

## Enzyme Preparations

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### DESCRIPTION

Enzyme Preparations used in food processing are derived from animal, plant, or microbial sources (see *Classification*, below). They may consist of whole cells, parts of cells, or cell-free extracts of the source used, and they may contain one active component or, more commonly, a mixture of several, as well as food-grade diluents, preservatives, antioxidants, and other substances consistent with good manufacturing practices. The individual preparations usually are named according to the substance to which they are applied, such as *Protease* or *Amylase*. Traditional names such as *Malt*, *Pepsin*, and *Rennet* also are used, however. The color of the preparations—which may be liquid, semiliquid, or dry—may vary from virtually colorless to dark brown. The active components consist of the biologically active proteins, which are sometimes conjugated with metals, carbohydrates, and/or lipids. Known molecular weights of the active components range from approximately 12,000 to several hundred thousand. The activity of enzyme preparations is measured according to the reaction catalyzed by individual enzymes (see below) and is usually expressed in activity units per unit weight of the preparation. In commercial practice (but not for *Food Chemicals Codex* purposes), the activity of the product is sometimes also given as the quantity of the preparation to be added to a given quantity of food to achieve the desired effect. Additional information relating to the nomenclature and the sources from which the active components are derived is provided under *Enzyme Assays*, Appendix V.

**Function:** Enzyme (see discussion under *Classification*, below)

**Packaging and Storage:** Store in well-closed containers in a cool, dry place.

### IDENTIFICATION

#### Classification

##### • ANIMAL-DERIVED PREPARATIONS

**Catalase, Bovine Liver:** Produced as partially purified liquid or powdered extracts from bovine liver. Major active principle: *catalase*. Typical application: used in the manufacture of certain cheeses.

**Chymotrypsin:** Obtained from purified extracts of bovine or porcine pancreatic tissue. Produced as white to tan, amorphous powders soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *chymotrypsin*. Typical application: used in the hydrolysis of protein.

**Lipase, Animal:** Obtained from the edible forestomach tissue of calves, kids, or lambs; and from animal pancreatic tissue. Produced as purified edible tissue preparations or as aqueous extracts dispersible in water, but insoluble in alcohol. Major active principle: *lipase*. Typical applications: used in the manufacture of cheese and in the modification of lipids.

**Lysozyme:** Obtained from extracts of purified chicken egg whites. Generally prepared and used in the hydrochloride form as a white powder. Major active principle: *lysozyme*. Typical application: used as an antimicrobial in food processing.

**Pancreatin:** Obtained from porcine or bovine (ox) pancreatic tissue. Produced as a white to tan, water-soluble powder. Major active principles: (1)  $\alpha$ -amylase; (2) protease; and (3) lipase. Typical applications: used in the preparation of precooked cereals, infant foods, and protein hydrolysates.

**Pepsin:** Obtained from the glandular layer of hog stomach. Produced as a white to light tan, water-soluble powder; amber paste; or clear, amber to brown, aqueous liquids. Major active principle: *pepsin*. Typical applications: used in the preparation of fishmeal and other protein hydrolysates and in the clotting of milk in the manufacture of cheese (in combination with rennet).

**Phospholipase A<sub>2</sub>:** Obtained from porcine pancreatic tissue. Produced as a white to tan powder or pale to dark yellow liquid. Major active principle: *phospholipase A<sub>2</sub>*. Typical application: used in the hydrolysis of lecithins.

**Rennet, Bovine:** Aqueous extracts made from the fourth stomach of bovines. Produced as a clear, amber to dark brown liquid or a white to tan powder. Major active principle: *protease* (pepsin). Typical application: used in the manufacture of cheese. Similar preparations may be made from the fourth stomach of sheep or goats.

**Rennet, Calf:** Aqueous extracts made from the fourth stomach of calves. Produced as a clear, amber to dark brown liquid or a white to tan powder. Major active principle: *protease* (chymosin). Typical application: used in the manufacture of cheese. Similar preparations may be made from the fourth stomach of lambs or kids.

**Trypsin:** Obtained from purified extracts of porcine or bovine pancreas. Produced as white to tan, amorphous powders soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *trypsin*. Typical applications: used in baking, in the tenderizing of meat, and in the production of protein hydrolysates.

##### • PLANT-DERIVED PREPARATIONS

**Amylase:** Obtained from extraction of ungerminated barley. Produced as a clear, amber to dark brown liquid or a white to tan powder. Major active principle:  $\beta$ -amylase. Typical applications: used in the production of alcoholic beverages and sugar syrups.

**Bromelain:** The purified proteolytic substance derived from the pineapples *Ananas comosus* and *Ananas bracteatus* L. (Fam. Bromeliaceae). Produced as a white to light tan, amorphous powder soluble in water (the solution is usually colorless to light yellow and somewhat opalescent), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *bromelain*. Typical applications: used in the chillproofing of beer, in the tenderizing of meat, in the preparation of precooked cereals, in the production of protein hydrolysates, and in baking.

**Ficin:** The purified proteolytic substance derived from the latex of *Ficus* sp. (Fam. Moraceae), which includes a variety of tropical fig trees. Produced as a white to off-white powder completely soluble in water. (Liquid fig latex concentrates are light to dark brown.) Major active principle: *ficin*. Typical applications: used in the chillproofing of beer, in the tenderizing of meat, and in the conditioning of dough in baking.

**Malt:** The product of the controlled germination of barley. Produced as a clear amber to dark brown liquid preparation or as a white to tan powder. Major active principles: (1)  $\alpha$ -amylase and (2)  $\beta$ -amylase. Typical applications: used in baking, in the manufacture of alcoholic beverages and of syrups.

**Papain:** The purified proteolytic substance derived from the fruit of the papaya *Carica papaya* L. (Fam. Caricaceae). Produced as a white to light tan, amorphous powder or a liquid soluble in water (the solution is usually colorless or light yellow and somewhat opalescent), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *papain* and (2) *chymopapain*. Typical applications: used in the chillproofing of beer, in the tenderizing of meat, in the preparation of precooked cereals, and in the production of protein hydrolysates.

#### • MICROBIAL-DERIVED PREPARATIONS

**$\alpha$ -Acetolactatedecarboxylase:** (*Bacillus subtilis* containing a *Bacillus brevis* gene) Produced as a brown liquid by controlled fermentation using the modified *Bacillus subtilis*. Soluble in water (the solution is usually a light yellow to brown). Major active principle: *decarboxylase*. Typical application: used in the preparation of beer.

**Aminopeptidase, Leucine:** (*Aspergillus niger* var., *Aspergillus oryzae* var., and other microbial species) Produced as a light tan to brown powder or as a brown liquid by controlled fermentation using *Aspergillus niger* var., *Aspergillus oryzae* var., or other microbial species. The powder is soluble in water (the solution is usually light yellow to brown). Major active principles: (1) *aminopeptidase*, (2) *protease*, and (3) *carboxypeptidase* activities in varying amounts. Typical applications: used in the preparation of protein hydrolysates and in the development of flavors in processed foods.

**Carbohydrase:** (*Aspergillus niger* var., including *Aspergillus aculeatus*) Produced as an off-white to tan powder or a tan to dark brown liquid by controlled fermentation using *Aspergillus niger* var. (including *Aspergillus aculeatus*). Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1)  $\alpha$ -amylase, (2) *pectinase* (a mixture of enzymes, including *pectin depolymerase*, *pectin methyl esterase*, *pectin lyase*, and *pectate lyase*), (3) *cellulase*, (4) *glucoamylase* (amyloglucosidase), (5) *amylase*, (6) *hemicellulase* (a mixture of enzymes, including *poly(galacturonate) hydrolase*, *arabinosidase*, *mannosidase*, *mannanase*, and *xylanase*), (7) *lactase*, (8)  $\beta$ -glucanase, (9)  $\beta$ -D-glucosidase, (10) *pentosanase*, and (11)  $\alpha$ -galactosidase. Typical

applications: used in the preparation of starch syrups and dextrose, alcohol, beer, ale, fruit juices, chocolate syrups, bakery products, liquid coffee, wine, dairy products, cereals, and spice and flavor extracts.

**Carbohydrase:** (*Aspergillus oryzae* var.) Produced as an off-white to tan, amorphous powder or a liquid by controlled fermentation using *Aspergillus oryzae* var. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1)  $\alpha$ -amylase, (2) *glucoamylase* (amyloglucosidase), and (3) *lactase*. Typical applications: used in the preparation of starch syrups, alcohol, beer, ale, bakery products, and dairy products.

**Carbohydrase:** (*Bacillus acidopullulyticus*) Produced as an off-white to brown, amorphous powder or a liquid by controlled fermentation using *Bacillus acidopullulyticus*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *pullulanase*. Typical applications: used in the hydrolysis of amylopectins and other branched polysaccharides.

**Carbohydrase:** (*Bacillus stearothermophilus*) Produced as an off-white to tan powder or a light yellow to dark brown liquid by controlled fermentation using *Bacillus stearothermophilus*. Soluble in water, but practically insoluble in alcohol, in ether, and in chloroform. Major active principle:  $\alpha$ -amylase. Typical applications: used in the preparation of starch syrups, alcohol, beer, dextrose, and bakery products.

**Carbohydrase:** (*Candida pseudotropicalis*) Produced as an off-white to tan, amorphous powder or a liquid by controlled fermentation using *Candida pseudotropicalis*. Soluble in water (the solution is usually light yellow to dark brown) but insoluble in alcohol, in chloroform, and in ether. Major active principle: *lactase*. Typical applications: used in the manufacture of candy and ice cream and in the modification of dairy products.

**Carbohydrase:** (*Kluyveromyces marxianus* var. *lactis*) Produced as an off-white to tan, amorphous powder or a liquid by controlled fermentation using *Kluyveromyces marxianus* var. *lactis*. Soluble in water (the solution is usually light yellow to dark brown), but insoluble in alcohol, in chloroform, and in ether. Major active principle: *lactase*. Typical applications: used in the manufacture of candy and ice cream and in the modification of dairy products.

**Carbohydrase:** (*Mortierella vinaceae* var. *raffinoseutilizer*) Produced as an off-white to tan powder or as pellets by controlled fermentation using *Mortierella vinaceae* var. *raffinoseutilizer*. Soluble in water (pellets may be insoluble in water), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle:  $\alpha$ -galactosidase. Typical application: used in the production of sugar from sugar beets.

**Carbohydrase:** (*Rhizopus niveus*) Produced as an off-white to brown, amorphous powder or a liquid by controlled fermentation using *Rhizopus niveus*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1)  $\alpha$ -

*amylase* and (2) *glucoamylase*. Typical application: used in the hydrolysis of starch.

**Carbohydrase:** (*Rhizopus oryzae* var.) Produced as a powder or a liquid by controlled fermentation using *Rhizopus oryzae* var. Soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1)  $\alpha$ -*amylase*, (2) *pectinase*, and (3) *glucoamylase* (amyloglucosidase). Typical applications: used in the preparation of starch syrups and fruit juices, vegetable purees, and juices and in the manufacture of cheese.

**Carbohydrase:** (*Saccharomyces* species) Produced as a white to tan, amorphous powder by controlled fermentation using a number of species of *Saccharomyces* traditionally used in the manufacture of food. Soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *invertase* and (2) *lactase*. Typical applications: used in the manufacture of candy and ice cream and in the modification of dairy products.

**Carbohydrase:** [(*Trichoderma longibrachiatum* var.) (formerly *reesei*)] Produced as an off-white to tan, amorphous powder or as a liquid by controlled fermentation using *Trichoderma longibrachiatum* var. Soluble in water (the solution is usually tan to brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *cellulase*, (2)  $\beta$ -*glucanase*, (3)  $\beta$ -D-*glucosidase*, (4) *hemicellulase*, and (5) *pentosanase*. Typical applications: used in the preparation of fruit juices, wine, vegetable oils, beer, and baked goods.

**Carbohydrase:** (*Bacillus subtilis* containing a *Bacillus megaterium*  $\alpha$ -*amylase* gene) Produced as an off-white to brown, amorphous powder or liquid by controlled fermentation using the modified *Bacillus subtilis*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle:  $\alpha$ -*amylase*. Typical applications: used in the preparation of starch syrups, alcohol, beer, and dextrose.

**Carbohydrase:** (*Bacillus subtilis* containing a *Bacillus stearothermophilus*  $\alpha$ -*amylase* gene) Produced as an off-white to brown, amorphous powder or a liquid by controlled fermentation using the modified *Bacillus subtilis*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: maltogenic *amylase*. Typical applications: used in the preparation of starch syrups, dextrose, alcohol, beer, and baked goods.

**Carbohydrase and Protease, Mixed:** (*Bacillus licheniformis* var.) Produced as an off-white to brown, amorphous powder or as a liquid by controlled fermentation using *Bacillus licheniformis* var. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1)  $\alpha$ -*amylase* and (2) *protease*. Typical applications: used in the preparation of starch syrups, alcohol, beer, dextrose, fishmeal, and protein hydrolysates.

**Carbohydrase and Protease, Mixed:** (*Bacillus subtilis* var. including *Bacillus amyloliquefaciens*) Produced as an off-white to tan, amorphous powder or as a liquid by controlled fermentation using *Bacillus subtilis* var.

Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1)  $\alpha$ -*amylase*, (2)  $\beta$ -*glucanase*, (3) *protease*, and (4) *pentosanase*. Typical applications: used in the preparation of starch syrups, alcohol, beer, dextrose, bakery products, and fishmeal, in the tenderizing of meat, and in the preparation of protein hydrolysates.

**Catalase:** (*Aspergillus niger* var.) Produced as an off-white to tan, amorphous powder or as a liquid by controlled fermentation using *Aspergillus niger* var. Soluble in water (the solution is usually tan to brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *catalase*. Typical applications: used in the manufacture of cheese, egg products, and soft drinks.

**Catalase:** (*Micrococcus lysodeikticus*) Produced by controlled fermentation using *Micrococcus lysodeikticus*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *catalase*. Typical application: used in the manufacture of cheese, egg products, and soft drinks.

**Chymosin:** (*Aspergillus niger* var. *awamori*, *Escherichia coli* K-12, and *Kluyveromyces marxianus*, each microorganism containing a calf *prochymosin* gene) Produced as a white to tan, amorphous powder or as a light yellow to brown liquid by controlled fermentation using the above-named genetically modified microorganisms. The powder is soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *chymosin*. Typical application: used in the manufacture of cheese and in the preparation of milk-based desserts.

**Glucose Isomerase:** (*Actinoplanes missouriensis*, *Bacillus coagulans*, *Streptomyces olivaceus*, *Streptomyces olivochromogenes*, *Microbacterium arborescens*, *Streptomyces rubiginosus* var., or *Streptomyces murinus*) Produced as an off-white to tan, brown, or pink amorphous powder, granules, or liquid by controlled fermentation using any of the above-named organisms. The products may be soluble in water, but practically insoluble in alcohol, in chloroform, and in ether; or if immobilized, may be insoluble in water and partially soluble in alcohol, in chloroform, and in ether. Major active principle: *glucose* (or *xylose*) *isomerase*. Typical applications: used in the manufacture of high-fructose corn syrup and other fructose starch syrups.

**Glucose Oxidase:** (*Aspergillus niger* var.) Produced as a yellow to brown solution or as a yellow to tan or off-white powder by controlled fermentation using *Aspergillus niger* var. Soluble in water (the solution is usually light yellow to brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *glucose oxidase* and (2) *catalase*. Typical applications: used in the removal of sugar from liquid eggs and in the deoxygenation of citrus beverages.



**Lipase:** (*Aspergillus niger* var.) Produced as an off-white to tan, amorphous powder by controlled fermentation using *Aspergillus niger* var. Soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *lipase*. Typical application: used in the hydrolysis of lipids (e.g., fish oil concentrates and cereal-derived lipids).

**Lipase:** (*Aspergillus oryzae* var.) Produced as an off-white to tan, amorphous powder or a liquid by controlled fermentation using *Aspergillus oryzae* var. Soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *lipase*. Typical applications: used in the hydrolysis of lipids (e.g., fish oil concentrates) and in the manufacture of cheese and cheese flavors.

**Lipase:** (*Candida rugosa*; formerly *Candida cylindracea*) Produced as an off-white to tan powder by controlled fermentation using *Candida rugosa*. Soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *lipase*. Typical applications: used in the hydrolysis of lipids, in the manufacture of dairy products and confectionery goods, and in the development of flavor in processed foods.

**Lipase:** [*Rhizomucor (Mucor) miehei*] Produced as an off-white to tan powder or as a liquid by controlled fermentation using *Rhizomucor miehei*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *lipase*. Typical applications: used in the hydrolysis of lipids, in the manufacture of cheese, and in the removal of haze in fruit juices.

**Phytase:** (*Aspergillus niger* var.) Produced as an off-white to brown powder or as a tan to dark brown liquid by controlled fermentation using *Aspergillus niger* var. Soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) 3-*phytase* and (2) *acid phosphatase*. Typical applications: used in the production of soy protein isolate and in the removal of phytic acid from plant materials.

**Protease:** (*Aspergillus niger* var.) Produced by controlled fermentation using *Aspergillus niger* var. The purified enzyme occurs as an off-white to tan, amorphous powder. Soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: used in the production of protein hydrolysates.

**Protease:** (*Aspergillus oryzae* var.) Produced by controlled fermentation using *Aspergillus oryzae* var. The purified enzyme occurs as an off-white to tan, amorphous powder. Soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical applications: used in the chillproofing of beer, in the production of bakery products, in the tenderizing of meat, in the production

of protein hydrolysates, and in the development of flavor in processed foods.

**Rennet, Microbial:** (nonpathogenic strain of *Bacillus cereus*) Produced as a white to tan, amorphous powder or a light yellow to dark brown liquid by controlled fermentation using *Bacillus cereus*. Soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: used in the manufacture of cheese.

**Rennet, Microbial:** (*Endothia parasitica*) Produced as an off-white to tan, amorphous powder or as a liquid by controlled fermentation using nonpathogenic strains of *Endothia parasitica*. The powder is soluble in water (the solution is usually tan to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: used in the manufacture of cheese.

**Rennet, Microbial:** [*Rhizomucor (Mucor)* sp.] Produced as a white to tan, amorphous powder by controlled fermentation using *Rhizomucor miehei*, or *pusillus* var. Lindt. The powder is soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: used in the manufacture of cheese.

**Transglutaminase:** (*Streptovorticillium mobaraense* var.) Produced as an off-white to weak yellow-brown, amorphous powder by controlled fermentation using *Streptovorticillium mobaraense* var. Soluble in water but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *transglutaminase*. Typical applications: used in the processing of meat, poultry, and seafood; production of yogurt, certain cheeses, and frozen desserts; and manufacture of pasta products and noodles, baked goods, meat analogs, ready-to-eat cereals, and other grain-based foods.

#### • REACTIONS CATALYZED

[NOTE—The reactions catalyzed by any given active component are essentially the same, regardless of the source from which that component is derived.]

**$\alpha$ -Acetolactatedecarboxylase:** Decarboxylation of  $\alpha$ -cetolactate to acetoin

**Amino peptidase, Leucine:** Hydrolysis of *N*-terminal amino acid, which is preferably leucine, but may be other amino acids, from proteins and oligopeptides, yielding free amino acids and oligopeptides of lower molecular weight

**$\alpha$ -Amylase:** Endohydrolysis of  $\alpha$ -1,4-glucan bonds in polysaccharides (starch, glycogen, etc.), yielding dextrans and oligo- and monosaccharides

**$\beta$ -Amylase:** Hydrolysis of  $\alpha$ -1,4-glucan bonds in polysaccharides (starch, glycogen, etc.), yielding maltose and betalimit dextrans

**Bromelain:** Hydrolysis of polypeptides, amides, and esters (especially at bonds involving basic amino acids, leucine, or glycine), yielding peptides of lower molecular weight

**Catalase:**  $2\text{H}_2\text{O}_2 \leftrightarrow \text{O}_2 + 2\text{H}_2\text{O}$

**Cellulase:** Hydrolysis of  $\beta$ -1,4-glucan bonds in such polysaccharides as cellulose, yielding  $\beta$ -dextrans

**Chymosin (calf and fermentation derived):** Cleaves a single bond in kappa casein

**Ficin:** Hydrolysis of polypeptides, amides, and esters (especially at bonds involving basic amino acids, leucine, or glycine), yielding peptides of lower molecular weight

**$\alpha$ -Galactosidase:** Hydrolysis of terminal nonreducing  $\alpha$ -D-galactose residues in  $\alpha$ -D-galactosides

**$\beta$ -Glucanase:** Hydrolysis of  $\beta$ -1,3- and  $\beta$ -1,4-linkages in  $\beta$ -D-glucans, yielding oligosaccharides and glucose

**Glucoamylase (amyloglucosidase):** Hydrolysis of terminal  $\alpha$ -1,4- and  $\alpha$ -1,6-glucan bonds in polysaccharides (starch; glycogen, etc.), yielding glucose (dextrose)

**Glucose Isomerase (xylose isomerase):** Isomerization of glucose to fructose, and xylose to xylulose

**Glucose Oxidase:**  $\beta$ -D-glucose + O<sub>2</sub>  $\leftrightarrow$  D-glucono- $\delta$ -lactone + H<sub>2</sub>O<sub>2</sub>

**$\beta$ -D-Glucosidase:** Hydrolysis of terminal, nonreducing  $\beta$ -D-glucose residues with the release of  $\beta$ -D-glucose

**Hemicellulase:** Hydrolysis of  $\beta$ -1,4-glucans,  $\alpha$ -L-arabinosides,  $\beta$ -D-mannosides, 1,3- $\beta$ -D-xylans, and other polysaccharides, yielding polysaccharides of lower molecular weight

**Invertase ( $\beta$ -fructofuranosidase):** Hydrolysis of sucrose to a mixture of glucose and fructose (invert sugar)

**Lactase ( $\beta$ -galactosidase):** Hydrolysis of lactose to a mixture of glucose and galactose

**Lysozyme:** Hydrolysis of cell-wall polysaccharides of various bacterial species leading to the breakdown of the cell wall most often in Gram-positive bacteria

**Maltogenic Amylase:** Hydrolysis of  $\alpha$ -1,4-glucan bonds

**Lipase:** Hydrolysis of triglycerides of simple fatty acids, yielding mono- and diglycerides, glycerol, and free fatty acids

#### **Pancreatin**

**$\alpha$ -Amylase:** Hydrolysis of  $\alpha$ -1,4-glucan bonds

**Protease:** Hydrolysis of proteins and polypeptides

**Lipase:** Hydrolysis of triglycerides of simple fatty acids

#### **Pectinase**

**Pectate lyase:** Hydrolysis of pectate to oligosaccharides

**Pectin depolymerase:** Hydrolysis of 1,4 galacturonide bonds

**Pectin lyase:** Hydrolysis of oligosaccharides formed by pectate lyase

**Pectinesterase:** Demethylation of pectin

**Pepsin:** Hydrolysis of polypeptides, including those with bonds adjacent to aromatic or dicarboxylic L-amino acid residues, yielding peptides of lower molecular weight

**Phospholipase A<sub>2</sub>:** Hydrolysis of lecithins and phosphatidylcholine, producing fatty acid anions

#### **Phytase**

**3-Phytase:**  $\text{myo-Inositol hexakisphosphate} + \text{H}_2\text{O} \leftrightarrow 1,2,4,5,6\text{-pentakisphosphate} + \text{ortho-phosphate}$

**Acid Phosphatase:** Orthophosphate monoester + H<sub>2</sub>O  $\leftrightarrow$  an alcohol + orthophosphate

**Protease (generic):** Hydrolysis of polypeptides, yielding peptides of lower molecular weight

**Pullulanase:** Hydrolysis of 1,6- $\alpha$ -D-glycosidic bonds on amylopectin and glycogen and in  $\alpha$ - and  $\beta$ -limit dextrins, yielding linear polysaccharides

**Rennet (bovine and calf):** Hydrolysis of polypeptides; specificity may be similar to pepsin

**Transglutaminase:** Binding of proteins

**Trypsin:** Hydrolysis of polypeptides, amides, and esters at bonds involving the carboxyl groups of L-arginine and L-lysine, yielding peptides of lower molecular weight

## **ASSAY**

### **• PROCEDURE**

**Analysis:** The following procedures, which are included under *Enzyme Assays*, Appendix V, are provided for application as necessary in determining compliance with the declared representations for enzyme activity<sup>1</sup>:  $\alpha$ -Acetolactatedecarboxylase Activity, Acid Phosphatase Activity,  $\alpha$ -Amylase Activity (Nonbacterial); Bacterial  $\alpha$ -Amylase Activity (BAU); Catalase Activity; Cellulase Activity; Chymotrypsin Activity; Diastase Activity (Diastatic Power);  $\alpha$ -Galactosidase Activity,  $\beta$ -Glucanase Activity; Glucoamylase Activity (Amyloglucosidase Activity); Glucose Isomerase Activity; Glucose Oxidase Activity;  $\beta$ -D-Glucosidase Activity; Hemicellulase Activity; Invertase Activity; Lactase (Neutral) ( $\beta$ -Galactosidase) Activity; Lactase (Acid) ( $\beta$ -Galactosidase) Activity; Lipase Activity; Lipase/Esterase (Forestomach) Activity; Maltogenic Amylase Activity; Milk-Clotting Activity; Pancreatin Activity; Pepsin Activity; Phospholipase Activity; Phytase Activity; Plant Proteolytic Activity; Proteolytic Activity, Bacterial (PC); Proteolytic Activity, Fungal (HUT); Proteolytic Activity, Fungal (SAP); Pullulanase Activity; and Trypsin Activity.

**Acceptance criteria:** NLT 85.0% and NMT 115.0% of the declared units of enzyme activity

## **IMPURITIES**

### **• LEAD, Lead Limit Test, Appendix IIIB**

**Control:** 5  $\mu$ g Pb (5 mL of *Diluted Standard Lead Solution*)

**Acceptance criteria:** NMT 5 mg/kg

## **SPECIFIC TESTS**

### **• MICROBIAL LIMITS**

[NOTE—Current methods for the following tests may be found in the Food and Drug Administration's Bacteriological Analytical Manual online at [www.cfsan.fda.gov/](http://www.cfsan.fda.gov/).]

**Acceptance criteria**

**Coliforms:** NMT 30 CFU/g

**salmonella:** Negative in 25 g

## **OTHER REQUIREMENTS**

Enzyme preparations are produced in accordance with good manufacturing practices. Regardless of the source of derivation, they should cause no increase in the total

<sup>1</sup>Because of the varied conditions under which pectinases are employed, and because laboratory hydrolysis of a purified pectin substrate does not correlate with results observed with the natural substrates under use conditions, pectinase suppliers and users should develop their own assay procedures that would relate to the specific application under consideration.

microbial count in the treated food over the level accepted for the respective food.

Animal tissues used to produce enzymes must comply with the applicable U.S. meat inspection requirements and must be handled in accordance with good hygienic practices.

Plant material used to produce enzymes or culture media used to grow microorganisms consist of components that leave no residues harmful to health in the finished food under normal conditions of use.

Preparations derived from microbial sources shall be obtained using a pure culture fermentation of a non-pathogenic and non-toxigenic strain and are produced by methods and under culture conditions that ensure a controlled fermentation, thus preventing the introduction of microorganisms that could be the source of toxic materials and other undesirable substances.

The carriers, diluents, and processing aids used to produce the enzyme preparations shall be substances that are acceptable for general use in foods, including water and substances that are insoluble in foods but removed from the foods after processing.

Although limits have not been established for mycotoxins, appropriate measures should be taken to ensure that the products do not contain such contaminants.

## Enzyme-Modified Fats

First Published: Prior to FCC 6

### DESCRIPTION

Enzyme-Modified Fats occur as light to medium tan liquids, pastes, or powders with a strong fatty acid odor and flavor. They are produced by enzyme lipolysis of fats obtained from milk, refined beef fat, or steam-rendered chicken fat, using suitable food-grade enzymes. Enzyme-modified milkfat may be prepared from milk, concentrated milk, dry whole milk, cream, concentrated cream(s), dry cream, butter, butter oil, dried butter, or anhydrous milkfat. For enzyme-modified milkfat, optional dairy ingredients such as skim milk, concentrated skim milk, nonfat dry milk, buttermilk, concentrated buttermilk, dried buttermilk, liquid whey, concentrated whey, and dried whey may be used to adjust the concentration of the flavors. Fat emulsions are reacted with suitable food-grade enzymes under controlled conditions to increase the flavor components. Thermoprocessing is then used to destroy the enzyme activity and provide acceptable microbiological quality. Suitable preservatives, emulsifiers, buffers, stabilizers, and antioxidants as well as sodium chloride may be added. The resulting product is concentrated or dried.

**Function:** Flavoring agent

**Packaging and Storage:** Store in tight containers in a cool place.

### IDENTIFICATION

#### • PROCEDURE

**Acceptance criteria:** A sample has a very strong fatty acid odor.

### IMPURITIES

#### Inorganic Impurities

- **LEAD,** *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method II, Appendix IIIB*  
**Acceptance criteria:** NMT 1 mg/kg

### SPECIFIC TESTS

- **ACID VALUE,** *Method II, Appendix VII*  
**Sample:** 5 g  
**Acceptance criteria:** NLT 98.0% and NMT 102.0% of the labeled value
- **LOSS ON DRYING,** *Appendix IIC: 105° for 48 h*  
**Acceptance criteria:** NMT 4.0% for the dry product
- **MICROBIAL LIMITS**  
[NOTE—Current methods for the following tests may be found by accessing the Food and Drug Administration's Bacteriological Analytical Manual (BAM) online at [www.cfsan.fda.gov](http://www.cfsan.fda.gov).]  
**Acceptance criteria**  
**Aerobic plate count:** NMT 10,000 CFU/g  
**Coliforms:** NMT 10 CFU/g  
**Salmonella:** Negative in 25 g  
**Staphylococcal enterotoxins:** Negative in 1 g  
**Staphylococcus aureus:** NMT 100 CFU/g  
**Yeasts and molds:** NMT 10 CFU/g

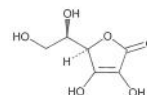
### OTHER REQUIREMENTS

- **LABELING:** Indicate the *Acid Value*.

## Erythorbic Acid

First Published: Prior to FCC 6

D-Araboascorbic Acid



$C_6H_8O_6$

Formula wt 176.13

INS: 315

CAS: 89-65-6

### DESCRIPTION

Erythorbic Acid occurs as white or slightly yellow crystals or powder. It gradually darkens when exposed to light. In the dry state, it is reasonably stable in air, but in solution, it rapidly deteriorates in the presence of air. It melts between 164° and 171° with decomposition. One g is soluble in about 2.5 mL of water and in about 20 mL of alcohol. It is slightly soluble in glycerin.

**Function:** Preservative; antioxidant

**Packaging and Storage:** Store in tight, light-resistant containers.

### IDENTIFICATION

#### • A. PROCEDURE

**Sample solution:** 20 mg/mL