 0.2 | 0.1 | 0.1 |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 |
| Limestone | 1.0 | 1.1 | 1.1 | 1.0 |
| Dicalcium Phosphate | 3.5 | 3.0 | 2.7 | 2.0 |
| Poultry Vitamins and Micro-minerals | 0.4 | 0.4 | 0.4 | 0.4 |

By way of example only the diet specification for turkeys may be as set out in the Table below:

| **Diet specification** | | | | |
|---|---|---|---|---|
| Crude Protein (%) | 29.35 | 26.37 | 22.93 | 20.00 |
| Metabolizable Energy Poultry (kcal/kg) | 2.850 | 2.900 | 2.950 | 3.001 |
| Calcium (%) | 1.43 | 1.33 | 1.22 | 1.02 |
| Available Phosphorus (%) | 0.80 | 0.71 | 0.65 | 0.53 |
| Sodium (%) | 0.16 | 0.17 | 0.17 | 0.17 |
| Dig. Lysine (%) | 1.77 | 1.53 | 1.27 | 1.04 |
| Dig. Methionine (%) | 0.79 | 0.71 | 0.62 | 0.48 |
| Dig. Methionine + Cysteine (%) | 1.12 | 1.02 | 0.90 | 0.74 |
| Dig. Threonine (%) | 1.03 | 0.89 | 0.73 | 0.59 |

By way of example only a feedstuff for piglets may be comprises of one or more of the ingredients listed in the table below, for example in the % ages given in the table below:

| Ingredient | Phase 1 (%) | Phase 2 (%) |
|---|---|---|
| Maize | 20.0 | 7.0 |
| Wheat | 25.9 | 46.6 |
| Rye | 4.0 | 10.0 |
| Wheat middlings | 4.0 | 4.0 |
| Maize DDGS | 6.0 | 8.0 |
| Soyabean Meal 48% CP | 25.7 | 19.9 |
| Dried Whey | 10.0 | 0.0 |
| Soyabean Oil | 1.0 | 0.7 |
| L-Lysine HCl | 0.4 | 0.5 |
| DL-methionine | 0.2 | 0.2 |
| L-threonine | 0.1 | 0.2 |
| L-tryptophan | 0.03 | 0.04 |
| Limestone | 0.6 | 0.7 |
| Dicalcium Phosphate | 1.6 | 1.6 |
| Swine Vitamins and Micro-minerals | 0.2 | 0.2 |
| Salt | 0.2 | 0.4 |

By way of example only the diet specification for piglets may be as set out in the Table below:

| Diet specification | | |
|---|---|---|
| Crude Protein (%) | 21.50 | 20.00 |
| Swine Digestible Energy (kcal/kg) | 3380 | 3320 |
| Swine Net Energy (kcal/kg) | 2270 | 2230 |
| Calcium (%) | 0.80 | 0.75 |
| Digestible Phosphorus (%) | 0.40 | 0.35 |
| Sodium (%) | 0.20 | 0.20 |
| Dig. Lysine (%) | 1.23 | 1.14 |
| Dig. Methionine (%) | 0.49 | 0.44 |
| Dig. Methionine + Cysteine (%) | 0.74 | 0.68 |
| Dig. Threonine (%) | 0.80 | 0.74 |

By way of example only a feedstuff for grower/finisher pigs may be comprises of one or more of the ingredients listed in the table below, for example in the % ages given in the table below:

| Ingredient | Grower/ Finisher (%) |
|---|---|
| Maize | 27.5 |
| Soyabean Meal 48% CP | 15.4 |
| Maize DDGS | 20.0 |
| Wheat bran | 11.1 |
| Rice bran | 12.0 |
| Canola seed meal | 10.0 |
| Limestone | 1.6 |
| Dicalcium phosphate | 0.01 |
| Salt | 0.4 |
| Swine Vitamins and Micro-minerals | 0.3 |
| Lysine-HCl | 0.2 |
| Vegetable oil | 0.5 |

By way of example only the diet specification for grower/finisher pigs may be as set out in the Table below:

| Diet specification | |
|---|---|
| Crude Protein (%) | 22.60 |
| Swine Metabolizable Energy (kcal/kg) | 3030 |
| Calcium (%) | 0.75 |
| Available Phosphorus (%) | 0.29 |
| Digestible Lysine (%) | 1.01 |
| Dig. Methionine + Cysteine (%) | 0.73 |
| Digestible Threonine (%) | 0.66 |

Feed Dosing

The feed additive composition according to the present invention may be designed for one-time dosing or may be designed for feeding on a daily basis.

The optimum amount of the composition (and each component therein) to be used in the combination of the present invention will depend on the product to be treated and/or the method of contacting the product with the composition and/or the intended use for the same.

The amount of enzymes used in the compositions should be a sufficient amount to be effective and to remain sufficiently effective in improving the performance of the animal fed feed products containing said composition. This length of time for effectiveness should extend up to at least the time of utilisation of the product (e.g. feed additive composition or feed containing same).

Combination with Other Components in Feed Applications

The enzyme(s) described herein may be used in combination with other components.

The components may be prebiotics. Prebiotics are typically non-digestible carbohydrate (oligo- or polysaccharides) or a sugar alcohol which is not degraded or absorbed in the upper digestive tract. Known prebiotics used in commercial products and useful in accordance with the present invention include inulin (fructo-oligosaccharide, or FOS) and transgalacto-oligosaccharides (GOS or TOS). Suitable prebiotics include palatinoseoligosaccharide, soybean oligosaccharide, alginate, xanthan, pectin, locust bean gum (LBG), inulin, guar gum, galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), non-degradable starch, lactosaccharose, lactulose, lactitol, maltitol, maltodextrin, polydextrose (i.e. Litesse®), lactitol, lactosucrose, soybean oligosaccharides, palatinose, isomalto-oligosaccharides, gluco-oligosaccharides and xylo-oligosaccharides.

The prebiotic may be administered simultaneously with (e.g. in admixture together with or delivered simultaneously by the same or different routes) or sequentially to (e.g. by the same or different routes) the feed additive composition (or constituents thereof) according to the present invention.

Other components of the combinations of the present invention include polydextrose, such as Litesse®, and/or a maltodextrin and/or lactitol. These other components may be optionally added to the feed additive composition to assist the drying process.

Further examples of other suitable components include one or more of: thickeners, gelling agents, emulsifiers, binders, crystal modifiers, sweeteners (including artificial sweeteners), rheology modifiers, stabilisers, anti-oxidants, dyes, enzymes, carriers, vehicles, excipients, diluents, lubricating agents, flavouring agents, colouring matter, suspending agents, disintegrants, granulation binders etc. These other components may be natural. These other components may be prepared by use of chemical and/or enzymatic techniques.

In one preferred embodiment the enzymes for use in the present invention may be used in combination with one or more lipids.

For example, the enzymes for use in the present invention may be used in combination with one or more lipid micelles. The lipid micelle may be a simple lipid micelle or a complex lipid micelle.

The lipid micelle may be an aggregate of orientated molecules of amphipathic substances, such as a lipid and/or an oil.

The present teachings further provide a method of increasing weight gain in a subject, e.g. poultry or swine, comprising feeding said subject a feedstuff comprising a feed additive composition according to the present invention.

The invention can be further understood by reference to the following Examples, which are provided by way of illustration and not meant to be limiting.

## EXAMPLES:

### Example 1

**Particle size distribution and smoothness index of sodium sulfate cores.**

Seven samples of anhydrous crystalline sodium sulfate core particles were obtained from two suppliers: Minera de Santa Marta (Villarubio, Spain) and Saltex (Forth Worth, Texas),

Particle size distributions of the samples were obtained by laser light scattering using a Malvern Mastersizer 2000 laser diffraction particle size analyzer. Envelope specific surface areas for these particle size distributions were calculated using an algorithm as described in the Methods section, using the true density of anhydrous sodium sulfate (2.664 g/cm$^3$). BET specific surface area was determined using a Micromeritics model ASAP 2420 Accelerated Porosimetry and Surface Area System, as described in the Methods section. The smoothness index for each sample is calculated as the ratio of these two specific surface areas. Representative images for two of the lots of cores are shown in Figure 1.

Table 1

| Core Lot | $D_{10}$ (um) | $D_{50}$ (um) | $D_{90}$ (um) | Envelope Specific Surface Area ($m^2/g$) | BET Specific Surface Area $m^2/g$ | Smoothness Index (BET/Env Area Ratio) | Particle Size Dispersity Index (PDSI) $D_{90}/D_{10}$ |
|---|---|---|---|---|---|---|---|
| MSM #1 | 257 | 353 | 480 | 0.00659 | 0.0145 | 2.2 | 1.87 |
| MSM #2 | 225 | 309 | 424 | 0.00751 | 0.0153 | 2.0 | 1.88 |
| MSM #3 | 182 | 258 | 362 | 0.00907 | 0.0096 | 1.1 | 1.99 |
| MSM #4 | 234 | 320 | 431 | 0.00726 | 0.0134 | 1.8 | 1.84 |
| MSM #5 | 164 | 240 | 345 | 0.00981 | 0.0194 | 2.0 | 2.10 |
| MSM #6 | 138 | 204 | 300 | 0.01160 | 0.0316 | 2.7 | 2.17 |
| Saltex #1 | 171 | 232 | 316 | 0.00843 | 0.0330 | 3.9 | 1.85 |

**Example 2**

**Production of a sample of detergent enzyme granules using sodium sulfate cores.**

79.8 kg of sodium sulfate cores from Minera de Santa Marta (MSM Lot #3 in Table 1 of Example 1) were charged into a 150 kg batch size pilot scale fluid bed coater. By light scattering, the cores had a mass median diameter ($D_{50}$) of 258 microns, a D10 of 182 microns and a D90 of 358 microns; by sieve screening, a $D_{50}$ of 210 microns. Based upon this, MSM Lot #3 had a PSDI of 362/182 = 1.99.

According to Table 1 of Example 1, the smoothness index of the MSM Lot #3 cores was 1.1, very close to the 1.0 smoothness index of perfectly smooth particles. Optical and scanning electron micrographs of the cores are shown in Figure 1. It can be perceived in the same figure that these cores are much smoother than a comparative sample of cores from Saltex. Table 1 shows that Saltex Lot #1 had a smoothness index of 3.9, indicating a significant deviation from a perfect smoothness index of 1.0.

The cores were fluidized at a bed temperature of 41 degrees C, and an aqueous protease enzyme solution (Purafast) at a concentration of 20% w/w solids (3450 PU/g) , containing 1% w/w partially hydrolyzed polyvinyl alcohol (final solids % w/w loading of the enzyme/PVA of 16%)and 0.5% w/w antifoam (Polyglycol EP 436E (lot 1L05091501) was sprayed onto the cores at an atomization air pressure ramping from 3.5 (initial) to 3.9 (final) bar.

After the enzyme spray was complete, a solution containing a mixture of sodium sulfate and magnesium sulfate (MgSO4 7H20, 30.5%, Na2SO4, 15%) was sprayed onto the cores to deliver a net 20% w/w – on the basis of the final granule weight -- onto the cores. The salt solution was sprayed at a bed temperature of 45 degrees C, with an atomization air pressure of 3.5 bar.

A final coating solution composed of partially hydrolyzed polyvinyl alcohol (Celvol 5-88), titanium dioxide and Lutensol non-ionic surfactant, was sprayed onto the salt-coated enzyme cores, to deliver a net 11% w/w total coating weight. The coating solution was sprayed at a bed temperature of 55 degrees C, with an initial atomization air pressure of 5.3 bar, ramping down to 4.8 bar.

After cooling down the batch of coated granules, 150 kg of product was harvested from the fluid bed coater. The final granules had a D50 of 308 um (by sieve analysis) and a bulk density of 1.18 g/cm3. The granules had a Hunter whiteness L-value of 73, and a Heubach enzyme total dust of 3.2 mg/pad

**Example 3**

Four additional batches of Purafast granules were prepared, following the recipe and process described in Example 2, altering only the amount of Purafast enzyme concentration solution coated onto the cores, the core particle size, and adjusting the core charge as necessary to accommodate the different levels of enzyme solids. Additional coatings and process parameters were not altered.

In Table 2 the granule of Example 2 is shown as Batch B2. Table 2. Composition of Granule Batches

| Example Number | Granule Batch | Enzyme layer solids (% w/w) | Core solids (%w/w) | Core Batch | Core Type | Core size D50 (um) by sieve | Core size D50 (um) by laser |
|---|---|---|---|---|---|---|---|
| 3 | A1 | 8 | 61 | | standard | 298 | 284 |
| 3 | B1 | 16 | 53 | | standard | 298 | 284 |
| 2 | B2 | 16 | 53 | MSM#3 | Small | 210 | 258 |
| 3 | C1 | 20 | 49 | | standard | 298 | 284 |
| 3 | C2 | 20 | 49 | MSM#3 | Small | 210 | 258 |

The below enzyme samples were storage tested in a Laundry Powder Detergent under different 'stressed' storage conditions. Stability was assessed via performance and biochemical analyses.

Storage testing: sample preparation

Corresponding amount of enzyme (see below) were added to 10 g of a Laundry detergent and put in plastic cups (150 ml), which were stored, open, at 70% Relative Humidity in acclimatized rooms at 37, 45 and 60°C.

A. 10 g Laundry Powder Detergent + Sample A1
B. 10 g Laundry Powder Detergent + Sample B1
C. 10 g Laundry Powder Detergent + Sample B2
D. 10 g Laundry Powder Detergent + Sample C1
E. 10 g Laundry Powder Detergent + Sample C2

Samples were analyzed fresh and after storage of 1 and 2 hours, and 3 days (at 60°C); and after 1, 3 and 8 days (at 37 and 45°C)

Performance assessment.

Wash Testing was carried out in a so-called Laundr-O-meter at 30 C, 25 FH and 15 minutes.

The 10 g stored sample was dissolved in 2.5 liters of 25°FH water (see below) in a stirred beaker for 2 minutes. 300 ml of this solution is put into a LOM beaker and below stains were added to give a Liquor to cloth ratio L/C ~ 90 (without steel balls).

To make 25 l of 25°FH = 250 ppm $Ca^{+2}/Mg^{+2}$=2/1, dilute 416.75 ml of a 15.000 ppm stock solution with 24583.25 ml de-ionized water (to make 25 liter) Stock solution 15.000ppm: 14.70 gram CaCl2.2H2O + 10.165 gram MgCl2.6H2O in 1 liter DI water.

Stains per beaker (initial reflectance [L*a*b, D65 illuminant] of the swatches previously measured with the reflectometer):

| Stain | Batch | Size (cm) | Weigh (g) | Per beaker | |
|---|---|---|---|---|---|
| E117 Blood/Milk/Ink on poly cotton | 12-18 | 6 1/2 x 6 1/2 | 0.7 | 2 | |
| E116 Blood/Milk/Ink on cotton | 20-15 | 6 1/2 x 6 1/2 | 0.7 | 2 | |
| CS08 Grass on cotton | 83 | 6 1/2 x 6 1/2 | 0.9 | 2 | |
| Total | | | 4.6 | 6 | |

After washing stains were rinsed for 3 minutes at running tap water (17 FH, Ca/Mg=4/1), spin dried and air dried at ambient conditions overnight (covered with dark clothes).

Triple measurement of the final reflectance [L*a*b, D65 illuminant] of the swatches were done by using the reflectometer. % Delta Soil Removal(% dE) is calculated according to:

$$\% \text{ dE} = \frac{\text{Soil Removal}}{\text{Initial Soil}} = \frac{\sqrt{(L_{after} - L_{before})^2 + (a_{after} - a_{before})^2 + (b_{after} - b_{before})^2}}{\sqrt{(L_{ref} - L_{before})^2 + (a_{ref} - a_{before})^2 + (b_{ref} - b_{before})^2}} * 100\%$$

Where $L_{ref} = 96.0$
$a_{ref} = 0.55$
$b_{ref} = 1.95$

Biochemical assessment

This method was used to determine proteolytic activity on fermentation, recovery, and final product samples (liquids and granules) of Properase. The assay is colorimetric and monitors the rate of degradation of N-succinyl-ala-ala-pro-phe-p-nitroanalide substrate. The release of the substrate's p-nitroanalide was measured at 405nm on a Konelab analyzer. The assay is calibrated against an assigned standard.

Samples preparation:

- 100 g of Tris Buffer (0.1M TRIS, 0.01M $CaCL_2$, 0.005% Triton X-100, pH 8.6) were added to the 10g of detergent samples and stirred for 30 min. After that 2ml of the previous dissolution was taken in an eppendorf tube and centrifuge for 10 min at 14000 rpm.
- A second dissolution (10X) was made using the HAMILTON diluter machine and Tris Buffer.
- Vortexed the samples after diluting, and poured them in the corresponding cuvettes for the Konelab. Included also the controls (in these case PU/g). Placed the cuvettes on sample rack(s). Insert rack(s) on instrument.

To run the Konelab assay:

- Prepared working substrate solution [N-succinyl-ala-ala-pro-phe-p-nitroanalide (AAPF) (Sigma # S7388)] and poured into reagent vessel and place onto reagent wheel (substrate = 20ml vessel, code = AAPF).

Reagent Preparation

1. Stock substrate solution (100mg/ml): Dissolve 500mg AAPF in 5ml DMSO. Stored at room temperature protected from light. Discard is solution turns yellow. Stable for 1 month.
2. Intermediate substrate solution (20mg/ml): Add 800ul stock substrate solution to 3.2ml DMSO and mix. Stored at room temperature protected from light. Discard if solution turns yellow. Stable for1 month.
3. Working substrate solution (1mg/ml):Add 250ul intermediate substrate solution to 4.75ml assay buffer and mix. Prepared as needed. Typically stable for 1 hour if stored on ice. Discarded if solution turns yellow.

- Labeled the sample cups in the software and define the test to be run in the Konelab (six replicates). Run and check the calibration. To calculate the activity of the granular samples (This equation assumes that 1g = 1ml as the specific gravity of the dissolution mixture):

PU/g granule = [(PU/ml)*(working dilution)]*[weight of dissolution buffer + granules(ml)/weight of granules(g)]

- Calculated the mean value for the replicates of each sample. Calculated the %CV (coefficient of variance).

In this Example, the "Mini" granules are those that contain small smooth cores according to the pres teachings.

Data are shown in Figure 2.

**Example 4**

**Production of three representative batches of phytase**

45 kg of sodium sulfate cores from Minera de Santa Marta (MSM Lot 41418.B) were charged into 150kg batch size pilot scale fluid bed coater. By light scattering, the cores had a mass median diameter 199 microns.

The cores were fluidized and an aqueous phytase enzyme solution (batch 488115613) at a concentration of 17,5% w/w solids, containing 2% w/w partially hydrolyzed polyvinyl alcohol (Celvol 5-88) and 0,5% w/w sodium phytate solution (Dr. Straetmans; Dermofeel PA-3; Lot NA2062) was sprayed onto the cores.

After the enzyme spray, a solution containing sodium sulfate (MSM, lot 13130405) was sprayed onto the cores to deliver a net 50% w/w – on the basis of the final granule weight.

A final coating solution composed of partially hydrolyzed polyvinyl alcohol (Celvol 5-88) and talc (Microtalc FC CG-AW, lot 13/00328), was sprayed onto the salt-coated enzyme cores to deliver a net 9% w/w total coating weight.

After cooling down each batch of coated granules, 136kg, 140kg and 134kg of product was harvested from the fluid bed coater. By light scattering, the final granules had a D50 of 362 μm on the average and bulk density of 1,27g/cm3.

Figure 4 shows the pelleting results of 3 representative granule batches 3115, 3116 and 3117. Pelleting trials were carried out at the Danish Teknological Institute.

**Example 5**

**Production of three representative batches of phytase standard TPT granules in pilot scale from the same concentrate batch**

65 kg of sodium sulfate cores from Minera de Santa Marta (MSM Na- G. III-EE, Lot 41235,12) were charged into 150kg batch size pilot scale fluid bed coater. By light scattering, the cores typically have a mass median diameter 290 microns.

Table 3: Part of Product Specification of MSM Na-G. III EE.

| GRANULOMETRY | | | |
|---|---|---|---|
| Above to 1000 µm | 0% | | IT19B-PA05 |
| Above to 355 µm | 20% maximum | | |
| Between 250 and 355 µm | 60% minimum | | |
| Below to 212 µm | 10% maximum | | |
| Mean size | 365 ± 40 microns | | IT20-PA05 |
| D.F.R | 150 ± 40 ml/s | | |

The cores were fluidized and an aqueous (batch 488115613) phytase enzyme solution at a concentration of 17,5% w/w solids, containing 2% w/w partially hydrolyzed polyvinyl alcohol (Celvol 5-88) and 0,5% w/w sodium phytate solution (Dr. Straetmans; Dermofeel PA-3; Lot NA2062) was sprayed onto the cores.

After the enzyme spray, a solution containing sodium sulfate (MSM, lot 13130405) was sprayed onto the cores to deliver a net 40% w/w – on the basis of the final granule weight.

A final coating solution composed of partially hydrolyzed polyvinyl alcohol (Celvol 5-88) and talc (Microtalc FC CG-AW, lot 13/00328), was sprayed onto the salt-coated enzyme cores to deliver a net 9% w/w total coating weight.

After cooling down each batch of coated granules, 151kg, 151kg and 150kg of product was harvested from the fluid bed coater. By light scattering the final granules had a D50 of 388 µm on the average and bulk density of 1,39g/cm3.

The pelleting of the three representative phytase standard TPT batches are shown in Figure 5.

## CLAIMS

1. A population of granules, wherein the granules comprise;

a small smooth core particle, and

an active agent,

wherein the small smooth core particles contained in said population have:

a smoothness index of less than 3,

a mass median diameter of less than 300 microns;

a particle size dispersity index of less than 2.5.

2. The population of granules in claim 1 wherein the active agent is an enzyme.

3. The population of granules of any of the preceding claims wherein the active agent is in an active agent coating surrounding the smooth core particle.

4. The population of any of the preceding claims further comprising at least one additional coating.

5. The population of any of the preceding claims further comprising at least one additional coating, wherein each of the at least one additional coating comprises at least 5% w/w of the final granule.

6. The population of any of the preceding claims wherein the small smooth core particle comprises a salt crystal.

7. The population of any of the preceding claims wherein the small smooth core particle comprises a sodium sulfate crystal.

8. The population of any of the preceding claims wherein the active agent comprises an enzyme, wherein the enzyme is at least one of phytase, protease, or amylase.

9. The population of any of the preceding claims wherein the small smooth core particles comprise;

a smoothness index of less than 2.8, less than 2.5, or less than 2,

a mass median diameter of less than 250 microns, less than 225 microns, less than 200 microns, less than 175 microns, less than 150 microns, less than 125 microns, or less than 100 microns; and,

a particle size dispersity index of less than 2.5, less than 2, or less than 1.5.

10. The population of any of the claims further comprising at least one additional coating, wherein each of the at least one additional coating comprises at least 6%, 7%, 8%, 9%, or 10% w/w of the final granule.

11. A population of granules comprising;

small smooth core particles, wherein the small smooth core particles comprise

a smoothness index of less than 2.5 but no less than 1.25,

a mass median diameter of less than 250 microns but no less than 100 microns,

a particle size dispersity index of less than 2.5 but no less than 1.5;

an active agent coating comprising an enzyme, wherein the enzyme is at least one of a phytase, a protease, or an amylase; and,

at least one additional coating, wherein each of the at least one additional coatings comprises at least 7% but no more than 10% w/w of the final granule.

12. The population of granules according to any of the preceding claims further comprising;

a first moisture barrier coating comprising a moisture barrier material; and,

a moisture hydrating coating comprising a moisture hydrating material surrounding the first moisture barrier coating.

13. The population of granules according to any of the preceding claims wherein the moisture barrier material is selected from barrier polymers, proteins, lipids, fats and oils, fatty acids, and gums.

14. The population of granules according to any of the preceding claims wherein the moisture hydrating material is selected from starch, inorganic salts, and sugar.

15. An animal feed pellet comprising the population of granules of any of claims 1-14.

16. An animal feed unpelleted mixture comprising the population of granules of any of claims 1-14.

17. A laundry detergent composition comprising the population of granules of any of claims 1-14.

18. A dish detergent composition comprising the population of granules of any of claims 1-14.

19. A textile treatment composition comprising the population of granules of any of claims 1-14.

20. A baking composition comprising the population of granules of any of claims 1-14.

21. A method of making a population of granules comprising;

coating a small smooth core particle with an active agent coating comprising an enzyme, wherein the small smooth core particle comprises,

a smoothness index of less than 2.5 but no less than 1.25,

a mass median diameter of less than 300 microns but no less than 150 microns, and,

a particle size dispersity index of less than 3.0 but no less than 1.5.

22. A process for producing an animal feed composition, comprising:

preparing the population of granules of any of claims 1-14;

mixing the granules together with a feed stuff to obtain an unpelleted mixture; and,

pelleting the unpelleted mixture at a temperature of 70°C-95°C.

23. A method of preparing a feed additive population of granules, comprising preparing the population of granules of any of claims 1-14

admixing the granules with a feed acceptable carrier, diluent or excipient,

and (optional) packaging.

24. The use of a population of granules according to any of claims 1-14 in a steam-treating or pelleting process.

25. A method of improving storage stability comprising making the granule of any of claims 1-14, and, comparing storage stability to a representative sample of a granule population lacking a small smooth core particle.

26. A method of reducing dust comprising making the granule of any of claims 1-14, and, comparing dust to a representative sample of a granule population lacking a small smooth core particle.

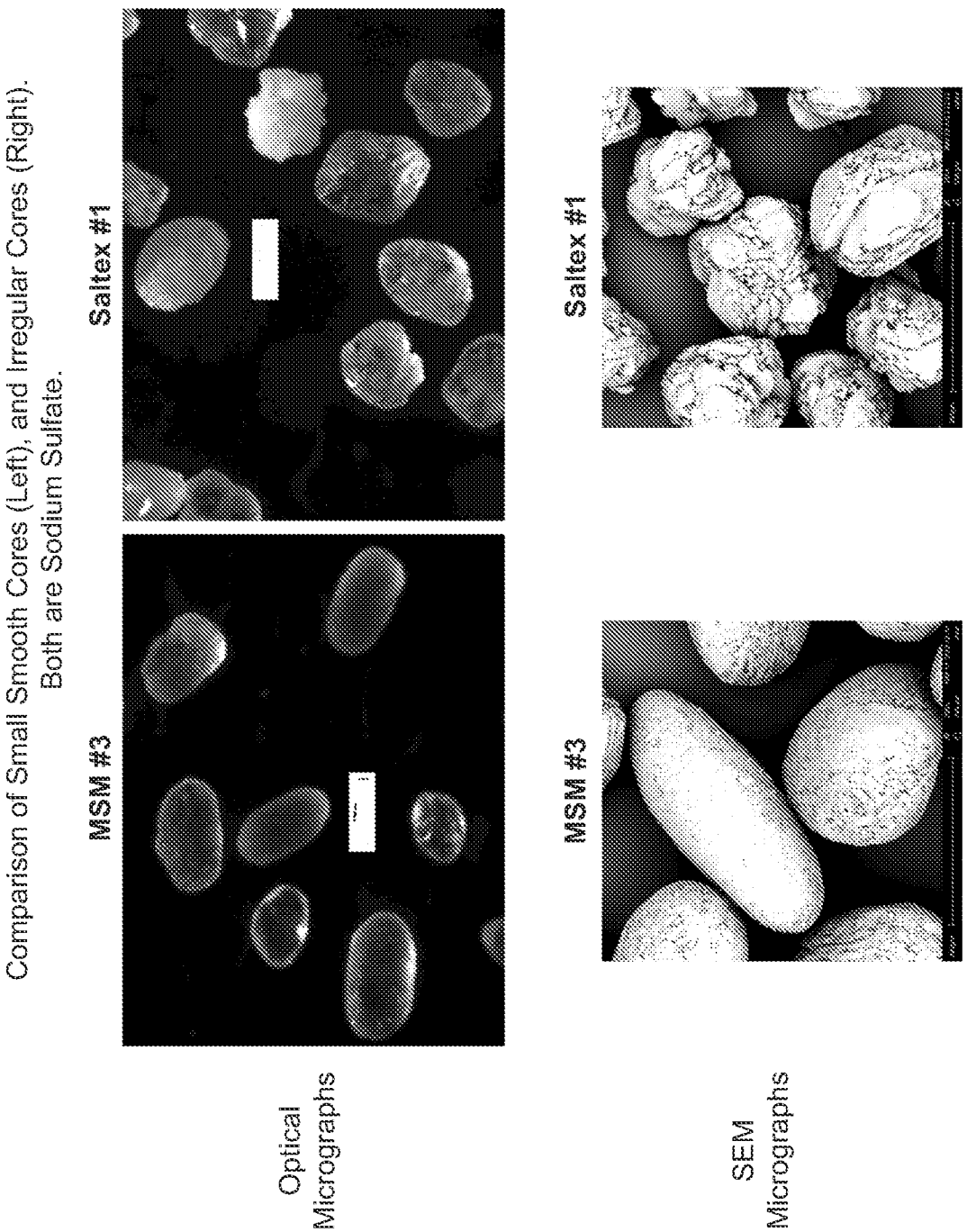
Comparison of Small Smooth Cores (Left), and Irregular Cores (Right).
Both are Sodium Sulfate.
MSM #3
Saltex #1
Optical Micrographs
MSM #3
Saltex #1
SEM Micrographs

FIG. 1

## Results I: Storage Conditions (60°C, 70%RH, op)

### PROTEASE PERFORMANCE ON BLOOD/MILK/INK (E116) %SR

| | | | | |
|---|---|---|---|---|
| A1 | 42 | 45 | 46 | 41 |
| B1 | 43 | 45 | 45 | 41 |
| B2 | 44 | 46 | 45 | 42 |
| C1 | 42 | 43 | 45 | 40 |
| C2 | 42 | 42 | 45 | 41 |

### BIOCHEMICAL ANALYSIS - PROTEOLYTIC ACTIVITY ON AAPF (PU/ml $*10^{-2}$)

| | | | | |
|---|---|---|---|---|
| A1 | 1.9 | 1.9 | 1.9 | 1.8 |
| B1 | 1.9 | 2.6 | 2.3 | 2.1 |
| B2 | 2.4 | 2.4 | 2.0 | 1.9 |
| C1 | 1.9 | 2.0 | 2.0 | 1.8 |
| C2 | 1.7 | 1.7 | 1.7 | 1.9 |

### % RESIDUAL PROTEASE PERFORMANCE ON BLOOD/MILK/INK (E116)

| | | | | |
|---|---|---|---|---|
| A1 | 100 | 107 | 108 | 97 |
| B1 | 100 | 105 | 105 | 97 |
| B2 | 100 | 106 | 104 | 96 |
| C1 | 100 | 101 | 105 | 95 |
| C2 | 100 | 100 | 106 | 99 |

### % RESIDUAL PROTEOLYTIC ACTIVITY ON AAPF - BIOCHEMICAL ANALYSIS

| | | | | |
|---|---|---|---|---|
| A1 | 100 | 103 | 98 | 94 |
| B1 | 100 | 131 | 116 | 110 |
| B2 | 100 | 97 | 82 | 77 |
| C1 | 100 | 102 | 106 | 95 |
| C2 | 100 | 101 | 99 | 110 |

FIG. 2

## Results II: Storage Conditions (45°C, 70%RH, op)

### PROTEASE PERFORMANCE ON BLOOD/MILK/INK (E116) %SR

| | | | |
|---|---|---|---|
| A1 | 42 | 43 | 45 |
| B1 | 43 | 41 | 45 |
| B2 | 44 | 43 | 45 |
| C1 | 42 | 43 | 39 |
| C2 | 42 | 42 | 42 |

### BIOCHEMICAL ANALYSIS - PROTEOLYTIC ACTIVITY ON AAPF (PU/ml $*10^{-2}$)

| | | | |
|---|---|---|---|
| A1 | 1.9 | 1.9 | 1.8 |
| B1 | 1.9 | 2.1 | 1.9 |
| B2 | 2.4 | 1.8 | 1.7 |
| C1 | 1.9 | 1.9 | 2.1 |
| C2 | 1.7 | 1.7 | 1.9 |

### % RESIDUAL PROTEASE PERFORMANCE ON BLOOD/MILK/INK (E116)

| | | | |
|---|---|---|---|
| A1 | 100 | 100 | 107 |
| B1 | 100 | 97 | 105 |
| B2 | 100 | 98 | 103 |
| C1 | 100 | 102 | 92 |
| C2 | 100 | 100 | 99 |

### % RESIDUAL PROTEOLYTIC ACTIVITY ON AAPF - BIOCHEMICAL ANALYSIS

| | | | |
|---|---|---|---|
| A1 | 100 | 100 | 97 |
| B1 | 100 | 109 | 100 |
| B2 | 100 | 75 | 68 |
| C1 | 100 | 99 | 111 |
| C2 | 100 | 102 | 112 |

FIG. 3

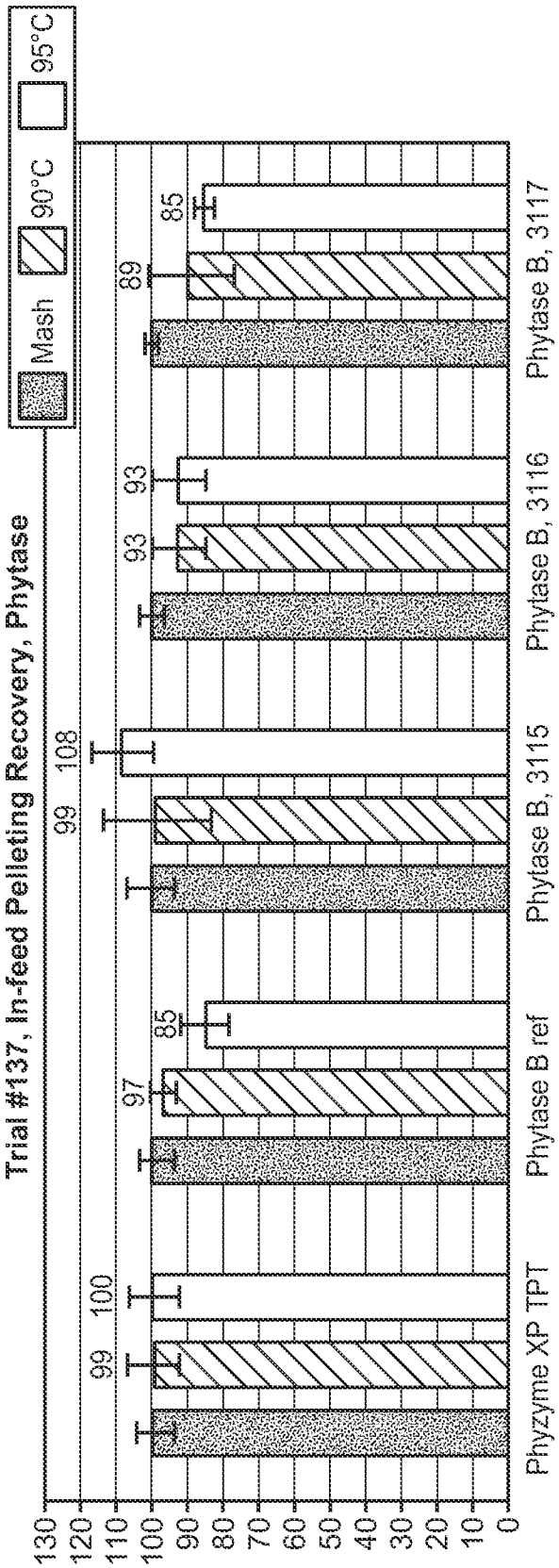
Trial #137, In-feed Pelleting Recovery, Phytase
Mash
90°C
95°C
Relative Residual Activity, %
130
120
110
100
90
80
70
60
50
40
30
20
10
0
99
100
99
108
85
97
85
93
93
89
85
Phyzyme XP TPT
Phytase B ref
Phytase B, 3115
Phytase B, 3116
Phytase B, 3117

FIG. 4

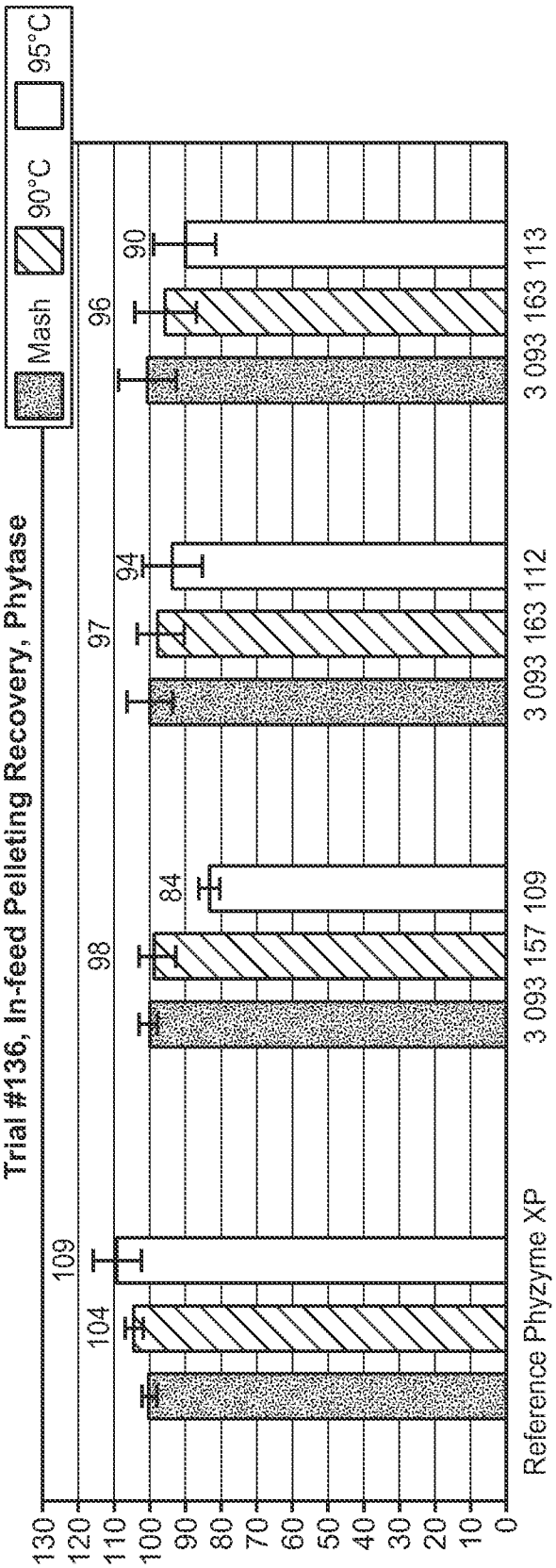
Trial #136, In-feed Pelleting Recovery, Phytase
Mash
90°C
95°C
Relative Residual Activity, %
130
120
110
100
90
80
70
60
50
40
30
20
10
0
109
104
98
84
97
96
94
90
Reference Phyzyme XP
3 093 157 109
3 093 163 112
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FIG. 5

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/043084

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23K1/00 A61K9/50 C12N9/98 C11D3/386 C11D17/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23K A61K C12N C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 9 October 2014 | 22/10/2014 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Culmann, J |

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Information on patent family members

International application No
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