United States Department of Agriculture
Agricultural Marketing Service | National Organic Program
Document Cover Sheet
https://www.ams.usda.gov/rules-regulations/organic/petitioned-substances

Document Type:

☐ **National List Petition or Petition Update**

A petition is a request to amend the USDA National Organic Program's National List of Allowed and Prohibited Substances (National List).

Any person may submit a petition to have a substance evaluated by the National Organic Standards Board (7 CFR 205.607(a)).

Guidelines for submitting a petition are available in the NOP Handbook as NOP 3011, National List Petition Guidelines.

Petitions are posted for the public on the NOP website for Petitioned Substances.

☒ **Technical Report**

A technical report is developed in response to a petition to amend the National List. Reports are also developed to assist in the review of substances that are already on the National List.

Technical reports are completed by third-party contractors and are available to the public on the NOP website for Petitioned Substances.

Contractor names and dates completed are available in the report.

Enzymes, Microorganisms, and Yeast

Handling/Processing

Summary of Petitioned Use

This limited scope technical report provides updated information to the National Organic Standards Board (NOSB) in support of the sunset reviews of the following materials listed at 7 CFR 205.605(a):

- (11) Enzymes— must be derived from edible, nontoxic plants, nonpathogenic fungi, or nonpathogenic bacteria.
- (19) Microorganisms—any food grade bacteria, fungi, and other microorganism.
- (30) Yeast— When used as food or a fermentation agent in products labeled as "organic," yeast must be organic if its end use is for human consumption; nonorganic yeast may be used when organic yeast is not commercially available. Growth on petrochemical substrate and sulfite waste liquor is prohibited. For smoked yeast, nonsynthetic smoke flavoring process must be documented.

Enzymes and yeast were both included on the National List of Allowed and Prohibited Substances (hereafter referred to as the "National List") with the first publication of the National Organic Program (NOP) Final Rule (65 FR 80548). Bacterial-derived (NOSB, 1995b), fungal-derived (NOSB, 1996b), plant-derived (NOSB, 1996c), and animal-derived (NOSB, 2000) enzymes were covered in separate technical reports. Microorganisms were added to the National List, effective September 12, 2006 (71 FR 53299).

The annotation for yeast was later reformatted to condense separate lines into a single entry, but otherwise the change did not affect the meaning or language (68 FR 61987). The listings for enzymes and yeasts were reformatted without any changes to the annotations (72 FR 58469). Finally, the National List entry for yeast was updated to include the current annotation which includes a clause requiring organic yeast, unless commercially unavailable (77 FR 33290).

This technical report focuses on the fermentation processes used to create these substances, with specific attention given to the use of excluded methods in their development and manufacture. However, it is not practical to evaluate the fermentation processes and the potential use of allowed and excluded methods for every enzyme, microorganism, and yeast product on the market within one technical report. Instead, we provide an overview of the fermentation processes and possible ways both allowed and excluded methods are used to produce these materials, with examples and considerations. An example list of manufacturers and brand names for enzymes, microorganisms, and yeasts is included in *Table 4*, within the *Appendix* at the end of this report. Furthermore, a list of enzymes, their uses, CAS RNs, and EC identification numbers are included in *Table 5*.

The request for this technical report included a list of excluded methods, based on the current NOSB recommendations, which are based on the definitions at 7 CFR 205.2. Current NOSB recommendations also refer to some technologies that were not considered prior to the publication of the NOP Final Rule (NOSB, 2022). The TR also includes examples of microorganisms produced with conjugation, which is mentioned in § 205.2 as a non-excluded method and is therefore not considered to be excluded from organic production and handling.

This TR provides examples of some of the better-known uses and methods of production for enzymes, microorganisms, and yeasts, and offers explanations as to why they are allowed, excluded, otherwise prohibited, or require NOSB consideration for classification. A list of food use microorganisms, and whether excluded methods are used in their production are listed in *Table 6* within the *Appendix*.

This report gives a broad overview of fermentation process design and certain common elements involved in fermentation technology. The examples provided illustrate specific fermentation processes and are not intended to cover all possible processes used to make ingredients intended for use in organic foods. It is beyond the scope of this TR to provide a comprehensive list of all products, every method by which they were produced, an exhaustive list of their uses, or information about whether any specific product is currently used in organic processing.

Plant enzymes at § 205.605(a)(11) are not produced from fermentation. Therefore, we are not addressing the conventional production processes used to produce the plant material, even though some enzymes are obtained from plants that have been genetically modified using excluded methods. Animal enzymes are cited separately at § 205.605(a)(3). Animal enzymes may also involve the use of excluded methods and synthetic substances that are not on § 205.605(b) in their extraction, purification, and packaging. These National List entries encompass many individual substances and fermentation processes.

This report contains a glossary of technical terms (at the end of the report) used to describe fermentation, enzyme production, and the culturing of yeast and other microorganisms. The glossary also includes terms related to genetic engineering and other methods excluded by the USDA organic regulations. Terms can have subtly different meanings depending on the context of the product and the organism, process, native language, and location.

Background

Excluded Methods

The USDA organic regulations prohibit the use of substances and ingredients made by excluded methods in organic production and handling [7 CFR 205.105(e)]. The regulation defines excluded methods as follows (§ 205.2):

> A variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes and are not considered compatible with organic production. Such methods include cell fusion, microencapsulation and macroencapsulation, and recombinant DNA technology (including gene deletion, gene doubling, introducing a foreign gene, and changing the positions of genes when achieved by recombinant DNA technology). Such methods do not include the use of traditional breeding, conjugation, fermentation, hybridization, in vitro fertilization, or tissue culture.

This technical report evaluates whether microorganisms—including yeast—and microbial products such as enzymes are produced by excluded methods under the above definition. The most recent version (2016) of the NOSB's formal guidance document titled "Excluded Methods Terminology" is part of this evaluation (NOSB, 2016c).

The definition of excluded methods originally focused on the use of recombinant DNA (rDNA) technologies used to genetically modify plants grown as agricultural crops for the food and feed supply. The examples given in the definition, such as gene doubling, gene deletion, removing a gene from a donor organism and inserting it into a recipient organism, and changing the positions of genes can also be performed on bacteria and fungi.

Not all excluded methods involve rDNA techniques. One example is cell fusion, which is a technique that merges individual cells into a bi- or multi-nucleate complex. The process generally involves propylene glycol or other synthetic chemicals. Plants bred using somatic cell hybridization transfer cytoplasmic male sterility into parent lines used in breeding programs to produce F1 hybrids, such as in cabbage or broccoli (NOP, 2013). The fusion of the gametes *in vitro* produces embryos that would not occur in nature. Tissue culture can also be used to asexually reproduce somatic embryos that develop into normal plants. Cell fusion can be used to produce cultured meat from animal skeletal muscle stem cells (Shaikh et al., 2021; Thorrez & Vandenburgh, 2019).

Micro- and macro-encapsulation refers to the use of synthetic polymers in the delivery systems for various fermentation organisms. Microencapsulation involves the encapsulation of a single cell in a polymeric semipermeable membrane. Macroencapsulation fills a membrane with multiple cells in a polymeric matrix. The organisms themselves are not necessarily made by excluded methods, but such delivery systems are excluded. Many micro- and macro-encapsulated bacteria can be used in various food applications. Probiotics and lactic acid bacteria can be microencapsulated before being added to food. Advances in nanotechnology have created the possibility to nanoencapsulate enzymes. Even though nanoencapsulation is not mentioned in the definition, it is implicitly excluded as microencapsulation (Gibbs et al., 1999; Nedovic et al., 2011; S. Ray et al., 2016).

NOP Policy Memos related to excluded methods

The National Organic Program (NOP) issued Policy Memo 11-13 in April 2011 (NOP, 2011b). Titled *Clarification of Existing Regulations Regarding the Use of Genetically Modified Organisms in Organic Agriculture*, the policy memo mostly addresses inadvertent contamination of crops in production by excluded methods and does not directly address the issues of non-organic, non-agricultural ingredients produced by excluded methods. In February 2013, the NOP issued Policy Memo 13-1 *Cell Fusion Techniques Used in Seed Production* (NOP, 2013). The memo is specific to a particular method used in plant breeding and does not mention genetically modified fermentation microorganisms that are used as food additives.

NOSB recommendations related to excluded methods

The National Organic Standards Board (NOSB) has made numerous recommendations to the NOP regarding the use of genetically modified organisms and other excluded methods in organic production and handling. The NOSB recommended a definition of genetic engineering in 1995 (NOSB, 1995a). In 1996, the NOSB recommended that genetically engineered organisms and their products be prohibited for organic production and handling (USDA / AMS / NOSB, 1996). This recommendation was reaffirmed in 1998 following the first proposed NOP rule (NOSB, 1998).

Previous Technical Advisory Panel reviews and technical reports (TRs) informed the NOSB's recommendations. The NOSB evaluated enzymes for their compatibility with organic handling using the criteria in 7 U.S.C. 6518(m) and, later, 7 CFR 205.600 (NOP, 2015; NOSB, 1995b, 1996c, 1996b, 2003). The NOSB specifically considered a technical report of chymosin from microbial sources genetically altered through rDNA techniques (NOSB, 1996a). In September 1996, the NOSB recommended that chymosin from genetically modified sources not be added to the National List of allowed non-organic ingredients (USDA / AMS / NOSB, 1996). In December 1997, the USDA proposed adding chymosin from genetically modified sources to the National List. After receiving public comments opposing the addition, the USDA proposed and finalized the NOP Final Rule (65 FR 80548). These regulations did not include genetically engineered chymosin and excluded methods that involved genetic engineering.

Between 2013 and 2016, the NOSB drafted and circulated a discussion document that included a "To Be Determined (TBD)" chart of technologies (NOSB, 2016b). Following public comment, the NOSB issued the 2016 *Formal Recommendation on Excluded Methods Terminology* (NOSB, 2016c). Most of the NOSB's document addressed questions related to plant breeding or livestock production. The NOSB's document did not explicitly address techniques used in developing enzymes and microorganisms (including yeast) for organic processing and handling applications.

In 2019, the NOSB issued a Formal Recommendation on induced mutagenesis and embryo transfer (NOSB, 2019b). This recommendation also stated that "induced mutagenesis developed through exposure to UV light, chemicals, irradiation, or other stress-causing activities" should remain on the most current "To Be Determined (TBD)" chart for future discussion and review. The most current formal recommendation on excluded methods determinations was issued by the NOSB in 2022 (NOSB, 2022). Like the 2016 recommendation, the current recommendation lacks descriptions for some of the techniques used in the development of enzymes and microorganisms. NOSB and NOP history on excluded methods is compiled in the NOP's Petitioned Substances Database (NOP, 2023b).

The role of FDA in approving enzymes, microorganisms, and yeast

The FDA maintains a partial list of substances added to food, and the list includes items that are not necessarily *Generally Recognized as Safe* (GRAS) or approved by the FDA for food use (U.S. FDA, 2023d).

This partial list includes substances that are made from or contain microorganisms, including enzymes and yeast.

The Food, Drug, and Cosmetic Act (FD&C Act) requires that the FDA review and approve any substances that may be added to food prior to it being marketed, unless it is considered GRAS by qualified experts using scientific procedures [21 U.S.C. 321(s) and 348]. GRAS status criteria for food use requires that the specific strains of the microorganisms—including yeasts—added or used to produce the derivative additive be non-pathogenic and non-toxicogenic.

One wishing to have a food additive recognized as GRAS has three choices:

- Voluntarily petition the FDA under the provisions of 21 CFR 171;
- Voluntarily notify the FDA that the substance is GRAS and receiving a letter from FDA of no questions (US FDA, 2010);
- Self-affirm a substance as GRAS by a review of publicly available scientific data and the opinion of an expert panel (US FDA, 2017).

FDA GRAS notification is a voluntary program (Gaynor & Cianci, 2005). Substances, including various fermentation cultures and enzyme preparations used before the 1958 amendments to the FD&C Act, are grandfathered in by virtue of a substantial history of consumption by a significant number of consumers [21 CFR 170.30(c) and 170.3(f)].

GRAS Notifications include the following information (US FDA, 2010, 2017):

1. Identify and description of the substance;
2. Detailed description of the manufacturing process;
3. Specifications for identity and purity, including analytical methods;
4. Intended technical effects and specific food uses;
5. Intake estimates for the public based on consumption data;
   And for enzyme preparations
6. Whether the enzyme preparations are used in meat or poultry processing;
7. Any allergenic ingredients that may be contained in the enzyme preparations.

The FDA reformed the procedure for GRAS affirmations in 1997 (62 FR 18937) and again in 2016 (81 FR 54960). Petitioners can choose to notify the FDA that a given substance is GRAS and provide the scientific basis for that determination. These are known as GRAS Notices (GRNs). The FDA publishes those notifications in its publicly available inventory (US FDA, 2023b). The FDA reviews the declaration and issues a letter that notifies the declarer that either (1) the FDA does not question the basis for the notifier's GRAS conclusion, (2) the FDA concludes that the notice does not provide a sufficient basis for a GRAS conclusion, or (3) the FDA has ceased to evaluate the GRAS notice at the notifier's request (U.S. FDA, 2023a). The notifier may choose claim that data and information in parts 2-7 is exempt from public disclosure under the Freedom Of Information Act (FOIA) (US FDA, 2017).

Microorganisms can be improved through natural selection, classical improvement techniques, recombinant DNA (rDNA) techniques, protein engineering, or—more recently—gene editing techniques (Hanlon & Sewalt, 2021; Pariza & Johnson, 2001; Sewalt et al., 2016). In 1983, no enzymes had been produced by genetically modified organisms (Pariza & Johnson, 2001). One of the first enzymes to be produced from a genetically modified microorganism, and the first approved for use in food by the FDA, was fermentation-derived chymosin preparation, now included at 21 CFR 184.1685 (Olempska-Beer et al., 2006). The FDA affirmed chymosin from genetically modified microorganisms as GRAS in 1990 (55 FR 10932, March 23, 1990). Enzymes made by techniques used prior to that approval can be safely assumed to be produced by allowed methods, not excluded from organic production or handling under 7 CFR 205.105(e).

Enzymes

This section provides a description of enzymes, explains how they are named and categorized, and describes the regulatory process by which enzymes are approved for food use in the United States.

Description

Enzymes are proteins that act as biological catalysts in various biochemical reactions. They change the rate of chemical reactions at a cellular level without any chemical change themselves (Palmer, 1995). The word "enzyme" is derived from Greek, loosely translated as "in yeast" (Aehle, 2007). While all known living organisms have enzymes, their significance was first discovered from their role in the fermentation of sugars into alcohol. The first enzyme to be isolated was amylase, isolated from germinated barley (Lobedanz et al., 2016). There are numerous enzymes used in food production, with a wide range of applications (see *Table 5*, in the *Appendix*).

The substance upon which the enzyme acts is called the *substrate*. The word "substrate" carries a slightly different but related meaning for fermentation cultures. Many foods use enzymes produced by the fermentation cultures that are used to make them. These are called "endogenous" enzymes. Enzymes that are produced, isolated, purified, standardized, and prepared outside of the final food product and are added with other ingredients in making a food are referred to as "exogenous" enzymes.

Enzymes are produced by all living organisms; however, this report only focuses on enzymes produced by microorganisms (including fungi), as described in *Summary of Petitioned Use* (above). In some cases, enzymes are produced by microorganisms that are developed using excluded methods. *Table 6* in the *Appendix* (at the end of this report) can be used as a resource to help identify whether a given enzyme product may be made by excluded methods. This resource is not an official assignment of status. The final determination of the NOP rule compliance status of a given brand name enzyme product is the responsibility of the certifier of a handler using that product.

Naming and Classification

The International Union of Biochemistry and Molecular Biology (IUBMB) classifies enzymes based on the reactions that they catalyze (IUBMB, 2023). The system was developed by the international Enzyme Commission (EC) between 1961 and 1964 to resolve ambiguity and inconsistency in nomenclature (Palmer, 1995). The EC Number system now has seven recognized major groups (IUBMB, 2023; McDonald & Tipton, 2023):

1. oxidoreductases
2. transferases
3. hydrolases
4. lyases
5. isomerases
6. ligases
7. translocases

These categories are defined in the *Glossary* included in this report.

These major categories are subclassified by the catalytic functions carried out on the specific substrates they act upon (IUBMB, 2023). Subclasses are further divided into sub-subclasses based on rules specific to the kinds of reactions they perform. The third tier of classification is by the enzyme's molecular structure. The fourth tier is a serial number in the sub-subclass (IUBMB, 2023).

Many enzymes are referred to by multiple names, or may be described by the supplier with a lack of specificity (McDonald & Tipton, 2023). The standard nomenclature for specific enzymes involves a root word that identifies the substrate or molecular structure to which they bind. For example, glucase is an enzyme that binds to glucose. Most enzyme nomenclature ends in "-ase" but most proteases—those enzymes that act on protein substrates—commonly end in "-in" (Palmer, 1995).

Enzymes may be produced by methods that are allowed, excluded, or otherwise prohibited for use in organic handling and processing, depending on the source organism(s), manufacturing process, and various auxiliary additives that are included in the enzyme package. Development of synthetic foods and food ingredients has been a long-standing goal of many food scientists, resulting in long-established synthetic ingredients such as vitamins, amino acids, and artificial flavors (Pyke, 1970). Researchers have been less successful in synthesizing enzymes because of their complexity. However, researchers have synthesized artificial enzymes by non-biological means (Breslow, 2005). These researchers and food companies see the commercial potential for synthetic analogs of nonsynthetic enzymes currently derived from microorganisms, plants, and animals. Researchers also see the potential to design enzymes with a functionality previously unknown in nature. Many of these involve the use of nanotechnology and are referred to as "nanozymes." Synthetic enzymes would not meet the requirements of 7 CFR 205.605(a) or the annotation for enzymes. As such, they are outside the scope of this review.

Over 8,000 enzymes are listed in the International Union of Biochemistry and Molecular Biology (IUBMB) enzyme database, ExplorEnz (IUBMB, 2023). The number of enzymes identified and classified continues to increase every year. The database contained fewer than 5,000 enzymes in 2008 (McDonald et al., 2008), for an average of about 200 enzymes being added every year. Some enzymes are also regularly reclassified or deleted (McDonald & Tipton, 2023). Enzymes are often identified by numbers assigned by the Nomenclature Committee (NC) of the IUBMB. Older references cite the numbers assigned by the Enzyme Commission (EC) of the International Union of Biochemistry (IUB), but the current recognized authority prefers to be cited as the NC of the IUBMB (IUBMB, 2023).

GRAS Notification for Enzymes

Microbial enzymes used in food processing are typically sold as *enzyme preparations* that contain the desired enzyme activity, other metabolites of the production organism, and added materials such as preservatives and stabilizers (Pariza & Johnson, 2001). Since September 30, 1999, the FDA has affirmed the GRAS status of 118 enzymes (U.S. FDA, 2023b) Petitioners of 14 enzymes were pending a response from the FDA as of September 30, 2023 (U.S. FDA, 2023b). GRAS notifications for enzymes generally follow the FDA's guidance provided for that category of food additive (US FDA, 2010). The FDA recommends that food additive petitioners and GRAS notifiers include the following information (Sewalt et al., 2016; U.S. FDA, 2010):

- identity
- characterization of the enzyme source
- composition of the enzyme preparation
- manufacturing process
- specification for identity and purity
- intended technical effects and use
- intake estimate
- information specific to enzyme preparations used in meat, poultry, and egg products
- enzyme preparations containing any allergenic ingredients

The guidance suggests that the notifier identify the enzyme using the accepted name, systematic name, the EC number, and the Chemical Abstract Service (CAS) number if available. The FDA recommends that notifiers provide specific information on the enzyme's biological and chemical properties. The "identity" requirement must include a description of any "structural modifications introduced by chemical or genetic methods that affect the enzyme performance under the intended conditions of use" (U.S. FDA, 2010).

The FDA also recommends that the "enzyme source" information include taxonomic information about the microorganisms used in enzyme production to show that they are nonpathogenic and nontoxigenic (U.S. FDA, 2010).

An expert committee of food scientists concluded that the safety of the production organism is the primary consideration for designating an enzyme derived from microorganisms as safe (Pariza & Foster, 1983). Nonpathogenic and nontoxigenic are relative terms (Pariza & Foster, 1983; Pariza & Johnson, 2001). The importance of the source organism for food safety was established prior to the use of recombinant DNA (rDNA) technologies and was based on those organisms that were the traditional sources of enzymes used in food processing (Pariza & Johnson, 2001). The expert panels provide their opinions on the use of rDNA involving multiple species requires consideration of the safety of donor organisms that are the sources of the plasmid or genetic sequence that produces the desired effect, as well as the safety of the host organism that is fermented to produce the enzyme (Pariza & Johnson, 2001; Sewalt et al., 2016). Industry and academic experts acknowledge that genetically modified microorganisms (GMMs) merit specific consideration of safe strain lineage to prevent toxins and pathogens from being inadvertently introduced into foods (Pariza & Johnson, 2001). FDA Guidance states the policy to review GMMs as follows:

> Such microorganisms should be thoroughly characterized with respect to any introduced DNA. The source(s) of the introduced DNA including the gene(s) encoding the enzyme(s) of interest, any other genes (e.g., genes encoding selectable markers), and regulatory DNA sequences necessary for gene expression should be identified. The enzyme-encoding genes can be derived from known organisms, unidentified organisms sampled from the environment, or generated from a pool of genes from various sources via molecular evolution also known as gene shuffling. The enzyme-encoding genes can also be synthesized or modified by traditional or site-specific mutagenesis to adapt the enzyme properties to the specific food application conditions or to enhance the enzyme production.
>
> The host microorganisms can also be modified by inactivating or deleting certain endogenous genes, for example, to prevent the synthesis of potentially harmful secondary metabolites (e.g., mycotoxins) or to minimize the production of other enzymes that may interfere with the production of the enzyme of interest or its function in food processing. All approaches and steps involved in the production of enzymes from GMMs should be described (U.S. FDA, 2010)

The "composition" section of the notification must include information about the ingredients other than the enzyme, including diluents, stabilizers, and preservatives (U.S. FDA, 2010). Such ingredients are part of nearly every enzyme preparation (Pariza & Johnson, 2001). Preservatives are almost always added during the production process and are often added following isolation and before packaging to maintain a shelf-stable product (Pariza & Foster, 1983). The section also must include information on other enzymes that may be present, on residues of metabolites derived from the production organism(s), and on any residues from the isolation or purification process (U.S. FDA, 2010).

The "manufacturing process" section recommends that the manufacturer discloses how it follows Current Good Manufacturing Processes (cGMPs). Fermentation process descriptions are requested, with all steps and controls necessary to maintain the proper growth conditions, purity, and genetic stability of the culture. The guidance requests full disclosure of all materials used in fermentation—including antifoaming and flocculating agents used—as well as agents used to isolate the enzyme from either the cellular material or the fermentation broth, and any chemical or physical treatments or quality controls (U.S. FDA, 2010).

The FDA incorporates by reference the "Enzyme Preparations" monograph in the 6th or current editions of the Food Chemicals Codex as preferred purity specifications. Enzyme preparations obtained from microbial sources should not contain antibiotics, toxins, or any transformable DNA coding for protein toxins or proteins that inactivate therapeutic antibiotics (U.S. FDA, 2010).

Fermentation processes to produce enzymes vary by the production organism, enzyme, application, specifications, and other factors. The FDA's GRAS inventory contains examples of various fermentation processes. The inventory is not exhaustive, and summarizing all possible processes is beyond the scope of this technical report. However, the inventory provides several assurances. The production organisms are deemed nonpathogenic and nontoxigenic based on expert opinion within the tolerances established by FDA. All enzymes affirmed as GRAS typically follow cGMPs (Sewalt et al., 2016).

The guidance also asks the notifier to report all foods or groups of foods in which the enzyme preparation is used or is intended to be used, its technical or functional effect, and its fate. The FDA further requests the Estimated Dietary Intake (EDI) of the enzyme. Based on a Memorandum of Understanding with the

USDA's Food Safety Inspection Service (FSIS), the FDA has agreed to review enzymes for uses covered under the various statutes and regulations that govern the processing of animal products under USDA's authority without requiring a separate petition. Any potential allergens in the preparation need to be reported. The guidance is non-binding and notifiers can use an alternative approach that satisfies the requirements of applicable statutes and regulations (U.S. FDA, 2010).

Microorganisms

Fermented foods have traditional origins that involve complex cultural, biological, and chemical mechanisms (Steinkraus, 1983). The safe and effective use of microorganisms as modern food additives requires selection, isolation, growth, production, and harvesting (Doelle et al., 2012).

While production techniques and source organisms have evolved over the years, the basic techniques to produce traditional foods remain the same (Ray & Didier, 2014; Ray & Joshi, 2014; Steinkraus, 1983). Production methods are described in more detail in response to *Focus Question* #2. Foods that use microorganisms include (Bamforth & Cook, 2019; R. C. Ray & Montet, 2014; Steinkraus, 1983; Yamashita, 2021):

- Yogurt, made from milk and various dairy cultures.
- Tempeh, made from soybeans and *Rhizopus* bacteria.
- Tamari, produced from soybeans and various organisms described as "Koji."
- Vinegar, made with various fruits and *Acetobacter* spp.
- Kombucha, from brewed tea, sugar, and a symbiotic complex of bacteria and yeast, referred to as "SCOBY."
- Koji, a culture grown on various grains and legumes used to make various traditional Japanese food products such as shoyu, tamari, and miso.

The market for microorganisms used in food processing is more segmented and specialized than for yeast or enzymes. These are generally not sold as consumer products, and they are less likely to be branded.

Probiotics

Organic food processors use many different probiotic organisms in their functional foods. Some of these are produced on-site using naturally-occurring cultures and organic substrates. A complete review of such operations would require original research that is beyond the scope of this technical report. Probiotics also may be added. These include a wide range of beneficial microorganisms that help with digestive functions, immunity, and safe consumer product storage (Fenster et al., 2019).

Biotechnology companies have developed novel or artificial microorganisms not found in nature to process food following the introduction of recombinant DNA techniques. These organisms are marketed in starter cultures that are used to make various food products.

Microalgae

In addition to bacteria and single-cell fungi, the FDA also classifies some single-celled algae as microorganisms. Algae may be manufactured industrially with submerged fermentation or may be agriculturally produced in pond culture, so they can be either agricultural or non-agricultural. The NOSB received a petition to add chlorella to the National List as a non-organically produced agricultural product at 21 CFR 205.606 (Wright, 2007). The NOSB voted not to add chlorella to the National List in November 2007 (NOSB, 2007) The Organic Integrity Database listed 31 sources of certified organic spirulina and 18 sources of certified organic chlorella as of December 1, 2023 (NOP, 2023a). Algae do not explicitly appear on the National List annotation for microorganisms, which refers to "any . . . other microorganism" besides food grade bacteria and fungi [7 CFR 205.605(a)(19)]. The 2016 Technical Report on Marine Plants & Algae used as processing ingredients notes that the microalgae *Dunaliela salina* can be cultivated in ponds or grown in tanks (NOP, 2016). Microalgae produced by industrial methods in fermentation tanks may be considered as non-agricultural (non-organic) ingredients under the current listing for microorganisms at 7 CFR 205.605(a), while microalgae cultivated in open pond culture may be considered agricultural and thus subject to the requirement of being organic or on the National List at 7 CFR 205.606.

Viruses
Viruses that are specific to bacteria are known as bacteriophages, or "phages." Phages are also used as food additives, mainly as diagnostic tools and biological controls for food-borne pathogens such as *Salmonella enterica* spp (Wei et al., 2019).

Yeast

Description
Yeast can be certified as organically produced and handled under the USDA standard (NOP, 2011a). Yeast is also included at 7 CFR 205.605(a)(30):

> When used as food or a fermentation agent in products labeled as "organic," yeast must be organic if its end use is for human consumption; nonorganic yeast may be used when organic yeast is not commercially available. Growth on petrochemical substrate and sulfite waste liquor is prohibited. For smoked yeast, nonsynthetic smoke flavoring process must be documented.

Most yeast is produced for baking applications (Hutkins, 2006). Pre-historic, and pre-industrial artisan bakers domesticated and cultured natural yeasts selected from wild strains on a simple substrate of flour from a grain—usually wheat with water and salt—a practice that continued with artisanal sourdough starter that contains both yeasts and acid-forming bacteria (Kulp & Lorenz, 2003). The deliberate production of baker's yeast began in the 1800s, first with yeast cultured on grain mashes, and then with molasses (Athnasios & Quantz, 2012). Louis Pasteur is credited with developing techniques to isolate pure strains of *S. cerevisiae* in the 1870s (Boynton & Greig, 2014).

The number of microorganisms that can ferment food is also undefined and is the subject of on-going research (Bernini & Lindner, 2022). Microorganisms rapidly evolve through mutation, and researchers are still discovering new species of wild yeasts (Nguyen & Boekhout, 2017). Food scientists are exploring both naturally-occurring and genetically modified alternatives that may be commercialized in the near future (Bamforth & Cook, 2019; Binati et al., 2021). These may also include new genetically modified strains of transgenic *S. cerevisiae* (Binati et al., 2021).

Taxonomy
There are countless species and strains of yeast in many genera, families, and orders in the classes Ascomycetes and Basidomycetes (Kurtzman, 1994; Kurtzman et al., 2011). The word "yeast" is used to describe various single-celled and a few multicellular fungi that reproduce asexually by budding, and sexually by sporulation and conjugation (Athnasios & Quantz, 2012; Kurtzman et al., 2011). The taxonomy of yeasts is a complex subject that has undergone numerous changes over the past 30 years with the advent of genetic sequencing (Kurtzman, 1994; Kurtzman et al., 2011). Prior to that, yeasts were taxonomically classified by phenotypical traits such as physiological reactions and the morphology of the budding and sexual states (Kurtzman, 1994). Taxonomy and nomenclature of yeasts produced by hybridization, trans-conjugation, and other methods has further complicated the identification of various strains (Nguyen & Boekhout, 2017). New species and strains are being discovered and categorized by genetic sequencing (Bernini & Lindner, 2022). Novel strains can also be created through various genetic engineering and new genomic technologies (Hanlon & Sewalt, 2021; Johnson & Echavarri-Erasun, 2011; Nguyen & Boekhout, 2017; Żymańczyk-Duda et al., 2017). The use of marker genes appears to be the most reliable technique used to identify pure lines of natural and artificially produced strains (Nguyen & Boekhout, 2017).

Saccharomyces cerevisiae is the microorganism most frequently encountered in food and beverage processing applications (Bamforth & Cook, 2019; Hutkins, 2006). These yeasts are used in fermentation and baking, making them a subcategory of food microorganisms along with bacteria, non-yeast fungi, microalgae, and viruses. Alcohol produced from the action of yeast on fruit juice or malted grain is considered to be the first industrial fermentation product (Stanbury et al., 2013). The yeast used to make lager beer is sometimes classified as *S. pastorianus*, phenotypically and genotypically distinct from other yeasts (Bamforth & Cook, 2019). The other yeast species currently used in food and beverages include (Athnasios & Quantz, 2012; Bamforth & Cook, 2019):

- *Saccharomyces uvarum*
- *S. bayanus*
- *Candida utilis* (torula yeast)
- *Torulaspora delbreuckii* (flor yeast or)
- *Kluyvermyces fragilis*
- *Schizosaccharomyces pombe*
- *Zygosacchromyces bailii*

Genetic modification

Yeasts transformed by rDNA techniques have been commercially available and used in wine making since the 1990s (Grossmann et al., 2011). More recently, biotechnology companies have used CRISPR-Cas9 technology to perform gene editing of yeast, particularly for applications in brewing (Seibel et al., 2023). Examples are given in the Focus Question section. Commercial yeasts are produced from a relatively narrow range of domesticated strains (Gallone et al., 2016). Yeast manufacturers that seek to incorporate traits from wild strains have begun to use gene editing techniques, such as CRISPR, rather than natural selection and classical improvement methods.

Food applications of enzymes, microorganisms, and yeast

Enzymes, microorganisms, and yeasts are used in baking, juicing, and malting (Ahlawat et al., 2018, 2018; Fennema, 1996; Horsmans Poulsen et al., 2012). They are commonly used in dairy foods, alcoholic beverages, animal feeds and cured meat products. Enzymes also have indirect food and non-food applications, including use in detergents, cleaning products, deodorizers, and pharmaceuticals (Aehle, 2007; Copeland, 2000; Palmer, 1995).

Baked goods

Bread and other bakery products rely on enzymes, microorganisms, and yeast for their processing and production (Hutkins, 2006; Kulp & Lorenz, 2003; Stear, 1995; van Oort, 2010). Millers, bakers, and other grain processors use various enzymes to prepare conditioned flours, confectionaries, bread, and other baked goods.

Dough can be made to rise using baker's yeast (*Saccharomyces cerevisiae*), from the use of a sourdough starter culture, the use of various soda leavenings, or from other novel approaches (Stear, 1995). Millers grind grain into flour, which bakers make into a dough with water, salt, and a fermentation organism. The respiration of the fermentation organisms causes the dough to rise. The risen dough is proofed or punched down and allowed to rise again over a variable number of cycles, and then baked.

Most mass-produced breads produced over the past 100 years use baker's yeast developed as standardized industrial products to make a more results in a consistent product (Stear, 1995). Sourdough starter is distinguished from baker's yeast by the presence of various lactic acid-producing bacteria *Lactobacillus* spp. including *L. acidophilus*, *L. brevis, L. buchneri, L. casei, L. fermentum, L. farciminis, L. fructivorans,* and *L. plantarum* (Bamforth & Cook, 2019). Sourdough starters around the world show considerable diversity, and new species and strains are still being discovered (Huys et al., 2012). In addition to *S. cerevisiae,* yeasts present in sourdough starter may include *Candida crusei, Pichia* spp*, Kazachstania* spp., and *Torulopsis holmii* (Bamforth & Cook, 2019; Huys et al., 2012). Traditionally, sourdough breads were produced in small batches by artisan bakers, although some industrial bakers have scaled up the process to larger batches (Cappelle et al., 2012). The microbiomes of sourdough starters around the world are highly diverse, with a wide range of flavor and functionality (Landis et al., 2021).

Bakeries have used exogenous enzyme preparations made from microorganisms since the 1920s. One of the earliest uses of enzymes to condition dough was a combination of starch-degrading ("diastase" or alpha-amylase) and proteolytic enzymes prepared from a strain of *Aspergillus oryzae* (Kohman et al., 1928).While amylase occurs naturally in flour and yeast, some bakers add alpha-amylase or Taka-amylase to standardize flour, reduce dough viscosity, provide for a more homogenous crumb structure, and extend shelf life (Horsmans Poulsen et al., 2012).

Enzymes are also able to replace synthetic substances like hydrochloric acid in the production of hard-candy and soft-chocolate shelf-stable confectionaries, and to replace potassium bromate used to condition dough (ETA, 2001).

Fruit and vegetable processing

Processors who squeeze or crush fruits and vegetables find they can get higher yields and improve production speed and consistency when they use pectin lyase or pectinase. Using these enzymes breaks down pectin, which reduces viscosity, increases yield, and overall accelerates juice extraction (Aehle, 2007; Grassin & Coutel, 2010; Horsmans Poulsen et al., 2012).

Manufacturers use *A. niger* to produce pectinase enzyme preparations (Grassin & Coutel, 2010). Genetically modified microorganisms are increasingly used to produce enzymes for the fruit and vegetable industry, especially in apple processing (Grassin & Coutel, 2010). Specifically, various *Aspergillus* strains have been modified through a genetic engineering technique called "homologous recombination-mediated gene targeting" to produce pure enzymes without unwanted side activities (Grassin & Coutel, 2010). Side activities are catalytic reactions made by enzymes that act on substances other than the primary substrate, for example removal of substances that impart characteristic flavors or colors. Other enzymes used in fruit juice processing are amylases used to break down starches and cellulase to remove cellulose (Grassin & Coutel, 2010; Kuddus, 2018).

Enzymes are less common in vegetable processing. As vegetable juice production has increased the use of carrots, beets, celery, and other vegetables used to make pure juice blends, more processors have turned to cellulase enzymes to break down the cellulose and increase yields (Grassin & Coutel, 2010).

Malting, brewing, and distilling

Beer and ale are alcoholic beverages made from fermented grains (Lewis, 2015). Distillers also make various spirits and other beverages from fermented grains (Bujake, 2000). Barley is the preferred grain for many beers, but brewers may also use wheat, rice, oats, rye, or almost any other available grains suitable to be malted and fermented (Thomas, 2014).

The malting process is as follows (Thomas, 2014):

1. Manufacturers begin the process by germinating grain.
2. They then dry or "kiln" the germinated grain at a temperature that is high enough to remove free moisture, but low enough not to denature their enzymatic activity.
3. The resulting product is malt.
4. Brewers and other end-users such as distillers can make the malt into a mash by adding water and a fermentation organism.
5. The mash ferments until the alcohol level reaches the level desired by the manufacturer. It is then prepared for consumption or marketing.

Maltsters rely on enzymes to prepare grains into malt. Traditionally, the process relied upon naturally existing enzymes within the grains that are released during the malting process. Hydration of sprouted grains introduce enzymes in the endosperm to play a primary role in malt modification in most cases (Thomas, 2014). By adding additional enzymes, modern maltsters can accelerate the malting process and get a more consistent product.

Beers can be made by traditional methods with malted grains and hops with no added enzymes (Lewis, 2015). Some brewers add enzymes with various technical and functional effects to make specific products, such as low alcohol or light beers (Uhlig, 1998). The primary added enzymes used for beer production are mostly alpha- and beta-amylases (Uhlig, 1998). Added enzymes can promote, facilitate, and terminate fermentation, and remove chill haze after fermentation (Kuddus, 2018; Lewis, 2015).

Dairy products

Some consider grain and dairy fermentation technologies to be interrelated. Yogurt and kefir are made from fluid milk through fermentation using specific dairy cultures (Hutkins, 2006; Steinkraus, 1983). While some cheeses can be made without added fermentation organisms or enzymes—such as cottage cheese, paneer, and farmer's cheese—most familiar cheeses such as cheddar or blue cheese rely on either an enzyme that coagulates and clots the fluid milk, or a fermentation organism, or both (Scott et al., 1998).

Traditional cheesemaking uses rennet: a mixture containing chymosin (also known as renin), pepsin, lipase, and other enzymes. These are traditionally retrieved from the abomasum (fourth stomach) of slaughtered calves, but some of these enzymes can also be obtained from plants and microorganisms. Enzymes in rennet coagulate milk through the hydrolyzing the amino acid bonds of the κ-Casein surrounding the protein globules in fluid milk (Lobedanz et al., 2016; Uhlig, 1998).

The dairy industry began to use protease enzymes derived from various natural microorganisms as substitutes for calf rennet beginning in the 1970s. These include mucorpepsin derived from *Mucor miehei* and *Mucor pusillus,* and endothiapepsin from *Cryphonectria parasitica* (Horsmans Poulsen et al., 2012). The pepsins have different coagulating properties and heat tolerances compared to calves' rennet, limiting their use in making certain specific styles of cheese (Scott et al., 1998).

Enzyme manufacturers are now producing dairy enzymes like chymosin from transgenic microorganisms, genetically modified with genes from cows and other mammals (Harboe et al., 2010; Law, 2009). Biotechnology companies have patented and produced chymosin and the related proteases prochymosin, preprochymosin, and pseudochymosin from the following organisms using recombinant DNA techniques:

- *E. coli* strain K-12 (Franke, 1990; Maat et al., 1998)
- *S. cerevisiae* (Goff et al., 1984)
- *Aspergillus nidulans* (Cullen et al., 1987)
- *Kluyveromyces lactis* (Van den Berg et al., 1989, 1990)

Other enzymes used in cheesemaking include (Scott et al., 1998):

- catalase
- reductases
- phosphatases
- lactoperoxidases
- oxidases

These have technical and functional effects on the quality of the cheeses, such as catalyzing reduction / oxidation reactions during fermentation. Many of these are naturally present in milk but are deactivated by pasteurization (Scott et al., 1998).

Besides coagulation, enzymes are also used in the cheesemaking process to aid with cheese ripening, impart characteristic flavor, improve whey separation, and create a more homogenous texture (Aehle, 2007; Horsmans Poulsen et al., 2012; Lobedanz et al., 2016). Cheese ripening is characterized by the proteolytic breakdown of the casein protein (Aehle, 2007) and the formation of fatty acids, methyl ketones, and α-ketoacids from milk fat (Uhlig, 1998). While many ripe, soft cheeses are fermentation products, the ripening process can be accelerated and made more predictable through the introduction of exogenous enzymes to the batch, such as plasmin (Horsmans Poulsen et al., 2012). Cheese ripening also involves the use of lipases (Nagodawithana et al., 2013). Dairy product manufacturers use the enzyme lactase (ß-galactosidase) to reduce the amount of lactose in milk-based products (Lobedanz et al., 2016). In addition to adding lactase to raw milk, it can also be added to whey and post-fermentation dairy products (Uhlig, 1998).

Some cheeses are made from acid-curdling (without added enzymes)—such as cottage cheese, paneer, and ricotta. These have characteristic textures and flavors that differ from enzymatically-curdled cheeses. Dairy manufacturers commonly use vinegar, which is itself a fermentation product, to make acid-curdled cheeses. However, lactic acid produced by bacterial cultures is the preferred acid for most cheeses.

Enzymes or probiotics may also be directly added to acid-curdled cheeses, but they are not essential (Scott et al., 1998).

Fermentation processes are commonly applied in dairy product manufacturing. For example, some aged cheeses are characterized by specific starter cultures that carry out a fermentation process. Blue cheese, camembert, and Roquefort cheeses are fermented products that are prepared with cultures of blue fungi of the genus *Penicillium* (Uhlig, 1998).

Other fermented dairy products include yogurt and kefir. Manufacturers also introduce probiotic bacteria into fermented and non-fermented dairy products such as yogurt and cottage cheese. Yogurt's standard of identity in the United States mandates the use of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (21 CFR 131.200). Yogurts that use only these two species are regarded as having poor sensory quality by researchers and most commercial products have a complex culture (Hutkins, 2006). The FDA permits other harmless bacteria are permitted as optional yogurt ingredients, provided the mandatory species are also used (U.S. FDA, 2023c). *Lactobacillus acidophilus* and other harmless lactic acid-producing bacteria may also be used in cultured milk products—including buttermilk (§ 131.112), sour cream (§ 131.160), cottage cheese (§ 131.128) (U.S. FDA, 2023c).

Lysozyme (an enzyme) added to milk has bactericidal properties (Uhlig, 1998). Some manufacturers will add lysozyme to milk to make various dairy products, such as St. Paulin (Aehle, 2007). While eggs and other animal products were the historical sources of lysozyme, the advent of rDNA technology has led to lysozyme produced by genetically modified yeasts and bacteria, including *E. coli, A. niger, S. cerevisiae. K. lactis,* and *Acremonium chrysogenum* (Ercan & Demirci, 2016).

Wine

Currently, vinters usually produce wine by inoculating fruit juice with yeast. Vintners crush fruit into a juice known as a "must" and use different combinations of yeast, enzymes, and other microorganisms to make specific wines. Grapes are the primary fruit made into wine, but other fruits can be vinted.

Before the isolation of yeast, vintners made wines without inoculation and relied on the wild fermentation organisms that naturally occurred on the fruit (Butzke, 2017). Winemakers have used added isolated fermentation organisms for about 150 years (Hutkins, 2006).

While *Saccharomyces cerevisiae* has long been the yeast species of choice for most wines made throughout the world, researchers that specialize in wine-making—oenologists — are actively exploring the use of other *Saccharomyces* species and other species and genera of yeasts (Alvarez et al., 2023). Wines inoculated with lactic bacteria (such as *Lactobacillus* spp., *Oenococcus* spp., and *Pediococcus* spp.) produce buttery notes desired in certain varietals like Chardonnay (Semon et al., 2001; UC Davis, 2023).

UC Davis scientists cloned the *L. delbruckii* gene for malolactic fermentation of wine into *S. cerevisiae* in the mid-1980s (Snow et al., 1984; Williams et al., 1984). Since then, yeast manufacturers have commercially released various genetically modified yeasts and notified the FDA that they are GRAS (U.S. FDA, 2023b). These are identified in the *Appendix* at the end of this report.

To break down pectin, increase yield, reduce fermentation time, and lower viscosity, vintners may introduce the enzyme pectinase to the wine must (Ahlawat et al., 2018; Kashyap et al., 2001; Mojsov, 2013; Uhlig, 1998). Organisms used to produce pectinase for wine are usually derived from fungi, including *A. niger, Penicillium notatum,* or *Botrytis cinerea* (Kashyap et al., 2001). *B. cinerea* is a grape pathogen responsible for gray mold and bunch rot, but is also known as noble rot because some vintners deliberately use infected fruit to provide a characteristic sweet flavor (Fournier et al., 2013). Enzymes may also be used to enhance color prior to fermentation, for maceration of red varietals that have low anthocyanins (a plant pigment) (Kelebek et al., 2007; Mojsov, 2013).

Some vintners add enzymes to wine following fermentation and prior to bottling. Young wines may have levels of ß-glucase that result in off flavors, undesirable aromas, cloudiness, mouthfeel, and other sensory defects. Such wines may be treated with ß-glucanase during the clarification process (Claus & Mojsov, 2018; Mojsov, 2013; Uhlig, 1998). Protease enzymes remove proteins responsible for off-flavors and lower the demand for fining agents such as bentonite clay during the clarification or fining process (Claus & Mojsov, 2018). Urease enzymes remove urea that can result in the contaminant ethyl carbamate and impart off-flavors (Claus & Mojsov, 2018; Mojsov, 2013; Ough & Trioli, 1988).

Vinegar and Pickling

Vinegar is a fermentation product that contains natural acetic acid. It has a pH in the range of 2-3.5 depending on the concentration of acetic acid (Webb, 2000). Traditional European vinegar is made from grape juice or wine, and the word is derived from the Old French phrase, "*vin egre*" or "sour wine" (Webb, 2000). Processors can ferment other fruits and grain into vinegar as well (Emde, 2014; Webb, 2000). For example, rice vinegar is a traditional ingredient in various East Asian cuisines (Ray & Didier, 2014; Webb, 2000).

Vinegar production is a two-step process (Emde, 2014; Webb, 2000):

1) Sugars from fruit or grains are converted to ethanol by yeast fermentation, and
2) The alcohol is converted to acetic acid by enzymes produced by bacteria capable of generating acetic acid.

Many different bacteria are capable of converting ethanol to acetic acid (Emde, 2014; Hutkins, 2006). Most acetic acid generating fermentation bacteria are in either the genera *Acetobacter* or *Gluconobacter* (Emde, 2014).The principal microorganisms responsible for converting fruit or grain substrates to vinegar in industrial settings are of the species *Acetobacter* (Emde, 2014; Hutkins, 2006). Traditional vinegar production from tropical fruit, cane sugar, or sorghum may use an intermediate fermentation step with lactic acid bacteria, and many rely on *Gluconobacter* or *Gluconoacetobacter* species capable of converting sugars directly into acetic acid (Gomes et al., 2018; Solieri & Giudici, 2009).

While vinegar is the preferred source for acid related to pickled fruits and vegetables, lactic acid produced from microbial fermentation is also common. Pickling is an ancient traditional method of food preservation in many cultures and has been used since before recorded history (Ray & Didier, 2014; Ray & Joshi, 2014; Steinkraus, 1983). Many cultures value acid-fermented vegetables and fruits as an essential part of their cuisines, such as kimchi in Korean cuisine, jeruk in Malaysia, and sauerkraut in German cuisine (Steinkraus, 1983).

Soy Products

Soybeans (*Glycine max*) can be fermented into several food products including soy sauce—a generic term that includes such traditional products as shoyu and tamari. Soybeans are also fermented to make soybean paste or miso, and tempeh. Soy sauces are produced from cooked soybeans fermented by a fungus that has been cultured on rice known in Japanese as "koji" (Allwood et al., 2021; Yamashita, 2021). Koji is a culture used to ferment rice in the production of sake (Allwood et al., 2021; Yamashita, 2021). The prevailing species used to make koji is *Aspergillus oryzae* (Steinkraus, 1983). Koji is mixed ith soy and an inoculum of *A. sojae* to make miso (Steinkraus, 1983).

Miso and its close relative natto are also fermentation products of soybeans, often made with barley, rice, or other grains as well (Ray & Didier, 2014; Shurtleff & Aoyagi, 1976; Steinkraus, 1983). The salt-tolerant fermentation organism *Pediococcus halophilus* takes part in the fermentation of soybeans into miso (Ray & Didier, 2014).

Another soy fermentation product is tempeh, made from partially-cooked soybeans fermented by various fungi, predominately *Rhizopus* spp. (Steinkraus, 1983). Industrial producers in North America have scaled up production using technologies different from those traditionally used in tropical climates based on similar biological and chemical processes (Hutkins, 2006).

Meat products

Animal slaughter products have a shorter history of fermentation and enzyme use than do grains and fruits. Historians believe that sausage making began in the Mediterranean region during the Roman era as a method to prepare animal slaughter products that are otherwise considered unpalatable, and to preserve meat that would otherwise rot (Hutkins, 2006). Fermented meats are less a part of Asian cuisine, but fermented fish pastes are staples of Southeast Asian cuisines (Ray & Didier, 2014; Steinkraus, 1983). Cured hams are also fermented meat products (Hutkins, 2006).

Processors use various enzymes to make meat products more tender, more palatable, and to accelerate cooking and provide characteristic texture. The three enzymes that are used in industrial scale meat production are all derived from fruit rather than microorganisms: bromelain, from pineapples; ficin, from figs; and papain, from papaya (Aehle, 2007). While not a microorganism, papaya genetically engineered to be resistant to papaya ringspot virus (PRSV) was the first genetically modified fruit to be commercially released (Gonsalves, 1998). By the early 2010s, genetically modified papaya varieties accounted for most domestic production in the United States. (Evans & Ballen, 2012). Genetically modified varieties account for a growing market share of papaya production world-wide (Akhtar et al., 2023).

<u>Fermentation methods</u>

Fermentation does the following (Bamforth & Cook, 2019; Hutkins, 2006):

- preserves perishable foods.
- serves to increase the nutritional value of foods.
- confers health benefits with probiotic organisms.
- produces foods that function differently from the raw foods in many cases.
- provides taste and other sensory qualities also not found in the raw foods.

Furthermore, using fermentation, microorganisms produce most commercial enzymes (Deckers et al., 2020). Only a small number of commercial enzymes come from plants, animals, or synthetic sources (Deckers et al., 2020).

The two key components of a fermentation system are (Bamforth & Cook, 2019):

1) the feedstock
2) the organism that acts upon it

The feedstock provides a substrate that the fermentation organism or organisms convert into a fermentation product (Bamforth & Cook, 2019). Organisms frequently require "priming sugars" for the fermentation process to begin. Fermentation organisms are usually bacterial or fungal species that have adapted to a given feedstock. Fermentation organisms also need water and nutrients. In most cases the feedstock provides sufficient moisture and nutrients, but in some cases the microorganisms need added water or supplemental nutrients to metabolize the feedstock. The feedstock and organism need to be placed in conducive environmental conditions for fermentation to occur. Most fermentation processes require warm and moist conditions. Some fermentation processes are requiring oxygen (aerobic), while others do not or benefit from reduced oxygen levels (anaerobic).[i] Producers and handlers prepare raw agricultural ingredients such as grains, fruit, vegetables, milk, meat, or legumes to make them biologically active and available to the organisms responsible for fermentation. In the case of grapes used for wine or vinegar production, this preparation means crushing the fruit and removing most of the solids. Another example is the malting of grains used (Bamforth & Cook, 2019).

Most fermentation processes use a liquid substrate, and are broadly referred to as "Liquid Fermentation" (LF in this report), "Submerged Fermentation" or "Submerged Liquid Fermentation" (Berenjian, 2019). However, a

[i] The term fermentation has different meanings, depending on the subject and audience. When discussing cellular respiration, biologists distinguish aerobic respiration processes (like the Krebs cycle) from anaerobic processes or fermentation. However, informally, fermentation is often used to describe both aerobic and anaerobic processes used by microorganisms.

growing number of fermentation processes involve "Solid-State Fermentation" (SF in this report) also known as "Solid Phase Fermentation" (Mienda et al., 2011).

Table 1: Comparison of submerged liquid and solid-state fermentations.

Factor	Submerged liquid fermentation	Solid-state fermentation
Substrates	Soluble (e.g., sugar)	Insoluble (e.g., cellulose, pectin, lignin)
Water	High volume of water	Low volume of water
Effluent	High volume of effluent pollution	Little or no effluent pollution
Aeration	Anaerobic or limited oxygen exchange without mechanical aeration	Aerobic with oxygen exchange
pH	Easy to control and modify pH	Buffered solid substrates resist rapid pH adjustment
Mixing	Culture and products are easily mixed and homogenized by agitation	Culture and products are less homogenous and many processes are static
Inoculation	Microorganisms are readily introduced and dispersed throughout the feedstock	Microorganisms are introduced as spores at the beginning of the batch
Temperature	Containers can have precise temperature controls	Mostly ambient temperatures with heat sometimes supplied by thermophilic organisms
Scale	Scalable to large, continuous processes	Small batch processes
Equipment cost	Medium to high	Low to medium
Concentration	Substrate / Products: 30-80 g/L	Substrate / Products: 100-300 g/L
Organisms	Mostly bacteria and yeasts	Mostly non-yeast fungi (e.g., *Rhizopus* and *Aspergillus*)

Sources: (Berenjian, 2019; Mienda et al., 2011)

Each process has advantages and disadvantages (see *Table 1*, above). LF production is more easily controlled in enclosed vessels and can be scaled up for large, continuous processing. LF requires special equipment for aeration and mixing, which can result in large loses if they fail (Hutkins, 2006). SF systems are limited in scale and can be used only for batch processing (Mienda et al., 2011). However, SF systems can be built with a lower capital investment and can use low-cost by-products as substrates more easily than LF systems (Berenjian, 2019). SF by-products can be readily composted, and composting itself is essentially a solid-state fermentation process (Viniegra-Gonzalez, 1997; Yafetto, 2022). LF uses more water and produces more effluent waste, while SF fermentation by-products are readily compostable (Mienda et al., 2011). LF by-products can be composted anaerobically, but aerobic composting usually requires drying out (Viniegra-Gonzalez, 1997).

Focus Questions Requested by the NOSB

1. What fermentation processes are used to derive enzymes, described by 7 CFR 205.605(a)(11)? Which products are derived using organisms developed by "excluded methods" (as described above in the scope of this review), and which products are derived using organisms developed through allowed methods?

Enzymes used in food production are produced from organisms that are developed through (Sewalt et al., 2016):

- natural selection
- classical improvement techniques
- recombinant DNA (rDNA) technologies

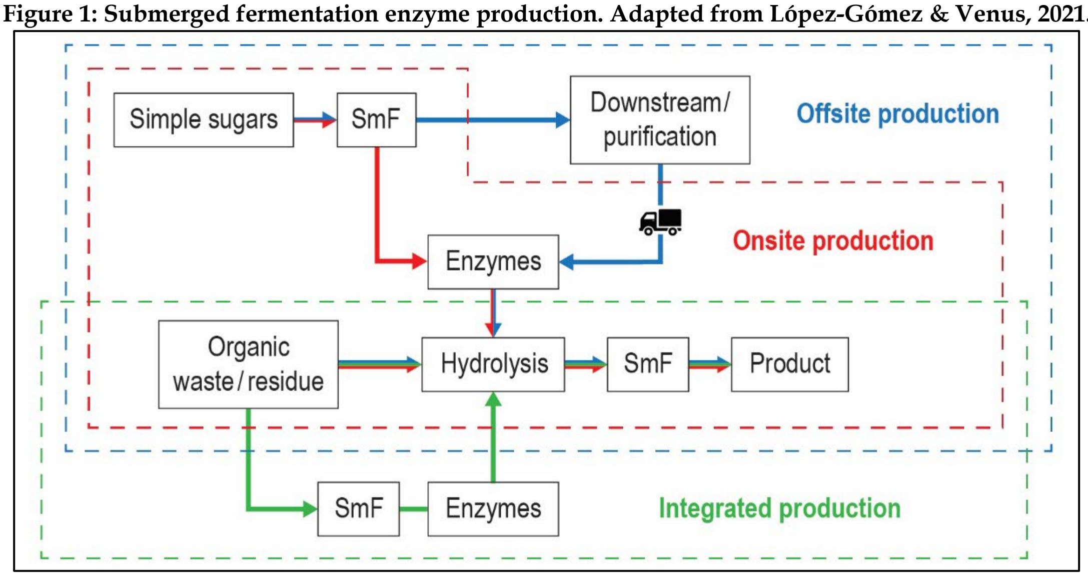
Enzymes from organisms that are naturally selected or derived from classical techniques are allowed as ingredients or processing aids in organic food [7 CFR 205.605(a)]. Enzymes from microorganisms developed through rDNA are prohibited for organic processing and handling [7 CFR 205.105(e)]. Enzymes may also be produced by synthetic methods (Breslow, 2005). These are also prohibited for use in organic food processing, given that enzymes do not appear as allowed synthetics on 7 CFR 205.605(b).

Fermentation processes used to derive enzymes

Food-grade enzymes are typically produced in pure culture fermentation using "Current Good Manufacturing Practices" for food (Sewalt et al., 2016). Almost all fermentation processes used to produce enzymes are aerobic (Lobedanz et al., 2016). Most industrial producers of food-grade enzymes use aerobic submerged fermentation or liquid fermentation (LF) (see *Fermentation Methods*, above) (Lobedanz et al., 2016; Sewalt et al., 2016). Fungi produce approximately 50% of the enzymes used globally, bacteria produce 35%, and the remaining 15% are produced from non-fermentation organisms like plants and animals (Deckers et al., 2020).

Simple sugars are introduced to a submerged fermentation vessel with media and culture (see *Figure 1*). The enzymes are purified and extracted by various means that are specific to the organism, the target enzyme, and the final market or use. These may be chemical, physical, or biological. Production methods vary widely, and a comprehensive review of the methods used for all enzymes extracted from all production organisms is beyond the scope of this review. Concerns over specific fermentation organisms, media ingredients, or extraction methods would involve a case-by-case review of each enzyme.

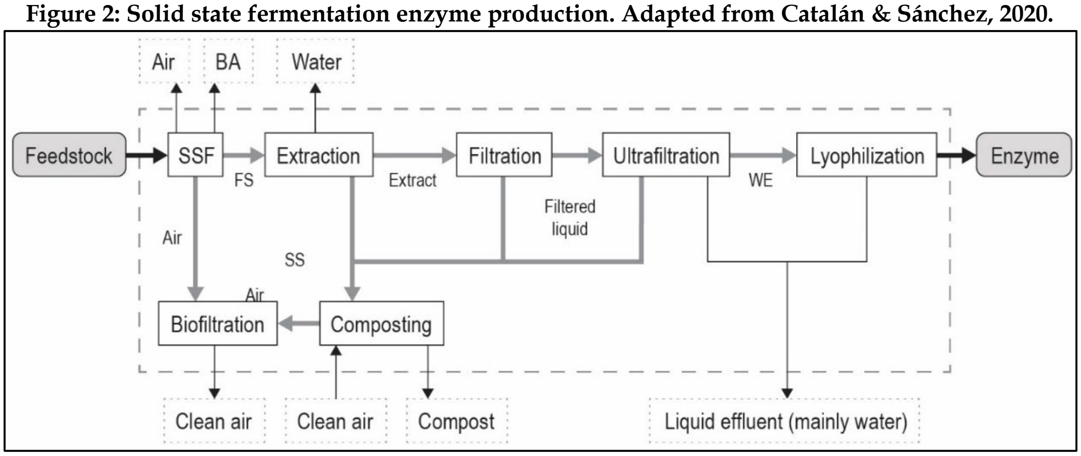
Figure 1: Submerged fermentation enzyme production. Adapted from López-Gómez & Venus, 2021.



There is a growing interest in solid-phase or solid-state fermentation (SSF) to culture enzyme-producing microorganisms (see *Fermentation methods*, above) (Mienda et al., 2011; Sewalt et al., 2016; Viniegra-Gonzalez, 1997; Yafetto, 2022).

Figure 2 shows a flow chart of a model SSF system based on coffee hull processing as an example (Catalán & Sánchez, 2020).

Figure 2: Solid state fermentation enzyme production. Adapted from Catalán & Sánchez, 2020.

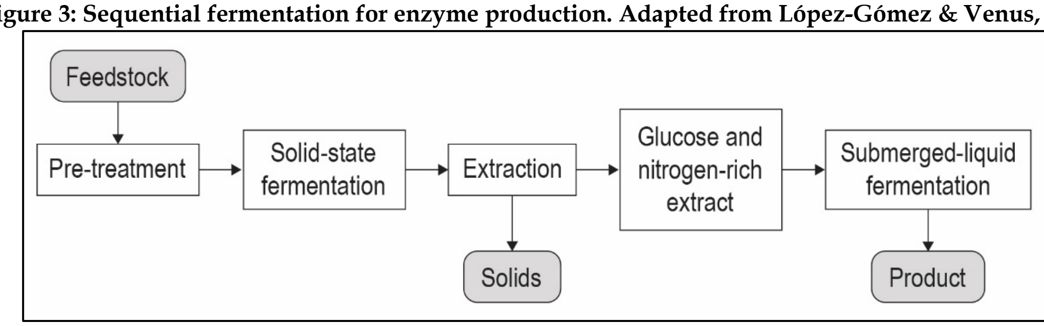


Most SSF fermentation processes involve the following steps (Catalán & Sánchez, 2020; López-Gómez & Venus, 2021; Viniegra-Gonzalez, 1997; Yafetto, 2022).

1. The feedstock is introduced to the solid-state fermentation vessel.
2. The organism inoculates the solid fermentation medium that may be in controlled or ambient conditions.
3. The fermentation solids and nutrients are periodically mixed with water.
4. Once the fermentation process is complete, the solids are extracted from the fermentation media via a variety of methods, mostly centrifugation, filtration, and other physical means.
5. Water and other solvents may be used to extract specific enzymes.
6. Enzyme preparations also often go through ultrafiltration. Once the final enzyme meets technical specifications, the manufacturer classifies, prepares, and packages for final shipment and sale (Catalán & Sánchez, 2020; López-Gómez & Venus, 2021; Viniegra-Gonzalez, 1997; Yafetto, 2022).

The two processes (LF and SF) are not mutually exclusive (López-Gómez & Venus, 2021). Sequential SSF-LF processes can optimize the benefits of both processes. Sequential processing for enzyme production involves a pre-treatment or "dry" stage of solid stage fermentation that replaces the grow-outs of starter cultures. The fermentation products of solid-state fermentation are then introduced into a nutrient-rich saturated environment of LF, with all subsequent steps the same as LF (López-Gómez & Venus, 2021).

Figure 3: Sequential fermentation for enzyme production. Adapted from López-Gómez & Venus, 2021



Scaling up production equipment from laboratory to industrial production is a challenge that requires an understanding of both microbiological production and chemical engineering (Aehle, 2007). LF systems use equipment able to contain liquids and control the various environmental parameters needed for fermentation to occur.

The main tank where fermentation occurs is called the fermenter. Before introducing the culture, the equipment is sterilized. Aerobic systems used air filters to prevent intake of contaminating microorganisms (Aehle, 2007).

The starter culture—sometimes referred to as the stock culture—is the preparation that contains a large number of the organism that accelerate the desired fermentation process (Behera et al., 2019). Stringent procedures and aseptic conditions need to be maintained to ensure that sufficient counts of the desired organism are present and that undesirable organisms are either within tolerance or not detectable (Hutkins, 2006). Quality control of microorganism growth media is also conducted with heat sterilization as the most common approach to sterilize the growth media, although hydrogen peroxide may also be used in some cases (Kuddus, 2018).

Once the growth media and starter culture are in place, manufacturers may introduce catalysts that accelerate the fermentation process. The production organism is fed the nutrients and given the conditions needed to maximize enzyme production (Dodge, 2009). The primary separation of enzymes from growth media and fermentation organisms is mostly physical, with centrifuging and filtration being the preferred techniques. Manufacturers may also use ion exchange resins, and various biological substrates are used to further purify the enzyme preparation. The organisms and media are mostly removed. It is rare for viable organisms to remain in the final enzyme product, but common for non-enzyme ingredients to remain in the final preparation provided they pose no food safety risk (Pariza & Johnson, 2001).

Most—though not all—GRAS notifications contain information on the growth media used (U.S. FDA, 2023b). Compiling a comprehensive list of all possible ingredients for every enzyme produced is beyond the scope of this review. Growth media used to make enzymes will vary according to the production organism's nutritional needs and the optimal nutritional program to maximize yield of the intended enzyme in the batch (Berenjian, 2019; Dodge, 2009). Some batches are optimized to produce multiple enzymes that are extracted through various substrates. Others are optimized for a single enzyme, and all other products of the process are sent to other value streams. Synthetic defoamers and flocculants are also commonly used in the fermentation process (Berenjian, 2019; Dodge, 2009).

The FDA issued guidance to the industry that requests that GRAS notices provide the fermentation techniques used to make their enzyme preparations (US FDA, 2010). Most of the GRNs reviewed follow this practice and provide a considerable amount of information on the steps carried out in the fermentation process (U.S. FDA, 2023b). Details and procedures vary, but most generally follow the cGMPs and the procedures developed to ensure GRAS status of enzymes (Pariza & Foster, 1983; Pariza & Johnson, 2001; U.S. FDA, 2010). A comprehensive description of all fermentation procedures used to produce food enzymes is beyond the scope of this technical report. However, all enzyme manufacturers follow some of the same basic procedures.

Antibiotics, such as Ampicillin, may be used to prevent undesirable bacteria from growing in the media (Clasado, 2013). This practice is more common with production organisms that have been genetically modified to be antibiotic resistant. If declared GRAS, such a step must be reported in the GRAS Notice (GRN,) along with the procedures used to remove antibiotics and test procedures to show the absence of antibiotics in the final product (U.S. FDA, 2010). One source says that antibiotics are prohibited in commercial enzyme production, but it does not provide a regulatory reference or cite FDA guidance (Sewalt et al., 2016).

Enzyme preparations derived from organisms developed by excluded methods

Within GRNs submitted to the FDA, we found 85 enzyme preparations that include references to excluded methods used to make the host organism (see *Table 6*, in the *Appendix*). Of these, the prevailing technique reported was rDNA, with 74 GRNs reporting use. Other excluded methods reported included gene doubling, gene deletion, and changing the position of genes. Several simply reported that the organism was genetically modified but did not disclose by what technique. Examples of enzymes identified as made from excluded methods are:

- amylases
- asparaginase
- cellulase
- lipase
- pectin esterase
- phospholipase
- proteases
- pullulanase

In addition, six enzymes were identified as "To Be Determined" based on Appendix A of the most recent NOSB's recommendations on excluded methods (see *Table 6*, in the *Appendix*). Three enzymes described in GRNB0085 are chemically mutated by methylation. Two are from a mutated strain of *Tayloromyces emersonii*, but the GRN (GRN0479) did not specify the mutagen. One does not disclose any method and does not make a statement that the organism is not genetically modified.

Most industrial production of food-use enzymes involves the growth of microorganisms through a fermentation process. Fermentation itself is not an "excluded method." The compliance risk for enzyme manufacturing is associated instead with the microorganism used in production.

Enzymes produced by excluded methods are, in most cases, indistinguishable from those produced from naturally occurring unmodified organisms (Barbau-piednoir et al., 2015; Deckers et al., 2020; Fraiture et al., 2020) (Barbau-Piednoir et al., 2015; Deckers et al., 2020; Fraiture et al., 2020). There are no currently available analytical methods that authorities can use to determine directly and conclusively whether an enzyme is produced by excluded methods (Deckers et al., 2022). Researchers are exploring whether analytical methods can be developed to distinguish whether a given enzyme is produced from a naturally-occurring or "wild-type" microorganism, or from a genetically modified strain (Deckers, 2022; Deckers et al., 2020).

Manufacturers and food safety authorities do not currently monitor production to make sure that unapproved genetically modified organisms are not used to make enzymes. Such an approach would (Fraiture et al., 2020):

1. Extract DNA from the food enzyme preparation and test for the presence of bacterial DNA and the presence of antimicrobial resistance (AMR) genes frequently present in food enzyme producing bacteria using polymerase chain reaction (PCR) techniques.
2. Analyze living microbial strains of the food enzyme producing microorganism isolated earlier for the presence of bacterial DNA and AMR genes using PCR techniques.
3. If the two strains match, the strain is not genetically modified. If the strains do not match and the strain in step 1 contains genes known to be present in genetically modified organisms, the analysis can be used as evidence that the production organism was made by excluded methods.

Enzyme preparations derived from organisms developed through allowed methods

Within GRNs submitted to the FDA (see *Table 6*, in the *Appendix*), 59 enzyme preparations are without evidence of excluded methods. These include all enzyme preparations listed as GRAS by the FDA prior to 1990. It also includes enzyme preparations declared as GRAS, where the GRN made a statement that the production organism was not genetically modified.

Enzymes that appear unlikely to be produced through excluded methods include:

- Aminopeptidase
- Arabinase
- Catalase
- Glucanase
- Lactase

2. What fermentation processes are used to derive *microorganisms*, described by 7 CFR 205.605(a)(19)? Which products are derived using organisms developed by "excluded methods" (as described above in the scope of this review), and which products are derived using organisms developed through allowed methods?

Fermentation processes used to derive microorganisms

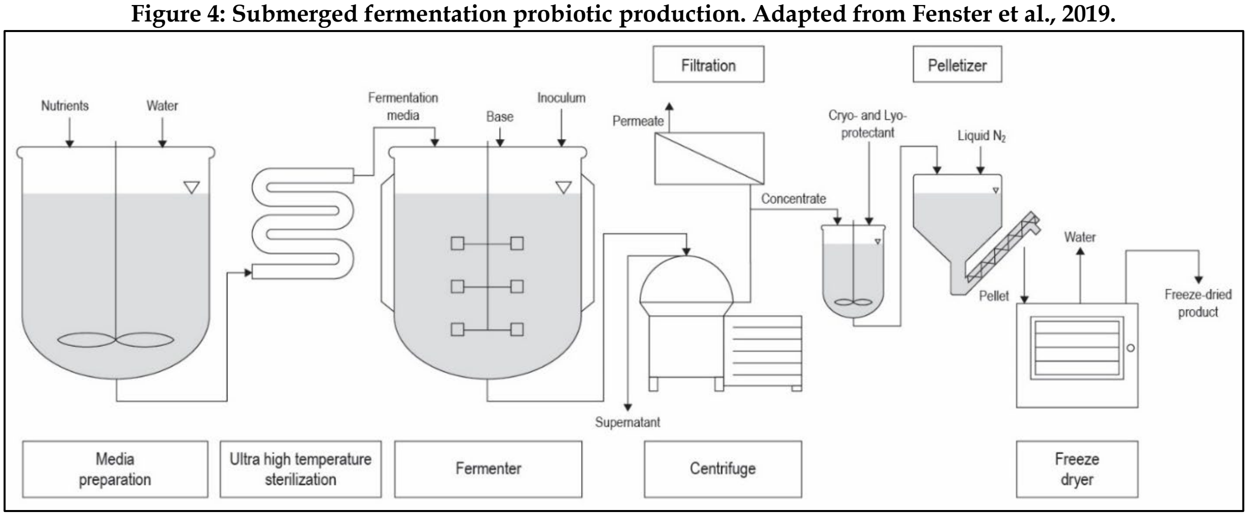
As stated in *Focus Question #1*, fermentation itself is not an "excluded method." The literature on the subject of microorganism production for food use is vast and growing (Bamforth & Cook, 2019; Doelle et al., 2012; Hutkins, 2006; Laranjo, 2021; Ray & Didier, 2014; Stanbury et al., 2013; Steinkraus, 1983). Many fermentation processes involve traditional methods practiced prior to recorded history (Ray & Didier, 2014; Steinkraus, 1983). Other microorganisms are relatively new and did not exist prior to the development of recombinant DNA techniques (Laranjo, 2021).

Fermentation technology is continuously evolving (Berenjian, 2019; Hutkins, 2006; Laranjo, 2021). Traditional fermentation processes span many foods using a wide variety of raw ingredients and many different microorganisms that are not easily classified (Ray & Didier, 2014; Steinkraus, 1983). Modern mass-production fermentation uses a narrower range of agricultural feedstocks, and fermentation organisms on a larger scale and more tightly controlled processes than do traditional and modern artisanal methods. Both submerged and solid-state fermentation methods as described above are used to produce microbial cultures for food use.

The media is prepared by adding nutrients and water to a mixing tank (see *Figure 4*, below). The figure shows the following step (Fenster et al., 2019):

1. Equipment, growth media and other adjuvants are often sterilized by various means as preparation for the introduction of isolated strains in a starter culture. Ultra-high temperature sterilization is the most common means. Manufacturers may also use ionizing radiation to sterilize food and other compact materials used in microorganism production (Doelle et al., 2012).
2. After sterilization, the media is then transferred to a fermentation tank and inoculated with the starter culture of the fermentation organism.
3. The fermentation organism is grown out in the fermentation tank.
4. The fermentation organism—probiotics in this example—are then filtered out and separated by centrifuge.
5. The concentrated probiotics are then transferred to a concentration vessel and pelletized.
6. The microbial product is then cryogenically freeze-dried with liquid nitrogen (N_2).
7. The freeze-dried microbial product is ready for packaging, bulk sale, or direct use.

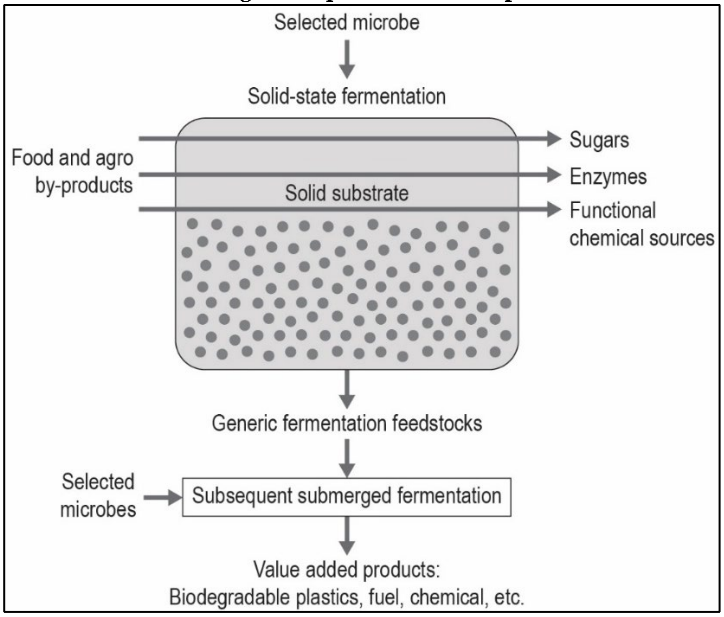
Figure 4: Submerged fermentation probiotic production. Adapted from Fenster et al., 2019.



Solid state fermentation is less common but may be used to produce various specialty microorganisms (see *Figure 5*, below). The figure shows the following steps (Srivastava, 2019):

1. A selected microorganism is introduced into a medium of biomass, usually of agricultural and food by-products with additional nutrient sources, and water.
2. The selected microorganism is grown out on a solid substrate.
3. The subsequent fermentation microorganism is isolated into a generic fermentation feedstock.
4. The fermentation feedstock is introduced to the subsequent food product as a fermentation organism or functional food additive.

Figure 5: Solid-state microorganism production. Adapted from Srivastava, 2019



Many of the new preparations are genetically modified. Of those, most involve the use of rDNA techniques: transferring plasmids and genetic sequences from a donor organism to a host organism that is used in fermentation production. The Appendix lists those organisms that are in the FDA's GRAS inventory, with the source / production organism and donor organism listed. In some cases, the host organism is the donor organism, and the technique may involve gene doubling or gene deletion. More recent notifications involve the use of gene editing through CRSPR and related techniques.

Like all life, microorganisms require an energy source, a protein source, vitamins, and minerals to grow. Growth media can be as simple as a single feedstock and water, or may be comprised of as many as 40 different components (Doelle et al., 2012). Components of microbial growth media may include (Doelle et al., 2012):

- Yeast
- Meat
- Carrot juice
- Coconut milk
- Wort
- Horse manure extract
- Peptone
- Whey permeate
- Corn steep liquor
- Soybean extract
- Molasses

Molasses tends to be a preferred energy source for many manufacturers because it is relatively low cost and has some mineral content. Yeast is a common protein source that is readily metabolized. Some media may be from commodity sources that are mostly produced by excluded methods, such as commodity corn to make corn steep liquor and soybeans to make soybean extract.

Growth media may also contain non-protein nitrogen such as synthetic ammonia that the organisms metabolize into protein. Commercial cultures of food microorganisms may have undigested media that includes non-protein nitrogen, depending on the isolation, extraction, and standardization methods used. Many GRNs report specific growth media ingredients. Some notifications omit proprietary information or claim only that they use ingredients that are commonly used in food.

Ancillary ingredients

Ancillary ingredients in microorganism products are often the culture in which they were grown (NOSB, 2016a). The Organic Trade Association reported that a variety of ancillary ingredients are used in microorganisms and dairy cultures used by organic handlers and processors (see *Table 2*, below) (NOSB, 2019a).

Table 2: Ancillary ingredients in microorganisms and dairy cultures.

Functional Class	Substance Name
Anti-caking & anti-stick agents	Magnesium stearate, calcium silicate, silicon dioxide
Carriers and fillers, agricultural or non-synthetic	Lactose, maltodextrins, sucrose, dextrose, potato starch, non-GMO soy oil, flour, milk, autolyzed yeast, inulin, cornstarch, sucrose
Carriers and fillers, synthetic	Micro-crystalline cellulose, propylene glycol, stearic acid, dicalcium phosphate
Preservatives	Sodium benzoate, potassium sorbate, ascorbic acid
Stabilizers	Maltodextrin
Cryoprotectants used to freeze-dry dairy cultures	Liquid nitrogen, maltodextrin, magnesium sulfate, dimethyl sulfoxide, sodium aspartate, mannitol, sorbitol
Substrate that may remain in final product	Milk, lactose, grain (rice, barley, wheat) flour, brewed black tea and sugar, soy

Source: (NOSB, 2019a))

The use of the ancillary ingredients in *Table 2* vary according to the specific needs of the fermentation organism, cultural methods used, and intended food application of the organism. Dairy cultures are commonly kept in skim milk powder (Bamforth & Cook, 2019). Freeze-dried media may have synthetic substances such as dimethyl sulfoxide or sodium aspartate used to protect organisms from being damaged by rapid cooling. Culture ingredients used for microorganism preparations are, in most cases, food ingredients or common food additives. These ingredients are often removed from target microorganism via consumption, filtration, or centrifugation (Bamforth & Cook, 2019; Hutkins, 2006). Synthetic nutrients such as ammonium phosphate may be present in small amounts (Bamforth & Cook, 2019) Agricultural ingredients cannot be assumed to be organically produced and standard industry practice relies on affidavits affirming that excluded methods, sewage sludge, or ionizing radiation were not used to prepare the ingredients (Wyard, 2015).

The processes manufacturers use to isolate, concentrate, package, and prepare shelf-stable microbial products vary. Most processes to isolate microorganisms from growth media involve centrifugation and physical filtration (Bamforth & Cook, 2019). Many microbial inoculants are freeze-dried to dehydrate and concentrate the organism at cryogenic temperatures. Freeze-dried cultures are shelf stable. Cultures stored for one month at 30°C (86°F) temperatures were still viable, but with poor survival of the culture and notable quality degradation. In the same experiment, freeze-dried yogurt culture was stored up to three months in climate-controlled conditions at about 4°C (39°F) with minimal loss of yogurt quality (Chutrtong, 2015).

Microorganisms developed by excluded methods

We found no direct evidence that microorganisms (other than yeast), that were declared as GRAS within a notice to the FDA, were produced by excluded methods (US FDA, 2023b). However, some of the GRAS Notices did not actually disclose how the strains were improved, specifically. Therefore, based on these GRAS Notices, it is not possible to say for sure whether all of these microorganisms are produced without excluded methods or not.

A search of the scientific literature showed that researchers and companies are interested in developing live genetically modified microorganisms other than yeast for direct food applications (Adrio & Demain, 2006; Hanlon & Sewalt, 2021; Meyer, 2008; Selle & Barrangou, 2015). However, most of the applications of bacteria and microfungi in the literature are for pharmaceutical production or non-food industrial applications (Adrio & Demain, 2006).

As noted in the enzymes section, dozens of *enzyme* production organisms *are* made with excluded methods. The separate yeast section identified those yeast strains that are made using excluded methods. However, we could find no reference to any microorganisms that are currently in use in the U.S. food supply. We searched both the scientific and trade literature, both in general and under specific product categories such as probiotics, acetic acid generating bacteria, and non-yeast fungi used to make koji. Internet reports that yogurt cultures using gene editing techniques such as CRSPR are on the market could not be independently verified through a search of the peer-reviewed literature, the patent literature, trade publications, or the GRAS Notices Inventory. One article published in 2020 said that only non-genetically modified lactic acid bacteria has been affirmed as GRAS by U.S. FDA (Plavec & Berlec, 2020). A more recent article states "(w)hile (Genetically Engineered Microorganisms) could also be incorporated as intact, live organisms into foods such as yogurt, kefir, or kombucha, this use falls outside the scope of the paper" (Hanlon & Sewalt, 2021). It is possible that such organisms have been developed and released, but the FDA has not been notified under the current voluntary system. We found no publicly available data for experts to support such a release.

Microorganisms developed by allowed methods
Table 6 in the *Appendix* is a taxonomically identified list of selected bacteria, yeasts, and other fungi used in food processing according to various sources (Hutkins, 2006; IDF, 2018; Steinkraus, 1983; US FDA, 2023b, 2023c, 2023b). Viruses in the form of bacterial phages are also included, but not taxonomically divided. The organisms are identified as bacteria, yeast, non-yeast fungi, microalgae, and viruses. *Table 6* also gives examples of the uses and applications of each organism. The table is further explained in the *Appendix*.

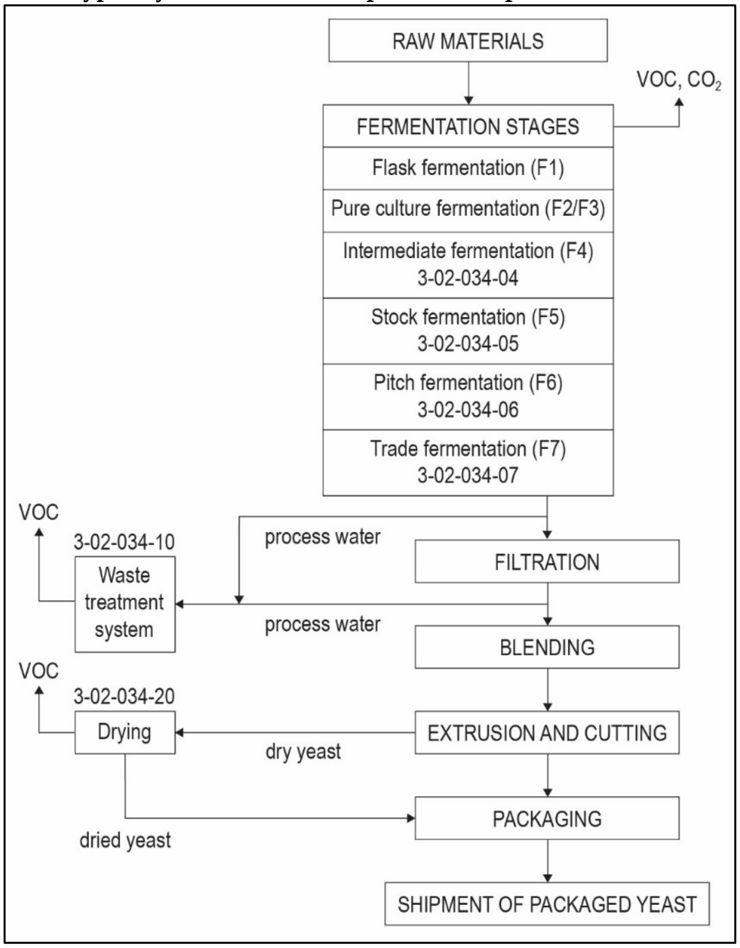
The list in *Table 6* is not intended to be exhaustive. Inclusion on the list does not mean that the FDA has affirmed GRAS status. There was no evidence that any non-yeast microorganisms or viruses were produced by Excluded Methods based on the NOP's definition of genetic engineering (7 CFR 205.2) or Appendix A of the most recent NOSB's recommendations on excluded methods (NOSB, 2022).

3. What fermentation processes are used to derive yeast, described by 7 CFR 205.605(a)(30)? Which products are derived using organisms developed by "excluded methods" (as described above in the scope of this review), and which products are derived using organisms developed through allowed methods?

Fermentation processes used to derive yeast
The technology used to manufacture yeast has evolved over the past 150 years, following its discovery by Louis Pasteur in the 1870s (Hutkins, 2006). Yeast production processes has advanced through the following innovations:

- In 1915, the German Institute for the Fermentation Industry developed the fed-batch process known as "Zulauf-Verfahren" (VH Berlin, 2023). The process used synthetic nitrogen in the form of ammonia to increase yields. The reference to "petrochemical substrate" in the yeast annotation apparently disallows yeasts that use ammonia produced from fossil fuels in the growth media, but the NOSB may need clarification of how the annotation is currently implemented with respect to synthetic ammonia. Yeast manufacturers continue to use incremental feeding method (Kulp & Lorenz, 2003).
- Active dry yeast was invented in the 1930s (Riley, 1935). The fermentation process resulted in a stabilized product that could be stored for long periods without spoiling and allowed manufacturers to scale up production. Once industrial scale was technologically feasible, manufacturers developed numerous processes to mass-produce food-grade yeasts (Athnasios & Quantz, 2012; Bekatorou et al., 2006; Żymańczyk-Duda et al., 2017).
- Yeast manufacturers innovated and invested in process automation from 1950 to the present to have greater control over media flow, pH, soluble oxygen, and ethanol content (Athnasios & Quantz, 2012).

Figure 6: Typical yeast fermentation process. Adapted from US EPA, 1995.



The process to produce yeast involves a series of steps to "scale up" the cultures (see Figure 6, above). Large-scale fermentation of baker's yeast will have at least three and sometimes as many as eight fermentation steps (Vaughan & Macreadie, 2000):

1) Fermentation begins with small flasks of less than 1 kg (about 2 lb).
2) The culture from the flasks is transferred to a pure culture fermenter, and second larger scale pure culture fermentation is performed in some cases up to about 120 kg (264 lb) capacity.
3) The pure culture fermentation is transferred to one or more progressively larger intermediate fermenters in some cases, and in some cases will go to the seed yeast or "pitch" fermenter that may a capacity of up to 15 tons.
4) The final production is in the largest tank, called the trade fermenter, some of which have a capacity of 100 tons (Vaughan & Macreadie, 2000).

Most of the sugar is provided by cane or beet molasses (Athnasios & Quantz, 2012; Vaughan & Macreadie, 2000). Additional nitrogen in the form of ammonia or ammonia salts, soluble phosphate, calcium, and magnesium, vitamins, and trace elements are also added (Athnasios & Quantz, 2012; Bamforth & Cook, 2019; Vaughan & Macreadie, 2000). Beet molasses has a more complete vitamin and mineral profile, and media that has only cane molasses requires the addition of thiamine and pantothenic acid (Athnasios & Quantz, 2012).

The yeast is then grown out in progressively larger-volume fermentation vessels through an intermediate scaling up, or directly into a stock fermentation vessel equipped with incremental feeding and good aeration. The intermediate vessel may either be continuously or batch fed. After the fermentation is complete, the yeast is separated from the bulk of the fermenter by centrifugation (US EPA, 1995).

The centrifuged biomass extracted from the stock fermenter is called the pitch. Molasses and other nutrients are incrementally fed into the pitch fermenter and the liquor is divided into several parts for pitching into the final fermentation stage. Final fermentation is performed in the trade fermenter. This vessel has the highest degree of aeration, with large air compressors. Molasses and nutrients are continuously fed until fermentation is complete. Final fermentation takes between 11 and 15 hours (U.S. EPA, 1995).

The yeast from the trade fermenter is then recovered by centrifuging out the solids and concentrating them in either a filter press or rotary vacuum unit (U.S. EPA, 1995). The yeast may also be washed to produce a yeast cream of 18-20% solids, which may be sold in bulk to industrial bakers or be further concentrated by drying and other physical means, and packaged in smaller units (Athnasios & Quantz, 2012; Vaughan & Macreadie, 2000). Such products may be compressed yeast cake, active dry yeast, or instant active dry yeast (Athnasios & Quantz, 2012).

Yeasts developed by excluded methods

All six of the microorganisms from GRAS notifications that were found to be produced with excluded methods use *S. cerevisiae* as the main production or host organism (see *Table 3*). Three used rDNA techniques and three used CRSPR gene editing techniques.

Table 3: Yeast strains identified as produced by excluded methods in FDA GRAS Notices. The table includes only those organisms where the FDA has been notified and has no questions for the notifier.

GRN	Recipient / Production / Host Organism	Donor Organism / Virus	Notifier	Method
120	*Saccharomyces cerevisiae* (strain ML01)	Oenococcus oeni and Schizosaccharomyces pombe	Lesaffre Yeast Corporation	rDNA
175	*Saccharomyces cerevisiae* (strain ECMo01)	Saccharomyces cerevisiae (strain ECMo01)	First Venture Technologies Corp.	rDNA
350	*Saccharomyces cerevisiae* (strain P1Y0)	S. cerevisiae (parent strain UCD2034)	Phyterra Yeast, Inc.	rDNA
798	*Saccharomyces cerevisiae* strain yBBS002	Mentha citrata and Occiumum basilicum	Berkeley Brewing Science, Inc.	Gene editing (CRSPR)
841	*Saccharomyces cerevisiae,* strain unspecified	Rhizopus oryzae	Mascoma LLC	rDNA
1062	*Saccharomyces cerevisiae* strain OYR-185	Undisclosed. Not derived from a *S. cerevisiae* strain	Omega Yeast Labs, LLC	Gene editing (CRSPR)
1096	Saccharomyces cerevisiae strain OYR-243	Saccharomyces cerevisiae strain S288C	Omega Yeast Labs, LLC	Gene editing (CRSPR)

Yeasts made by allowed methods

As noted in *Focus Question #1* and *Focus Question #2*, fermentation itself is not an "excluded method." Yeast manufacturers pioneered fermentation processes to mass-produce that resulted to methods to mass produce other microorganisms (Bamforth & Cook, 2019; Hutkins, 2006). Yeast requires fermentable sugars, protein-forming nutrients, minerals, and vitamins (Bamforth & Cook, 2019). Most industrial yeasts are manufactured from a medium made primarily with molasses produced during beet or cane sugar refining as the fermentable sugar source (Athnasios & Quantz, 2012; US EPA, 1995). Yeast media also contains many nutrients, including ammonia, phosphates, vitamins, minerals, and other ingredients, some of which may be synthetic or derived from petrochemicals or by-products of food processing, such as whey (Athnasios & Quantz, 2012; Bekatorou et al., 2006; US EPA, 1995).

Organically produced yeast

The fermentation process to produce certified organic yeast is more restrictive. The yeast must be made from certified organic inputs such as certified organic flour or certified organic corn steep liquor. Synthetic substances not on the National List, such as ammonia, would not be permitted. The starter culture does not need to be certified organic for a yeast product labeled as "Organic," but a "100% Organic" labeled yeast would require a certified organic starter culture. Any non-organic ingredients added to the yeast prior to packaging would need to appear on the National List as an allowed ingredient for processing and handling, and could not exceed 5% of the finished product by weight, net of water or salt (NOP, 2011a).

Report Authorship

The following individuals were involved in research, data collection, writing, editing, and/or final approval of this report:

- Brian Baker, Principal, Belcairn Concerns LLC
- Peter O. Bungum, Research and Education Manager, OMRI
- Doug Currier, Technical Director, OMRI
- Ashley Shaw, Technical Assistant, OMRI
- Amy Bradsher, Deputy Director, OMRI
- Meghan Murphy, Graphic Designer, OMRI

All individuals are in compliance with Federal Acquisition Regulations (FAR) Subpart 3.11—Preventing Personal Conflicts of Interest for Contractor Employees Performing Acquisition Functions.

References

Adrio, J. L., & Demain, A. L. (2006). Genetic improvement of processes yielding microbial products. *FEMS Microbiology Reviews*, *30*(2), 187–214. https://doi.org/10.1111/j.1574-6976.2005.00009.x

Aehle, W. (Ed.). (2007). *Enzymes in Industry: Production and Applications*. John Wiley & Sons.

Ahlawat, S., Kumawat, M., & Babele, P. K. (2018). Microbial enzymes in food technology. In M. Kuddus (Ed.), *Enzymes in food technology: Improvements and innovations* (pp. 1–17). Springer.

Akhtar, N., Perween, S., & Ansari, A. M. (2023). Resistance to papaya ringspot virus: A review. *Agriculture Association of Textile Chemical and Critical Reviews*, *11*(3), 11.

Allwood, J. G., Wakeling, L. T., & Bean, D. C. (2021). Fermentation and the microbial community of Japanese koji and miso: A review. *Journal of Food Science*, *86*(6), 2194–2207. https://doi.org/10.1111/1750-3841.15773

Alvarez, R., Garces, F., Louis, E. J., Dequin, S., & Camarasa, C. (2023). Beyond S. cerevisiae for winemaking: Fermentation-related trait diversity in the genus Saccharomyces. *Food Microbiology*, *113*.

Athnasios, A. K., & Quantz, M. (2012). Yeasts. In *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley Online Library.

Bamforth, C. W., & Cook, D. J. (2019). *Food, fermentation, and micro-organisms*. John Wiley & Sons.

Barbau-piednoir, E., De Keersmaecker, S. C. J., Delvoye, M., Gau, C., Philipp, P., & Roosens, N. H. (2015). Use of next generation sequencing data to develop a qPCR method for specific detection of EU-unauthorized genetically modified Bacillus subtilis overproducing riboflavin. *BMC Biotechnology*, *15*(1), 1–10.

Behera, S. S., Ray, R. C., Das, U., Panda, S. K., & Saranraj, P. (2019). Microorganisms in fermentation. In A. Berenjian (Ed.), *Essentials in fermentation technology* (pp. 1–39). Springer.

Bekatorou, A., Psarianos, C., & Koutinas, A. A. (2006). *Production of Food Grade Yeasts*. https://api.semanticscholar.org/CorpusID:2428533

Berenjian, A. (Ed.). (2019). *Essentials in Fermentation Technology*. Springer.

Bernini, V., & Lindner, J. D. D. (Eds.). (2022). *New Insights Into Food Fermentation*. MDPI-Multidisciplinary Digital Publishing Institute.

Binati, R. L., Salvetti, E., Bzducha-Wróbel, A., Bašinskienė, L., Čižeikienė, D., Bolzonella, D., & Felis, G. E. (2021). Non-conventional yeasts for food and additives production in a circular economy perspective. *FEMS Yeast Research*, *21*(7), 1–18.

Boynton, P. J., & Greig, D. (2014). The ecology and evolution of non-domesticated Saccharomyces species. *Yeast*, *31*(12), 449–462.

Breslow, R. (Ed.). (2005). Artificial enzymes. In *Artificial Enzymes* (pp. 1–35). Wiley-VCH.

Bujake, J. E. (2000). Beverage spirits, distilled. In *Kirk-Othmer Encyclopedia of Chemical Technology* (p. 22950). Wiley Blackwell.

Butzke, C. E. (2017). Wine. In *Kirk-Othmer Encyclopedia of Chemical Technology* (pp. 1–40). Wiley Blackwell.

Cappelle, S., Guylaine, L., Gänzle, M., & Gobetti, M. (2012). History and social aspects of sourdough. In *Handbook on Sourdough Biotechnology* (pp. 1–10). Springer.

Catalán, E., & Sánchez, A. (2020). Solid-state fermentation (SSF) versus submerged fermentation (SmF) for the recovery of cellulases from coffee husks: A life cycle assessment (LCA) based comparison. *Energies*, *13*(11), 1–20.

Chutrtong, J. (2015). Survival of probiotic bacteria in freeze—Dry yogurt starter cultures storage at 4 and 30 degree celsius. *Procedia - Social and Behavioral Sciences*, *191*, 2219–2225. https://doi.org/10.1016/j.sbspro.2015.04.514

Clasado. (2013). *GRAS exemption claim for BbgIV* Beta-*galactosidae enzyme preparation* (FDA GRAS Notification 485; pp. 1–50). Clasado, Inc.

Claus, H., & Mojsov, K. (2018). Enzymes for wine fermentation: Current and perspective applications. *Fermentation*, *4*(3), 1–19.

Copeland, R. A. (2000). *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis* (2nd ed.). Wiley-VCH.

Cullen, D., Gray, G. L., Wilson, L. J., Hayenga, K. J., Lamsa, M. H., Rey, M. W., Norton, S., & Berka, R. M. (1987). Controlled expression and secretion of bovine chymosin in Aspergillus nidulans. *Bio/Technology*, *5*(4), 369–376.

Deckers, M. (2022). *Strategies to control the presence of microbial impurities, including genetically modified microorganisms, in food enzymes* [PhD Thesis]. Ghent University.

Deckers, M., De Loose, M., Papazova, N., Deforce, D., Fraiture, M.-A., & Roosens, N. H. (2022). First monitoring for unauthorized genetically modified bacteria in food enzymes from the food market. *Food Control*, *135*, 1–12.

Deckers, M., Deforce, D., Fraiture, M.-A., & Roosens, N. H. C. (2020). Genetically modified micro-organisms for industrial food enzyme production: An overview. *Foods*, *9*(3), 326.

Dodge, T. (2009). Production of industrial enzymes. In R. J. Whitehurst & M. van Oort (Eds.), *Enzymes in Food Technology* (pp. 44–58). Wiley Online Library.

Doelle, H. W., Fiechter, A., Schlegel, G., Shimizu, S., Ulber, R., & Yamada, H. (2012). *Biotechnology: Cultivation of Microorganisms*. Ullmann's Encyclopedia of Industrial Chemistry; Wiley Online Library.

Emde, F. (2014). Vinegar. In *Ullmann's Encyclopedia of Industrial Chemistry* (pp. 1–24). Wiley-VCH.

Ercan, D., & Demirci, A. (2016). Recent advances for the production and recovery methods of lysozyme. *Critical Reviews in Biotechnology*, *36*(6), 1078–1088.

ETA. (2001). *Enzymes: A primer on use and benefits today and tomorrow* (pp. 1–34). Enzyme Technical Association. https://www.enzymetechnicalassociation.org/wp-content/uploads/2013/09/benefits_paper.pdf

ETA. (2023). *Enzyme Technical Association*. https://www.enzymetechnicalassociation.org/

Evans, E. A., & Ballen, F. H. (2012). An overview of global papaya production, trade, and consumption. *EDIS*, *2012*(9).

Fennema, O. R. (1996). *Food Chemistry* (3rd ed.). Dekker.

Fenster, K., Freeburg, B., Hollard, C., Wong, C., Rønhave Laursen, R., & Ouwehand, A. C. (2019). The production and delivery of probiotics: A review of a practical approach. *Microorganisms*, *7*(3), 1–16.

Fournier, E., Gladieux, P., & Giraud, T. (2013). The 'Dr Jekyll and Mr Hyde fungus': Noble rot versus gray mold symptoms of B otrytis cinerea on grapes. *Evolutionary Applications*, *6*(6), 960–969.

Fraiture, M.-A., Bogaerts, B., Winand, R., Deckers, M., Papazova, N., Vanneste, K., De Keersmaecker, S. C., & Roosens, N. H. (2020). Identification of an unauthorized genetically modified bacteria in food enzyme through whole-genome sequencing. *Scientific Reports*, *10*(1), 7094.

Franke, A. E. (1990). *Expression plasmids for improved production of heterologous protein in bacteria* (US Patent Office Patent 4,935,370).

Gallone, B., Steensels, J., Prahl, T., Soriaga, L., Saels, V., Herrera-Malaver, B., Merlevede, A., Roncoroni, M., Voordeckers, K., Miraglia, L., & others. (2016). Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts. *Cell*, *166*(6), 1397–1410.

Gaynor, P., & Cianci, S. (2005). *How U.S. FDA's GRAS notification program works*. https://www.food-safety.com/articles/4126-how-us-fdas-gras-notification-program-works

Gibbs, B. F., Kermasha, S., Alli, I., & Mulligan, C. N. (1999). Encapsulation in the food industry: A review. *International Journal of Food Sciences and Nutrition*, *50*(3), 213–224.

Goff, C. G., Moir, D. T., Kohno, T., Gravius, T. C., Smith, R. A., Yamasaki, E., & Taunton-Rigby, A. (1984). Expression of calf prochymosin in Saccharomyces cerevisiae. *Gene*, *27*(1), 35–46.

Gomes, R. J., de Fatima Borges, M., de Freitas Rosa, M., Castro-Gómez, R. J. H., & Spinosa, W. A. (2018). Acetic acid bacteria in the food industry: Systematics, characteristics and applications. *Food Technology and Biotechnology*, *56*(2), 139–151.

Gonsalves, D. (1998). Control of papaya ringspot virus in papaya: A case study. *Annual Review of Phytopathology*, *36*(1), 415–437.

Grassin, C., & Coutel, Y. (2010). Enzymes in fruit and vegetable processing and juice extraction. In R. J. Whitehurst & M. van Oort (Eds.), *Enzymes in Food Technology* (2nd ed., pp. 236–263). Wiley-Blackwell.

Grossmann, M., Kießling, F., Singer, J., Schoeman, H., Schröder, M.-B., & von Wallbrunn, C. (2011). Genetically modified wine yeasts and risk assessment studies covering different steps within the wine making process. *Annals of Microbiology*, *61*(1), 103–115. https://doi.org/10.1007/s13213-010-0088-2

Hanlon, P., & Sewalt, V. (2021). GEMs: Genetically engineered microorganisms and the regulatory oversight of their uses in modern food production. *Critical Reviews in Food Science and Nutrition*, *61*(6), 959–970.

Harboe, M., Broe, M. L., & Qvist, K. B. (2010). The production, action and application of rennet and coagulants. In B. A. Law & A. Y. Tamime (Eds.), *Technology of Cheesemaking* (pp. 98–129). Wiley-Blackwell.

Horsmans Poulsen, C., Kragh, K. M., Frisbak Sorensen, J., Grassin, C., Herweijer, M., Wilms, J., de Roos, A., & Borch Soe, J. (2012). Enzymes: Food Application. In *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley-VCH.

Hutkins, R. W. (2006). *Microbiology and technology of fermented foods*. IFT Press / Blackwell.

Huys, G., Daniel, H.-M., De Vuyst, L., Gobetti, M., & Gänzle, M. (2012). Taxonomy and biodiversity of sourdough yeasts and lactic acid bacteria. In *Handbook on Sourdough Biotechnology* (pp. 105–154). Springer.

IDF. (2018). *Inventory of microbial food cultures with safety demonstration in fermented food products* [Bulletin of the International Dairy Federation]. International Dairy Federation. https://shop.fil-idf.org/products/bulletin-idf-n-495-2018-inventory-microbial-food-cultures-safety-demonstration-fermented-food-products

IUBMB. (2023). *ExplorEnz – The Enzyme Database* [dataset]. International Union of Biochemistry and Molecular Biology. https://www.enzyme-database.org/

Johnson, E. A., & Echavarri-Erasun, C. (2011). Yeast biotechnology. In C. P. Kurtzman, J. W. Fell, & T. Boekhout (Eds.), *The Yeasts* (pp. 21–44). Elsevier.

Kashyap, D. R., Vohra, P. K., Chopra, S., & Tewari, R. (2001). Applications of pectinases in the commercial sector: A review. *Bioresource Technology*, *77*(3), 215–227.

Kelebek, H., Canbas, A., Cabaroglu, T., & Selli, S. (2007). Improvement of anthocyanin content in the cv. Öküzgözü wines by using pectolytic enzymes. *Food Chemistry*, *105*(1), 334–339.

Kohman, H. A., Irvin, R., & Stateler, E. S. (1928). *Production of enzymes for use in the manufacture of bread* (US Patent Office Patent 1,676,446).

Kuddus, M. (2018). *Enzymes in food technology: Improvements and innovations*. Springer.

Kulp, K., & Lorenz, K. (Eds.). (2003). *Handbook of Dough Fermentations* (p. 328). CRC Press.

Kurtzman, C. P. (1994). Molecular taxonomy of the yeasts. *Yeast*, *10*(13), 1727–1740.

Kurtzman, C. P., Fell, J. W., & Boekhout, T. (2011). Definition, classification and nomenclature of the yeasts. In C. P. Kurtzman, J. W. Fell, & T. Boekhout (Eds.), *The Yeasts* (pp. 3–5). Elsevier.

Landis, E. A., Oliverio, A. M., McKenney, E. A., Nichols, L. M., Kfoury, N., Biango-Daniels, M., Shell, L. K., Madden, A. A., Shapiro, L., & Sakunala, S. (2021). The diversity and function of sourdough starter microbiomes. *Elife*, *10*, 1–24.

Laranjo, M. (2021). *Fermentation: Processes, Benefits and Risks*. Intech Open.

Law, B. A. (2009). Enzymes in Dairy Product Manufacture. In R. J. Whitehurst & M. van Oort (Eds.), *Enzymes in Food Technology* (pp. 88–102). https://doi.org/10.1002/9781444309935.ch5

Lewis, M. J. (2015). *Beer and brewing*. Kirk-Othmer Encyclopedia of Chemical Technology; Wiley Online Library.

Lobedanz, S., Damhus, T., Borchert, T. V., Hansen, T. T., Lund, H., Lai, W., Lin, M., Leclerc, M., & Kirk, O. (2016). Enzymes in industrial biotechnology. In *Kirk-Othmer Encyclopedia of Chemical Technology* (pp. 1–73). Wiley Online Library.

López-Gómez, J. P., & Venus, J. (2021). Potential role of sequential solid-state and submerged-liquid fermentations in a circular bioeconomy. *Fermentation*, *7*(2), 1–76. https://doi.org/10.3390/fermentation7020076

Maat, J., Verrips, C. T., Ledeboer, A. M., & Edens, L. (1998). *DNA molecules comprising the genes for preprochymosin and its maturation forms, and microorganisms transformed thereby* (European Patent Office Patent 77,109).

McDonald, A. G., Boyce, S., & Tipton, K. F. (2008). ExplorEnz: The primary source of the IUBMB enzyme list. *Nucleic Acids Research*, *37*, D593–D597. https://doi.org/10.1093/nar/gkn582

McDonald, A. G., & Tipton, K. F. (2023). Enzyme nomenclature and classification: The state of the art. *The FEBS Journal*, *290*(9), 2214–2231.

Meyer, V. (2008). Genetic engineering of filamentous fungi—Progress, obstacles and future trends. *Biotechnology Advances*, *26*(2), 177–185.

Mienda, B. S., Idi, A., & Umar, A. (2011). Microbiological features of solid state fermentation and its applications-An overview. *Research in Biotechnology*, *2*(6), 21–26.

Mojsov, K. (2013). Use of enzymes in wine making: A review. *International Journal of Marketing and Technology (IJMT)*, *3*(9), 112–127.

Nagodawithana, T., Reed, G., & Taylor, S. (Eds.). (2013). *Enzymes in Food Processing* (3rd ed.). Elsevier Science. https://books.google.com/books?id=RabYBAAAQBAJ

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, *1*, 1806–1815.

Nguyen, H.-V., & Boekhout, T. (2017). Characterization of *Saccharomyces uvarum* (Beijerinck, 1898) and related hybrids: Assessment of molecular markers that predict the parent and hybrid genomes and a proposal to name yeast hybrids. *FEMS Yeast Research*, *17*(2), 1–18. https://doi.org/doi.org/10.1093/femsyr/foab052

NOP. (2011a). *Certification of organic yeast* (Guidance 5014; pp. 1–2). US Department of Agriculture, Agricultural Marketing Service, National Organic Program. https://www.ams.usda.gov/sites/default/files/media/5014.pdf

NOP. (2011b, April 15). *Policy Memo 11-13 Genetically modified organisms*. https://www.ams.usda.gov/sites/default/files/media/NOP-PM-11-13-GMOClarification.pdf

NOP. (2013, February 1). *Policy memo 13-1 Cell Fusion Techniques used in Seed Production*. https://www.ams.usda.gov/sites/default/files/media/NOP-PM-13-1-CellFusion.pdf

NOP. (2015). *Technical evaluation report handling/processing: Enzymes*. https://www.ams.usda.gov/sites/default/files/media/Enzymes%20Microbial%20TR%202015.pdf

NOP. (2016). *Marine Plants & Algae Technical Report*. https://www.ams.usda.gov/sites/default/files/media/Marine%20Plants%20and%20Algae%20TR.pdf

NOP. (2023a). *USDA Organic Integrity Database*. https://organic.ams.usda.gov/integrity/

NOP. (2023b, November 22). *Excluded Methods*. https://www.ams.usda.gov/rules-regulations/organic/petitioned-substances/excluded-methods

NOSB. (1995a). *Final minutes of the National Organic Standards Board full board meeting, Austin, TX, October 31-November 4, 1995*. USDA Agricultural Marketing Service. http://www.ams.usda.gov/sites/default/files/media/Thiram%20minutes%201995.pdf

NOSB. (1995b). *Technical Advisory Panel report, processing/handling: Enzymes*. https://www.ams.usda.gov/sites/default/files/media/Enzymes%20Microbial%20TR%201995.pdf

NOSB. (1996a). *Technical Advisory Panel report, processing/handling: Chymosin (microbial rennet)*. https://www.ams.usda.gov/sites/default/files/media/Chymosin%20TR.pdf

NOSB. (1996b). *Technical Advisory Panel report, processing/handling: Enzymes, Bacterial*. https://www.ams.usda.gov/sites/default/files/media/Enzymes%20Microbial%20TR%201995.pdf

NOSB. (1996c). *Technical Advisory Panel report, processing/handling: Enzymes, Plant and Fungal*. https://www.ams.usda.gov/sites/default/files/media/Microog%20Technical%20Advisory%20Panel%20Report%20%282003%29.pdf

NOSB. (1998). *Final Minutes of the National Organic Standards Board Full Board Meeting Ontario, CA March 16-20, 1998*. USDA National Organic Standards Board. https://www.ams.usda.gov/sites/default/files/media/NOSB%20Meeting%20Minutes%26Transcripts%201992-2009.pdf

NOSB. (2000). *Technical Advisory Panel report, processing/handling: Enzymes, Animal-derived*. https://www.ams.usda.gov/sites/default/files/media/Enzymes%20Animal%20TR%202000.pdf

NOSB. (2003). *Technical Advisory Panel report, processing/handling: Enzymes, Plant and Fungal*. https://www.ams.usda.gov/sites/default/files/media/Microog%20Technical%20Advisory%20Panel%20Report%20%282003%29.pdf

NOSB. (2007). *Final Minutes of the National Organic Standards Board Full Board Meeting Arlington, VA November 27-30, 2007* (pp. 10172–11614). USDA National Organic Standards Board. https://www.ams.usda.gov/sites/default/files/media/NOSB%20Meeting%20Minutes%26Transcripts%201992-2009.pdf

NOSB. (2016a, April 27). *Formal Recommendation: Ancillary Substances Procedure Proposal*. https://www.ams.usda.gov/sites/default/files/media/HS%20Ancillary%20Substance%20Proposal%20NOP.pdf

NOSB. (2016b, August 30). *Materials/GMO Subcommittee Excluded Methods Terminology – Third Discussion Document*. https://www.ams.usda.gov/sites/default/files/media/MSExcludedMethodsDiscussionDocumentNov2016.pdf

NOSB. (2016c, November 18). *Formal Recommendation Excluded Methods Terminology*. https://www.ams.usda.gov/sites/default/files/media/MSExcludedMethods.pdf

NOSB. (2019a). *Sunset 2021 meeting 2- Review handling substances §§205.605(a), 205.605(b), 205.606*. https://www.ams.usda.gov/sites/default/files/media/HS2021SunsetRvwFall2019.pdf

NOSB. (2019b, October 25). *Formal Recommendation Excluded Methods Determinations – Induced Mutagenesis and Embryo Transfer*. https://www.ams.usda.gov/sites/default/files/media/MSExcludedMethodsInducedMutagenesis.pdf

NOSB. (2022, April 28). *Formal Recommendation Excluded Methods Determinations*. https://www.ams.usda.gov/sites/default/files/media/MSExcludedMethodsFinalRecApril2022.pdf

Olempska-Beer, Z. S., Merker, R. I., Ditto, M. D., & DiNovi, M. J. (2006). Food-processing enzymes from recombinant microorganisms—A review. *Regulatory Toxicology and Pharmacology*, *45*(2), 144–158.

Ough, C. S., & Trioli, G. (1988). Urea removal from wine by an acid urease. *American Journal of Enology and Viticulture*, *39*(4), 303–307.

Palmer, T. (1995). *Understanding Enzymes* (4th ed.). Prentice-Hall.

Pariza, M. W., & Foster, E. M. (1983). Determining the safety of enzymes used in food processing. *Journal of Food Protection*, *46*(5), 453–468.

Pariza, M. W., & Johnson, E. A. (2001). Evaluating the safety of microbial enzyme preparations used in food processing: Update for a new century. *Regulatory Toxicology and Pharmacology*, *33*(2), 173–186.

Plavec, T. V., & Berlec, A. (2020). Safety aspects of genetically modified lactic acid bacteria. *Microorganisms*, *8*(2), 1–297.

Pyke, M. (1970). *Synthetic Food*. John Murray Publishers Ltd.

Ray, R. C., & Joshi, V. K. (2014). Fermented Foods: Past, Present and Future. In R. C. Ray & D. Montet (Eds.), *Microorganisms and Fermentation of Traditional Foods* (1st ed., pp. 1–36). CRC Press.

Ray, R. C., & Montet, D. (2014). *Microorganisms and Fermentation of Traditional Foods*. CRC Press.

Ray, S., Raychaudhuri, U., & Chakraborty, R. (2016). An overview of encapsulation of active compounds used in food products by drying technology. *Food Bioscience*, *13*, 76–83. https://doi.org/10.1016/j.fbio.2015.12.009

Riley, H. (1935). *Preserved and dried yeast* (US Patent Office Patent 1,991,629).

Scott, R., Robinson, R. K., & Wilbey, R. A. (1998). *Cheesemaking Practice*. Springer Science & Business Media.

Seibel, K., De Mesmaeker, M., & Weiland, F. (2023). CRISPR-Cas9 and its application potential in the brewing industry. *BrewingScience Yearbook*, 1–26.

Selle, K., & Barrangou, R. (2015). CRISPR-Based technologies and the future of food science. *Journal of Food Science*, *80*(11), R2367–R2372.

Semon, M. J., Forsyth, D., Edwards, C. G., & Dinn, C. (2001). Inducing malolactic fermentation in Chardonnay musts and wines using different strains of Oenococcus oeni. *Australian Journal of Grape and Wine Research*, *7*(1), 52–60.

Sewalt, V., Shanahan, D., Gregg, L., La Marta, J., & Carrillo, R. (2016). The generally recognized as safe (GRAS) process for industrial microbial enzymes. *Industrial Biotechnology*, *12*(5), 295–302.

Shaikh, S., Lee, E., Ahmad, K., Ahmad, S.-S., Chun, H., Lim, J., Lee, Y., & Choi, I. (2021). Cell types used for cultured meat production and the importance of myokines. *Foods*, *10*(10), 1–13.

Shurtleff, W., & Aoyagi, A. (1976). *The Book of Miso*. Autumn Press.

Snow, S. R., Kunkee, R. E., Hodges, R. A., & Williams, S. A. (1984). *Malolactic gene* (US Patent Office Patent 4,472,502).

Solieri, L., & Giudici, P. (2009). Vinegars of the World. In *Vinegars of the World* (pp. 1–16). Springer.

Srivastava, N. (2019). Production of food-processing enzymes from recombinant microorganisms. In M. Kuddus (Ed.), *Enzymes in Food Biotechnology* (pp. 739–767). Elsevier.

Stanbury, P. F., Whitaker, A., & Hall, S. J. (2013). *Principles of Fermentation Technology*. Elsevier.

Stear, C. A. (1995). *Handbook of Breadmaking Technology*. Springer Science & Business Media.

Steinkraus, K. (Ed.). (1983). *Handbook of Indigenous Fermented Foods*. Dekker.

Thomas, D. (2014). *Craft Maltsters' Handbook*. White Mule.

Thorrez, L., & Vandenburgh, H. (2019). Challenges in the quest for 'clean meat.' *Nature Biotechnology*, *37*(3), 215–216.

UC Davis. (2023). *Fermentation Management Guide*. University of California, Davis Department of Viticulture & Enology. https://wineserver.ucdavis.edu/industry-info/enology/fermentation-management-guides/wine-fermentation

Uhlig, H. (Ed.). (1998). *Industrial Enzymes and Their Applications*. Wiley.

US EPA. (1995). Yeast Production. In *AP 42, Fifth Edition, Volume I Chapter 9: Food and Agricultural Industries: Vol. I* (5th ed., pp. 1–7). US Enviromental Protection Agency Office of Air Quality Planning and Standards. https://www3.epa.gov/ttn/chief/ap42/ch09/final/c9s13-4.pdf

US FDA. (2010). *Guidance for Industry: Recommendations for Submission of Chemical and Technological Data for Food Additive Petitions and GRAS Notices for Enzyme Preparations* (pp. 1–11) [Guidance Document]. US Food and Drug Administration Center for Food Safety and Applied Nutrition. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-recommendations-submission-chemical-and-technological-data-food-additive-petitions

US FDA. (2017). *Regulatory framework for substances intended for use in human food or animal food on the basis of the Generally Recognized As Safe (GRAS) provision of the Federal Food, Drug, and Cosmetic Act: Guidance for industry* (pp. 1–28) [Guidance Document]. US Food and Drug Administration Center for Food Safety and Applied Nutrition. https://www.fda.gov/media/109117/download

US FDA. (2023a). *Generally Recognized as Safe (GRAS)*. https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras

US FDA. (2023b). *GRAS Notices Inventory*. https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances

US FDA. (2023c). *Microorganisms & Microbial-Derived Ingredients Used in Food (Partial List)*. https://www.fda.gov/food/generally-recognized-safe-gras/microorganisms-microbial-derived-ingredients-used-food-partial-list

US FDA. (2023d). *Substances Added to Food* (formerly EAFUS). https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances

USDA / AMS / NOSB. (1996). *Final Minutes of the National Organic Standards Board Full Board Meeting Indianapolis, IN September 18-20, 1996*. USDA National Organic Standards Board. https://www.ams.usda.gov/sites/default/files/media/NOSB%20Meeting%20Minutes%26Transcripts%201992-2009.pdf

Van den Berg, J. A., Van Ooyen, A. J., & Rietveld, K. (1989). *Kluyveromyces as a host strain.* (European Patent Office Patent EP0301670A1).

Van den Berg, J. A., Van Ooyen, A. J., & Rietveld, K. (1990). *Kluyveromyces as a host strain.* (US Patent Office Patent 4,943,529).

van Oort, M. (2010). Enzymes in Bread Making. In *Enzymes in Food Technology* (pp. 103–143). Wiley-Blackwell. https://doi.org/10.1002/9781444309935.ch6

Vaughan, P., & Macreadie, I. G. (2000). *Yeasts*. Kirk-Othmer Encyclopedia of Chemical Technology; Wiley Online Library.

VH Berlin. (2023). *History*. Research Institute for Baker's Yeast (*German: Versuchsanstalt Der Hefeindustrie e.V.*). https://www.vh-berlin.org/history/

Viniegra-Gonzalez, G. (1997). Solid State Fermentation: Definition, Characteristics, Limitations and Monitoring. In S. Roussos, B. Lonsane, M. Raimbault, & G. Viniegra-Gonzalez (Eds.), *Advances in solid state fermentation* (pp. 5–22). Springer Science & Business Media.

Webb, A. D. (2000). *Vinegar*. Kirk-Othmer Encyclopedia of Chemical Technology; Wiley Online Library.

Wei, S., Chelliah, R., Rubab, M., Oh, D.-H., Uddin, M. J., & Ahn, J. (2019). Bacteriophages as potential tools for detection and control of Salmonella spp. In food systems. *Microorganisms*, *7*(11), 1–570.

Williams, S. A., Hodges, R. A., Strike, T. L., Snow, R., & Kunkee, R. E. (1984). Cloning the gene for the malolactic fermentation of wine from Lactobacillus delbrueckii in Escherichia coli and yeasts. *Applied and Environmental Microbiology*, *47*(2), 288–293.

Wright, T. H. (2007). *Petition of Non-organically Produced Agricultural Products for Inclusion of Chlorella powder on the §205.606 National List* (pp. 1–8). Synergy Company.

Wyard, G. (2015). *Handling Subcommittee – Ancillary Substances Permitted in Microorganisms* (pp. 1–7). Organic Trade Association. https://ota.com/sites/default/files/indexed_files/OTA_MicroorganismsAncillary_AMS-NOP-15-0002.pdf

Yafetto, L. (2022). Application of solid-state fermentation by microbial biotechnology for bioprocessing of agro-industrial wastes from 1970 to 2020: A review and bibliometric analysis. *Heliyon*, *8*, 1–17.

Yamashita, H. (2021). Koji starter and Koji world in Japan. *Journal of Fungi*, *7*(7), 1–18.

Żymańczyk-Duda, E., Brzezińska-Rodak, M., Klimek-Ochab, M., Duda, M., & Zerka, A. (2017). Yeast as a versatile tool in biotechnology. *Yeast Ind Appl*, *1*, 3–40.

Appendix

Table 4: Selected list of enzyme, microorganism, and yeast manufacturers and examples of their brand names.

Company	Brand Name(s)
Associated British Foods, PLD	Corolase®, Fleischmann's Yeast, VERON®
Advanced Enzyme Technologies Ltd.	SEBMash, ClariSEB
Ajinomoto	Activa®
Amano Enzyme, Inc.	Amano, Thermoase
BASF	Nutrilife®
DSM Food Specialties	Accelerzyme®, Bakezyme, Cakezyme, Maxapal, MaxiBright™, Maxilact®, Meltamase™, PreventASem™
DuPont Nutrition & Biosciences	Chymostar, Danisco, Dyadics, FoodPro™, Genencor, GRINDAMYL™
Hayashibara International	DENABAKE™, DENAZYME™, Nagase
Kerry	Amylo™, Biobake™, Bioglucanase™, Biogox™, Biolactase™, Biolipase™, Profix™, Promalt™
Lallemand	Essential®
LeSaffre / ADM	Red Star YeastSaf Pro® Star-Zyme™
Novozymes / Chr. Hansen	Acrylaway®, Branchzyme®, CHY-MAX®, Glucanex®, Gluzyme®, Lipopan, Novozym®, Novozymes®, Ultraflo®
Shin Nihon Chemical Co., Ltd.	Sumizyme™, Takabio

Sources: (ETA, 2023; US FDA, 2023b)

Table 5: Enzyme identity and food applications. Enzyme names given are trivial and may represent groups of enzymes with multiple CAS and EC numbers. Where multiple enzyme molecular structures from different sources are reported, the EC number ends in the letter "X" to show that various structures are assigned different numbers based on Enzyme Commission criteria. Enzymes with CAS and EC numbers that refer to specific enzymes that had evidence they were produced by excluded methods are included where available and noted at the bottom of the table. The table was compiled from the GRAS Notices Inventory (U.S. FDA, 2023b) and Substances Used in Food (U.S. FDA, 2023d).

Common (Trivial) Name	Uses and Applications	CAS Number	EC Number
Acetolactate decarboxylase	Processing aid in the production of alcoholic malt beverages and distilled liquors.	9025-02-9	4.1.1.5
Prolyl oligopeptidase also known as prolyl endopeptidase	Used to degrade gluten and cereal protein, prevent chill hazing, and decrease foam production in brewing beer and other fermented beverages.	72162-84-6	3.4.21.26
Aminopeptidase	Flavor development in specific cheeses.	9031-94-1	3.4.11.22
Amylase	Liquefaction of starch in the production of syrups and thinning of starch in distilling mashes; brewing and baking; starches, cereals, and other cereal-based beverages.	9000-90-2	3.2.1.1 and others
Amyloglucosidase	Degrading gelatinized starch into constituent sugars.	9032-08-0	3.2.1.3
Amylomaltase	Starch treatment used in dairy, cheese analogues, bakery/cake mixes, emulsified low fat spreads, confectionary, and dressings or emulsified sauces.	9032-09-01	2.4.1.25
Arabinase	Fruit and vegetable-based purees, pastas, and juices, and in winemaking.	75432-96-1	3.2.1.99
Asparaginase	To reduce the levels of free L-asparagine, a precursor in the formation of acrylamide in grain-based, potato-based, products.	9015-68-3	3.5.1.1
Carboxypeptidase	Used in cheese production to accelerate ripening and as a debittering aid, and in fermented meat to accelerate the development of flavor during the ripening process.	9077-67-2	3.4.16.4
Catalase	Use in foods in general as an enzyme in accordance with current good manufacturing practices.	9001-05-2	1.11.1.6

Common (Trivial) Name	Uses and Applications	CAS Number	EC Number
Cellulase (general)	Used as an enzyme in brewing, processing of other cereal-based beverages, fruits and vegetables, starch and grain, and baked goods, increased starch recovery from potatoes and other starch sources, tenderizing fruits and vegetables prior to cooking, essential oil and flavor extraction, treatment of distillers mash, reduce wort viscosity and haze formation in beer production.	9012-54-8 and others	3.2.1.4 and others
Chymosin	For use as a processing aid in cheese production.	9001-98-3[ii] 977165-51-7[iii] 977165-50-6[iv] 977156-61-8[v]	3.4.23.4
Esterase lipase	Flavor enhancer in cheeses, fats and oils, and milk products.	9001-62-1	3.1.1.3
Galactosidase	For use as a processing aid in the production of galacto-oligosaccharides (GOS) and production of sucrose from sugar beets.	90025-35-8	3.2.1.22
1,4-α-Glucan 6-α-glucosyltransferase	For use in brewing, grain processing aid, high-fructose corn syrup, alcoholic beverages.	9030-12-0	2.4.1.24
Glucoamylase	Use in foods in general as an enzyme in accordance with current good manufacturing practices.	9032-08-0	3.2.1.3
Glucose isomerase	Production of high-fructose corn syrup.	9055-00-9	5.3.1.5
Glucose oxidase	Used in baking processes to strengthen the protein complexes and stabilize starch-based products. Also used in cheese, beer, carbonated beverages, and fruit juices.	9001-37-0	1.1.3.4
Glutaminase	Used to deamidate vegetable, milk, egg, and yeast proteins in baked goods, dairy foods, and egg-based foods.	9001-47-2 and others	3.5.1.X
Glycerophospholipid cholesterol acyltransferase (GCAT)	Used to modify phospholipids to lyso-phospholipids and cholesterol-ester in egg yolk to avoiding product separation in pasteurized mayonnaise production; to emulsify processed meat products; dairy products and baked goods.	9031-14-5	2.3.1.43
Glycosyltransferase	Used to obtain dextrins from starch with improved physical properties, such as higher solubility, lower viscosity, and reduced retrogradation.	9001-97-2	2.4.1.18
Invertase	Used in foods in general as an enzyme in accordance with current good manufacturing practices.	9025-57-4 and others	3.2.1.26 and others
Isoamylase	Hydrolyzes the 1,6-Î±-D-glucosidic branch linkages in glycogen, amylopectin, and their Î±-limit dextrins.		3.2.1.8
Laccase	Used in breath mints and chewing gum. Facilitates reactions of naturally occurring polyphenolic compounds in food and food extracts that interact with odor-causing compounds located in the mouth.	9015-68-3	3.5.1.1
Lactase	Processing aid in milk and whey products to hydrolyze lactose. Treated products are used in a variety of food products for lactose-intolerant people.	9031-11-2	3.2.1.23
Lipase	Edible fats and oils, dairy based flavoring preparations, cheeses, liquid and dried egg white, bread, flour, bakery products, hydrolyzed lecithin, and modified egg yolk. Fat-splitting oils into mono-, di-, and tri-glycerides.	9001-62-1	3.1.1.3

[ii] From bovine sources
[iii] From recombinant *Aspergillus niger* var. Awamori
[iv] From recombinant *Escherichia coli* K-12
[v] From recombinant *Kluyveromyces marxianus* var. Lactis

Common (Trivial) Name	Uses and Applications	CAS Number	EC Number
Lysozyme	Used as an ingredient in functional foods and beverages and medical foods, ingredients produced by microbial fermentation, such as xanthan gum, gellan gum, and yeast extracts, to assist in the removal of cellular debris.	9001-63-2	3.1.1.17
Mannanase (manna endo-1,4-ß-mannosidase)	Used in fruit and vegetable processing, oil processing, and coffee production.	37288-54-3	3.2.1.78
Pectin esterase	Used in the processing of fruits, vegetables, coffee, wine, and flavoring.	90025-98-3	3.1.1.11
Pectin lyase	Reduce viscosity in the processing of fruits and vegetables.	9033-35-6	4.2.2.10
Pectinases (Usually a mixture of different specific enzymes)	Used in fruit and vegetable processing.	9032-75-1 and others	3.2.1.15 and others
Peroxidases	Used in cheese-whey, soy milk, and cream.	9003-99-0	1.11.1.7
Phosphodiesterase	Used in the production of yeast extracts or yeast autolysates for soups, sauces, snacks, processed cheese, dressings, spreads, flavors, and seasonings.	9025-82-5	3.1.4.1
Phospholipases	Used as a processing aid in edible oil refining, degumming oils; cheese, yogurt, and other dairy products; mayonnaise and other egg products.	9001-84-7	3.1.1.4
Polygalacturonase	Used in fruit and vegetable processing, wine production; coffee production; and in grain processing.	9032-75-1	3.2.1.15
Protease (general)	Used to hydrolyze proteins in a wide variety of food and beverage products.	9014-01-1 and others	3.4.x.x
Proteases (Acid fungal)	Use in grain processing (corn steeping), manufacturing of alcoholic beverages, manufacturing of non-citrus juice (i.e., apple juice), and degumming of membranes during orange juice manufacturing.	9025-49-4	3.4.23.18
Proteases (Milk-clotting)	Used to coagulate milk to make cheeses and other dairy products.	977183-89-3[vi] 977017-74-5[vii] 977017-73-4[viii] 977017-76-7[ix] 977017-75-6[x]	3.4.23.22[c] 3.4.23.23[de]
Pullulanase	Used in the saccharification of liquified starch in the production of dextrose and maltose syrups used in bakery products and alcoholic beverages.	9075-68-7	3.2.1.41
Sterol esterase	Used as a processing aid for partial or extensive hydrolysis of lipids from plant sources and in bread making.	9026-00-0	3.1.1.13
Thermolysin	Used as a processing aid in the production of yeast extract, cooked fish, egg white hydrolysates, enzyme-modified dairy ingredients, and protein hydrolysates (soy, wheat, gluten, milk protein, fish) to improve the protein solubility, taste, and digestibility.	9073-78-3	3.4.24.27
Thermomycolin (Serine endopeptidase)	Used in the processing of partially or extensively hydrolyzed proteins from both animal and vegetable sources.	52233-31-5	3.4.21.65
Transglucosidase	Used in the production of isomalto-oliogsaccharide syrups from starch and potable ethanol from molasses.	9032-09-1	2.4.1.25

[vi] From recombinant *Aspergillus oryzae*
[vii] From *Bacillus cereus*
[viii] From *Endothia parasitica*
[ix] From *Mucor meihei*
[x] From *Mucor pusillus*

Common (Trivial) Name	Uses and Applications	CAS Number	EC Number
Transglutaminase	Used in meat products, fish products, dairy products, vegetable protein and soybean products, baked goods (including pastries) and bread products, pasta and noodles, grain mixtures, and ready-to-eat cereals.	80146-85-6	2.3.2.13
Triacylglycerol lipase	For use in cocoa butter substitutes, baked products and other cereal based processed.	9001-62-1	3.1.1.3
Xylanase	Used in bakery products, brewing, and potable alcohol.	9025-57-4 and others	3.2.1.X

Sources: (IUBMB, 2023; Pariza & Johnson, 2001; US FDA, 2023d, 2023b)

Table 6: A selection of enzyme sources and statuses of the source or production organisms, the donor organisms of the genetic material used to modify the production organism, the method of genetic modification, and whether the notification contains evidence that the method used is excluded under that USDA organic regulations, or there is no evidence. Items may also be identified as "To Be Determined" based on the NOSB's recommendations. Data obtained from the regulatory text of 21 CFR 173 and 21 CFR 184, the FDA's database of Substances Added to Food (U.S. FDA, 2023d), enzymes identified as having no questions by FDA in the FDA's GRAS Notices Inventory (U.S. FDA, 2023b) and a key reference to the GRAS process applied to enzymes (Pariza & Johnson, 2001).

GRN #. or 21 CFR §	Production / Recipient Organism	Donor Organism	Enzyme (Common or Trivial Name)	Method	Excluded?[xi]
§184.1372	*Actinoplane missouriensis*	None specified	Glucose isomerase	Undisclosed	N
GRN0088	*Aspergillus niger*	None specified	Pectinase	Undisclosed	N
GRN0088	*Aspergillus niger*	None specified	Catalase	Undisclosed	N
GRN0832	*Aspergillus niger*	*Aspergillus niger*	Acid prolyl endopeptidase	rDNA	X
GRN0214	*Aspergillus niger*	*Aspergillus niger*	Asparaginase	Classical mutagenesis, gene deletion, gene insertion	X
GRN0428	*Aspergillus niger*	*Aspergillus niger*	Asparaginase	rDNA	X
GRN0088	*Aspergillus niger*	*Aspergillus niger*	Carbohydrase	Not disclosed	N
GRN0089	*Aspergillus niger*	*Aspergillus niger*	Carbohydrase	Not disclosed	N
GRN0345	*Aspergillus niger*	*Aspergillus niger*	Carboxypeptidase	Classical mutagenesis, gene deletion, gene insertion	X
GRN0089	*Aspergillus niger*	*Aspergillus niger*	Catalase	Undisclosed	N
§173.120	*Aspergillus niger*	*None*	Cellulase	Undisclosed	N
GRN0088	*Aspergillus niger*	*Aspergillus niger*	Glucose oxidase	Undisclosed	N
GRN0089	*Aspergillus niger*	*Aspergillus niger*	Glucose oxidase	Undisclosed	N
GRN0132	*Aspergillus niger*	None	Lactase	Undisclosed	N
GRN0111	*Aspergillus niger*	None	Lipase	Naturally occurring strains	N
GRN0158	*Aspergillus niger*	*Candida antartica*	Lipase	rDNA	X
GRN0296	*Aspergillus niger*		Lipase	Classical mutagenesis, gene deletion, gene insertion	X
GRN0964	*Aspergillus niger*	*Trichoderma reesei*	Lysophospho-lipase	rDNA	X
GRN0089	*Aspergillus niger*	*Aspergillus niger*	Pectinase	Undisclosed	N
GRN0402	*Aspergillus niger*		Peroxidase	rDNA	X
GRN0402	*Aspergillus niger*		Peroxidase	rDNA	X
GRN0183	*Aspergillus niger*	*Sus scrofa (pig)*	Phospholipase	rDNA	X

[xi] N=No evidence of excluded methods; T=To Be Determined; X=Evidence of excluded methods.

GRN #. or 21 CFR §	Production / Recipient Organism	Donor Organism	Enzyme (Common or Trivial Name)	Method	Excluded?[xi]
GRN0857	*Aspergillus niger*	*Aspergillus niger*	Phospholipase A1	Gene multiplication	X
GRN0088	*Aspergillus niger*	*Aspergillus niger*	Protease	Undisclosed	N
GRN0089	*Aspergillus niger*	*Aspergillus niger*	Protease	Undisclosed	N
§173.120	*Aspergillus niger*	*None*	Carbohydrase	Undisclosed	N
GRN1030	*Aspergillus niger*	*Trichoderma reesei*	Cellulase	rDNA	X
§184.1685	*Aspergillus niger var. awamori*	*Bos taurus*	Chymosin	rDNA	X
GRN0653	*Aspergillus nishimurae*	*Trichoderma reesei.*	Lysophospho-lipase	rDNA	X
GRN0201	*Aspergillus oryzae*	*Aspergillus oryzae*	Asparaginase	rDNA	X
GRN0088	*Aspergillus oryzae*	None disclosed	Carbohydrase	Not disclosed	N
GRN0090	*Aspergillus oryzae*	None disclosed	Carbohydrase	Not disclosed	N
GRN0010	*Aspergillus oryzae*	*Aspergillus sojae and others*	Exopeptidase	rDNA	X
GRN0106	*Aspergillus oryzae*	None	Glucose oxidase	rDNA	X
GRN0122	*Aspergillus oryzae*	*Myceliophthora thermophila*	Laccase	rDNA	X
GRN0113	*Aspergillus oryzae*	None	Lipase	Naturally occurring strains	N
GRN0043	*Aspergillus oryzae*	*Thermomyces lanuginosus*	Lipase	rDNA	X
GRN0103	*Aspergillus oryzae*	*Thermomyces lanuginosus & Fusarium oxysporum*	Lipase	rDNA	X
GRN0142	*Aspergillus oryzae*	*Fusarium venenatum*	Phospholipase	rDNA	X
GRN0088	*Aspergillus oryzae*	None disclosed	Protease	Not disclosed	N
GRN0090	*Aspergillus oryzae*	None disclosed	Protease	Not disclosed	N
§173.150	*Aspergillus oryzae*	*Rhizomucor miehei*	Proteases (Milk-clotting)	rDNA	X
GRN0510	*Aspergillus oryzae*	*A, niger*	Acid lactase	rDNA	X
GRN0979	*Aspergillus oryzae*	*A. tubingensis*	Pectin esterase	rDNA	X
GRN0982	*Aspergillus oryzae*	*A. tubingensis*	Polygalacturonase	rDNA	X
GRN0965	*Aspergillus tubingensis*	*Aspergillus tubingensis*	Arabinase	Non-genetically modified	N
GRN0558	*Aspergillus tubingensis*	*Trichoderma reesei*	Pectin esterase	rDNA	X
GRN0557	*Aspergillus tubingensis*	*Trichoderma reesei*	Polygalacturonase	rDNA	X
GRN0507	*Bacillus amyloliquefaciens*	*Thermus thermophiles*	Amylomaltase	Rdna	X
§173.150	*Bacillus cereus*	*None*	Proteases (Milk-clotting)	Not identified as genetically modified	N
GRN0649	*Bacillus circulans*	*Bacillus subtilis*	Galactosidase	rDNA	X
§184.1372	*Bacillus coagulans*	None	Glucose isomerase	Undisclosed	N
GRN0861	*Bacillus deramificans*	*Bacillus subtilis*	Pullulanase	rDNA	X
GRN0079	*Bacillus licheniformis*	*Bacillus licheniformis*	Amylase	Homologous rDNA	X
GRN0975	*Bacillus licheniformis*	*Geobacillus stearothermophilus*	Amylase (maltogenic)	rDNA	X
GRN0645	*Bacillus licheniformis*	*Bacillus deramificans & Bacillus acidopullulyticus*	Pullulanase	rDNA	X
§184.1027	*Bacillus licheniformis*	None	Carbohydrase	Undisclosed	N

GRN #. or 21 CFR §	Production / Recipient Organism	Donor Organism	Enzyme (Common or Trivial Name)	Method	Excluded?[xi]
GRN0277	*Bacillus licheniformis*	*Pseudomonas stutzeri*	Maltotetrao-hydrolase	rDNA	X
§184.1027	*Bacillus licheniformis*	None	Protease	Undisclosed	N
GRN0564	*Bacillus licheniformis*	*Nocardiopsis prasina*	Protease	rDNA	X
GRN0265	*Bacillus licheniformis*	*Aeromonas salmonicida subsp. salmonicida*	Glycerophospholipid cholesterol acyltransferase (GCAT)	rDNA	X
GRN0472	*Bacillus licheniformis*	*B. licheniformis*	Xylanase	rDNA	X
GRN1055	*Bacillus licheniformis*	*Chryseobacterium*	Xylanase	rDNA	X
GRN0361	*Bacillus stearothermophilus*	None	Glucan	Non-genetically modified	N
§184.1012	*Bacillus stearothermophilus*	None	Amylase	Undisclosed	N
§173.115	*Bacillus subtilis*	*Bacillus brevis*	Acetolactate decarboxylase	rDNA	X
GRN0974	*Bacillus subtilis*	*Bacillus subtilis*	Amylase (maltogenic)	rDNA	X
GRN0476	*Bacillus subtilis*	*Bacillus subtilis*	Asparaginase	Homologous rDNA	X
GRN0476	*Bacillus subtilis*	*Bacillus subtilis*	Asparaginase	Homologous rDNA	X
GRN0861	*Bacillus subtilis*	*Bacillus deramificans*	Pullulanase	rDNA	X
GRN0114	*Bacillus subtilis*	None	Pectate lyase	Mutagenesis with NTG (methylation)	T
GRN0205	*Bacillus subtilis*	*B. acidopullulyticus*	Pullulanase	rDNA	X
GRN0020	*Bacillus subtilis*	*Bacillus naganoensis*	Pullulanase	rDNA	X
GRN1011	*Bacillus subtilis*	*Thermoactinomyces vulgaris*	Amylase	rDNA	X
GRN0274	*Bacillus subtilis*	*Rhodothermus obamensis*	Glycosyl-transferase	Genetically modified microorganism	X
GRN0406	*Bacillus subtilis (strain 168)*	*Aquifex aeolicus (strain VF5)*	Glucan	rDNA	X
GRN0801	*Camelus dromedarius*	*Aspergillus niger*	Chymosin	rDNA	X
§184.1387	*Candida pseudotropicalis*	None	Lactase	Not disclosed	N
GRN0081	*Candida rugosa*	None	Lipase	Selected strain not subjected to rDNA	N
GRN0267	*Chryseobacterium proteolyticum*	*Chryseobacterium proteolyticum*	Protein glutaminase	Not subjected to rDNA techniques	N
GRN0482	*Disporotrichum dimorphosporum*	None	Beta-glucanase	Isolation and culturing of a wild-type strain	N
GRN0482	*Disporotrichum dimorphosporum*	None	Xylanase	Isolation and culturing of a wild-type strain	N

GRN #. or 21 CFR §	Production / Recipient Organism	Donor Organism	Enzyme (Common or Trivial Name)	Method	Excluded?[xi]
§173.150	*Endothia parasitica*	None	Proteases (Milk-clotting)	Not identified as genetically modified	N
GRN0485	*Escherichia coli BL21(DE3)*	*Bifidobacterium bibidum*	Beta-galactosidase	rDNA	X
§184.1685	*Escherichia coli K-12*	*Bos taurus*	Chymosin	rDNA	X
GRN0631	*Fusarium oxysporum*	*Trichoderma reesei*	Triacylglycerol lipase	rDNA	X
GRN0563	*Fusarium venenatum*	*Fusarium oxysporum*	Protease	rDNA	X
GRN0598	*Geobacillus stearothermophilus*	Undisclosed	Thermolysin	Not stated in the GRN	T
GRN0746	*Geobacillus stearothermophilus*	*Bacillus subtilis*	Amylase	rDNA	X
GRN0405	*Geobacillus stearothermophilus (strain TRBE14)*	*Geobacillus stearothermophilus (strain TRBE14)*	Glucan	Not genetically modified	N
GRN0238	*Hansenula polymorpha*	*Fusarium heterosporum*	Lipase	rDNA	X
GRN0195	*Humicola insolens*	None	Glucanase	Not genetically modified	N
GRN0195	*Humicola insolens*	None	Xylanase	Not genetically modified	N
§184.1388	*Kluyveromyces lactis*	None	Lactase	Not disclosed	N
GRN0088	*Kluyveromyces marxianus*	*Kluyveromyces marxianus*	Lactase	Not disclosed	N
§184.1685	*Kluyveromyces marxianus var. lacti*	*Bos taurus*	Chymosin	rDNA	X
§184.1985	*Lactococcus lactis*	None	Amino-peptidase	Not disclosed	N
GRN0505	*Leptographium procerum*	None	Phosphodiesterase	Classical mutation and selection	N
GRN0817	*Malbranchea cinnamomea*	*Trichoderma reesei*	Serine endopeptidase	rDNA	X
§173.135	*Micrococcus lysodeikticus*	*None*	Catalase	Prior sanctioned	N
§173.145	*Morteirella vinaceae var. raffinoseutilizer*	*None*	Galactosidase	Prior sanctioned	N
§173.140	*Mucor miehei*	*None*	Esterase-lipase	Prior sanctioned	N
§173.150	*Mucor miehei*	*None*	Proteases (Milk-clotting)	Not identified as genetically modified	N
§173.150	*Mucor pusillus*	*None*	Proteases (Milk-clotting)	Not identified as genetically modified	N
GRN0292	*Myceliophthora thermophila*	*Myceliophthora thermophila*	Cellulase	Genetically modified microorganism	X
GRN0743	*Papiliotrema terrestris*	*Papiliotrema terrestris*	Galactosidase	Mutagenesis with NTG (methylation)	T
GRN0908	*Penicillium camemberti*	*Penicillium camemberti*	Lipase	Not genetically modified	N
GRN0068	*Penicillium camembertii*	*None*	Lipase	Undisclosed	N
GRN0509	*Penicillium chrysogenum*	*Penicillium chrysogenum*	Glucose oxidase	Non-genetically modified	N
GRN0707	*Penicillium spp.*	*Trichoderma reesei*	Glucose oxidase	rDNA	X

GRN #. or 21 CFR §	Production / Recipient Organism	Donor Organism	Enzyme (Common or Trivial Name)	Method	Excluded?[xi]
GRN1025	*Pichia pastoris*	*Saccharomyces cerevisiae* expressing a gene from *Sus scrofa*	Pepsin A	rDNA	X
GRN0204	*Pichia pastoris*	*Phospholipase C*	Phospholipase C	rDNA	X
GRN0085	*Pseudomonas amyloderamosa*	None—derived by classical mutation	Isoamylase	Classic NTG (methyl) mutation	T
GRN0462	*Pseudomonas fluorescens Biovar I*	*Pseudomonas fluorescens Biovar I*	Lipase	rDNA	X
GRN0126	*Pseudomonas fluorescens Biovar I*	*Three microorganisms within the order Thermococcales*	α-Amylase	rDNA	X
§184.1420	*Rhizopus niveus*	None	Lipase	Undisclosed	N
§173.110	*Rhizopus niveus*	None	Amylo-glucosidase	Prior sanctioned	N
GRN0088	*Rhizopus oryzae*	*Rhizopus oryzae*	Carbohydrase	Not disclosed	N
GRN0216	*Rhizopus oryzae*	None	Lipase	Not subjected to rDNA techniques	N
§173.130	*Rhizopus oryzae*	None	Carbohydrase	Prior sanctioned	N
GRN0708	*Rhizopus oryzae*	*Aspergillus niger*	Triacylglycerol lipase	rDNA	X
GRN0783	*Rhizopus oryzae*	*Aspergillus niger*	Triacylglycerol lipase	rDNA	X
GRN0090	*Rhizopus orzae*	*Rhizopus orzae*	Carbohydrase	Not disclosed	N
GRN0842	*Saccharomyces cerevisiae*	*Geobacillus stearothermophilus*	Amylase (maltogenic)	rDNA	X
GRN0088	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*	Invertase	Undisclosed	N
GRN0120	*Saccharomyces cerevisiae strain ML01*	*Oenococcus oeni and Schizosaccharomyces pombe*	Malate permease	rDNA	X
GRN1021	*Streptomyces mobaraensis*	*Streptomyces mobaraensis*	Trans-glutaminase	rDNA	X
§184.1372	*Streptomyces olivaceus*	Prior sanctioned	Glucose isomerase	Undisclosed	N
§184.1372	*Streptomyces olivochromogenes*	Prior sanctioned	Glucose isomerase	Undisclosed	N
§184.1372	*Streptomyces rubiginosus*	Prior sanctioned	Glucose isomerase	Undisclosed	N
GRN145	*Streptomyces violaceoruber*	None	Phospholipase	Not genetically modified	N
GRN0212	*Streptomyces violaceruber*	*S. violaceruber and S. cinnamoneum*	Phospholipase	rDNA	X
GRN0479	*Talaromyces emersonii*	*None*	Beta-glucanase	Classical mutagenesis	T
GRN0479	*Talaromyces emersonii*	*None*	Cellulase	Classical mutagenesis	T
GRN0739	*Tayloromyce leycettanus*	*Aspergillus niger*	Mannanase	rDNA	X
GRN0149	*Trichoderma harzianum*	None	Beta-glucanase	Not genetically modified	N
GRN0891	*Trichoderma reesei*	*Aspergillus fumigatus*	Cellulase	rDNA	X
GRN0756	*Trichoderma reesei*	*Trichoderma reesei*	Glucanase	Homologous rDNA	X
GRN0853	*Trichoderma reesei*	*Acremonium alcalophilum*	Lysozyme	rDNA	X
GRN0566	*Trichoderma reesei*	*Trichoderma reesei*	Mannanase	Homologous rDNA	X

GRN #. or 21 CFR §	Production / Recipient Organism	Donor Organism	Enzyme (Common or Trivial Name)	Method	Excluded?[xi]
GRN0032	*Trichoderma reesei*	*Aspergillus niger*	Pectin lyase	rDNA	X
GRN0490	*Trichoderma reesei*	*A. nishimurae*	Phospholipase	rDNA	X
GRN0675	*Trichoderma reesei*	*Talaromyces leycettanus*	Xylanase	rDNA	X
GRN0628	*Trichoderma reesei*	*Trichoderma reesei*	Xylanase	rDNA	X
GRN0230	*Trichoderma reesei*	*Prochymosin B (bovine)*	Chymosin	rDNA	X
GRN0372	*Trichoderma reesei*	*T. reesei (glucoamylase enzyme preparation)*	Glucoamylase	rDNA	X
GRN0524	*Trichoderma reesei*	*Aspergillus nishimurae*	Phospholipase	rDNA	X
GRN0333	*Trichoderma reesei*	*Trichoderma reesei*	Protease (Acid fungal)	rDNA	X
GRN0981	*Trichoderma reesei*	*Melanocarpus albomyces*	Sterol esterase	rDNA	X
GRN0315	*Trichoderma reesei*	*Aspergillus niger*	Transglucosidase	rDNA	X
GRN0940	*Yarrowia lipolytica*	*Sus scrofa*	Phospholipase	rDNA	X

Source: (US FDA, 2023b)

<u>Table 7: Selected microorganisms used in food and fiber processing</u>

Table 7 contains a list of bacteria, fungi, viruses, and microalgae that have been affirmed as GRAS by the FDA as of October 18, 2023, and have evidence in the GRAS notification of being produced by methods excluded by the NOP in the opinion of the reviewers. It is not intended to be an exhaustive list of such organisms. The list includes only those where FDA was notified. It does not include microorganisms where the FDA did not find a sufficient basis for the organism to be GRAS or those that are still pending. It also does not contain viruses and microalgae produced using excluded methods.

Table 7: Selected microorganisms used in food and fiber processing

Microorganism	Type[xii]	Uses / Applications
Acetobacter aceti	B	Vinegar
Acetobacter fabarum	B	Chocolate, coffee
Acetobacter lovaniensis	B	Pickling vegetables
Acetobacter malorum	B	Vinegar (apple cider)
Acetobacter orientalis	B	Pickling vegetables
Acetobacter pasteurianus	B	Chocolate, vinegar
Acetobacter pomorum	B	Vinegar (apple cider)
Acetobacter syzygii	B	Chocolate, vinegar
Acetobacter tropicalis	B	Chocolate, coffee
Arthrobacter arilaitensis	B	Cheese
Arthrobacter bergerei	B	Cheese
Arthrobacter globiformis	B	Cheese
Arthrobacter ilicis	B	Cheese
Arthrobacter protophrmiae	B	Cheese
Arthrospira platensis	A	Juices, milk, and other beverages; dairy, grain and plant protein products; processed fruits and vegetables; snack foods, soft candy, and soups
Aspergillus acidus	F	Tea
Aspergillus niger	F	Dairy products, liquor, citric acid, enzymes
Aspergillus fumigatus	F	chocolate
Aspergillus oryzae	F	Rice fermentation, koji, miso, soy sauces
Aspergillus sojae	F	Soy miso, soy sauces, koji
Bacillus cereus	B	Chocolate

[xii] A=Algae, B=Bacteria, F=Non-yeast fungi, V=Virus, Y=Yeast Fungi

Microorganism	Type[xii]	Uses / Applications
Bacillus coagulans	B	Chocolate
Bacillus licheniformis	B	chocolate
Bacillus stearothermophilus	B	chocolate
Bacillus subtilis	B	soy natto
Bacterial monophages	V	Fruits, vegetables.
Bifidobacterium adolescentis	B	Dairy products
Bifidobacterium animalis	B	Dairy products
Bifidobacterium bifidum	B	Dairy products
Bifidobacterium breve	B	Dairy and soy products
Bifidobacterium infantis	B	Dairy products
Bifidobacterium lactis	B	Dairy products
Bifidobacterium longum	B	Dairy products
Bifidobacterium pseudolongum	B	Dairy products
Bifidobacterium thermophilum	B	Dairy products
Brachybacterium alimentarium	B	Dairy products
Brachybacterium tyrofermentans	B	Dairy products
Brevibacterium antiquum	B	Dairy products
Brevibacterium aurantiacum	B	Cheese
Brevibacterium casei	B	Cheese
Brevibacterium linens	B	Cheese
Candida colliculosa	Y	Dairy, cheese and kefir
Candida krusei	Y	Wine
Candida milleri	Y	Sourdough bread
Candida mogii	Y	Soy products
Candida rugosa	Y	Dairy
Candida tropicalis	Y	Vegetables, chocolate
Candida valida(10)	Y	Sourdough bread
Candida vini(10)	Y	Wine and cheese
Candida zeylanoides	Y	Dairy products
Carnobacterium divergens	B	Dairy, fish, and meat products
Carnobacterium maltaromaticum	B	Dairy products
Carnobacterium mobile	B	Dairy products
Chlamydomonas reinhardtii	A	Protein supplementation
Chlorella sorokiniana	A	Nutrition bars, protein and nutritional powders, grain products
Chlorella vulgaris	A	Meal replacement bars and mixes; fruit juices, soy milk, and other beverages; medical foods
Corynebacterium ammoniagenes	B	Dairy products
Corynebacterium casei	B	Dairy products
Corynebacterium flavescens	B	Dairy products
Corynebacterium mooreparkense	B	Dairy products
Corynebacterium variabile	B	Dairy products
Cyberlindnera mrakii	B	Wine
Cystofilobasidium infirmominiatum	B	Cheese
Debaryomyces hansenii	B	Dairy products, meat, fish, vegetables
Dunaliella bardawil	A	Cheese, bread and rolls, mayonnaise, cookies, crackers, tofu, and soybean fermentation products
Enterococcus faecalis	B	Pickled vegetables, dairy products, soy sauce, miso, ham, sausages
Fusarium domesticum	F	Dairy products
Geotrichum candidum	F	Dairy products
Gluconacetobacter azotocaptans	B	Chocolate and coffee
Gluconacetobacter diazotrophicus	B	Chocolate and coffee
Gluconacetobacter entanii	B	Vinegar
Gluconacetobacter europaeus	B	Vinegar
Gluconacetobacter hansenii	B	Vinegar
Gluconacetobacter johannae	B	Chocolate and coffee
Gluconacetobacter oboediens	B	Vinegar

Microorganism	Type[xii]	Uses / Applications
Gluconacetobacter xylinus	B	Vinegar
Gluconobacter oxydans	B	Vinegar
Hafnia alvei	B	Dairy products
Halomonas elongata	B	Meat
Issatchenkia orientalis	F	Dairy kefir
Kazachstania exigua	F	Dairy kefir, sourdough bread
Kazachstania unispora	F	Dairy kefir
Kloeckera africana	F	Kombucha
Kloeckera apiculata	F	Wine
Kluyveromyces lactis	Y	Dairy products
Kluyveromyces marxianus	Y	Dairy products
Kocuria rhizophila	B	Dairy products
Kocuria rhizophila	B	Dairy and meat products
Kocuria varians	B	Dairy and meat products
Komagataeibacter hansenii	B	Vinegar
Lactobacillus acetotolerans	B	Pickled fruits and vegetables, sourdough bread
Lactobacillus acidifarinae	B	Sourdough bread
Lactobacillus acidipiscis	B	Dairy and fish products
Lactobacillus acidophilus	B	Yogurt, dairy products, pickled vegetables
Lactobacillus alimentarius	B	Meat and fish products
Lactobacillus brevis	B	Dairy products, pickled vegetables
Lactobacillus bucheri	B	Sourdough bread and wine
Lactobacillus cacaonum	B	Chocolate
Lactobacillus casei	B	Dairy products
Lactobacillus collinoides	B	Apple cider
Lactobacillus composti	B	Distilled alcoholic beverages
Lactobacillus coryniformis	B	Cheese
Lactobacillus crispatus	B	Sourdough bread
Lactobacillus curvatus	B	Dairy and meat products
Lactobacillus delbrueckii	B	Dairy products, pickled vegetables
Lactobacillus dextrinicus	B	Meat products
Lactobacillus diolivorans	B	Alcoholic beverages
Lactobacillus fabifermentans	B	Chocolate
Lactobacillus farciminis	B	Fish and soy products
Lactobacillus fermentum	B	Dairy products, sourdough bread, chocolate
Lactobacillus gasseri	B	Sourdough bread
Lactobacillus ghanensis	B	Chocolate
Lactobacillus hammesii	B	Sourdough bread
Lactobacillus harbinensis	B	Pickled vegetables
Lactobacillus helveticus	B	Dairy products, pickled vegetables
Lactobacillus hilgardii	B	Wine, chocolate
Lactobacillus homohiochii	B	Sourdough bread, alcoholic beverages
Lactobacillus homohiochii	B	Sourdough bread
Lactobacillus jensenii	B	Sourdough bread
Lactobacillus johnsonii	B	Sourdough bread
Lactobacillus kefiranofaciens	B	Dairy kefir
Lactobacillus kefiri	B	Dairy kefir
Lactobacillus kimchii	B	Pickled vegetables (kimchi)
Lactobacillus kisonensis	B	Pickled vegetables
Lactobacillus malefermentens	B	Apple cider, alcoholic beverages
Lactobacillus manihotivorans	B	Sourdough bread
Lactobacillus mindensis	B	Sourdough bread
Lactobacillus mucosae	B	Sourdough bread
Lactobacillus nagelii	B	Wine
Lactobacillus namuresis	B	Sourdough bread
Lactobacillus nantesis	B	Sourdough bread
Lactobacillus nodensis	B	Dairy products

Microorganism	Type[xii]	Uses / Applications
Lactobacillus oeni	B	Wine
Lactobacillus otakiensis	B	Pickled vegetables
Lactobacillus panis	B	Sourdough bread
Lactobacillus parabrevis	B	Dairy products, pickled vegetables
Lactobacillus parabuchneri	B	Sourdough bread
Lactobacillus paracasei	B	Dairy and meat products
Lactobacillus parakefiri	B	Dairy kefir
Lactobacillus paralimentarius	B	Sourdough bread
Lactobacillus paraplantarum	B	Dairy products, pickled vegetables
Lactobacillus pentosus	B	Dairy products, fish products, wine, fruit juices
Lactobacillus perolens	B	Dairy products, pickled vegetables
Lactobacillus plantarum	B	Dairy products, pickled vegetables, wine, beer, meat, fish
Lactobacillus pobuzihii	B	Fruit
Lactobacillus pontis	B	Sourdough bread
Lactobacillus rapi	B	Pickled vegetables
Lactobacillus reuteri	B	Sourdough bread
Lactobacillus rhamnosus	B	Dairy products, meat, pickled vegetables
Lactobacillus rossiae	B	Sourdough bread
Lactobacillus sakei	B	Alcoholic beverages, meat products
Lactobacillus salivarius	B	Dairy products
Lactobacillus sanfranciscensis	B	Sourdough bread
Lactobacillus satsumensis	B	Alcoholic beverages
Lactobacillus secaliphilus	B	Sourdough bread
Lactobacillus senmaizukei	B	Pickled vegetables
Lactobacillus siliginis	B	Sourdough bread
Lactobacillus similis	B	Alcoholic beverages
Lactobacillus spicheri	B	Sourdough bread
Lactobacillus suebicus	B	Fruit
Lactobacillus sunkii	B	Pickled vegetables
Lactobacillus tucceti	B	Meat and dairy products
Lactobacillus vaccinostercus	B	Pickled fruits and vegetables
Lactobacillus versmoldesis	B	Meat sausage
Lactobacillus yamanashiensis	B	Apple cider, wine
Lactococcus lactis	B	Dairy products, chocolate
Lactococcus raffinolactis	B	Cheese
Lecanicillium lecanii	F	Cheese
Leuconostoc carnosum	B	Meat
Leuconostoc citreum	B	Cheese
Leuconostoc citreum	B	Fish
Leuconostoc fallax	B	Sauerkraut
Leuconostoc holzapfelii	B	Coffee
Leuconostoc inhae	B	Pickled vegetables (kimchi)
Leuconostoc kimchii	B	Pickled vegetables (kimchi)
Leuconostoc lactis	B	Cheese
Leuconostoc mesenteroides	B	Dairy products, pickled vegetables, chocolate
Leuconostoc palmae	B	Alcoholic beverages
Leuconostoc pseudomesenteroides	B	Dairy products
Macrococcus caseolyticus	B	Meat sausage, cheese
Microbacterium foliorum	B	Dairy products
Microbacterium gubbeenense	B	Dairy products
Micrococcus luteus	B	Cheese
Micrococcus lylae	B	Meat sausage
Mucor hiemalis	F	Soy products
Mucor plumbeus	F	Cheese
Mucor racemosus	F	Dairy products
Neurospora sitophilia	F	Soy products
Neurospora intermedia	F	Soy products

Microorganism	Type[xii]	Uses / Applications
Neurospora sitophilia	F	Soy products
Oenococcus oeni	B	Wine
Pediococcus acidilactici	B	Pickled vegetables, meat sausage
Pediococcus acidilactici	B	Pickled vegetables
Pediococcus pentosaceus	B	Meat sausage
Penicillium album	B	Cheese
Penicillium camemberti	B	Cheese
Penicillium caseifulvum	B	Cheese
Penicillium chrysogenum	B	Cheese and meat sausage
Penicillium commune	B	Cheese
Penicillium nalgiovense	B	Cheese, meat products
Penicillium roqueforti	B	Cheese
Penicillium solitum	B	Meat
Pichia fermentans	B	Dairy products, wine
Propionibacterium acidipropionici	B	Cheese
Propionibacterium freudenreichii	B	Dairy products
Propionibacterium jensenii	B	Cheese
Propionibacterium thoenii	B	Cheese
Psychrobacter celer	B	Dairy products
Rhizopus microspores	F	Soy products
Saccharomyces bayanus	Y	Beer, cider, wine
Saccharomyces carlsbergensis	Y	Beer
Saccharomyces cerevisiae	Y	Bread, beer, wine, dairy products, chocolate
Saccharomyces pastorianus	Y	Beer
Saccharomyces rouxii	Y	Soy products
Staphylococcus carnosus	B	Dairy and meat products
Staphylococcus condimenti	B	Soy products
Staphylococcus equorum	B	Dairy and meat products
Staphylococcus fleurettii	B	Cheese
Staphylococcus piscifermentans	B	Fish
Staphylococcus saphrophyticus	B	Meat products
Staphylococcus sciuri	B	Dairy products
Staphylococcus simulans	B	Dairy and meat products
Staphylococcus succinus	B	Dairy and meat products
Staphylococcus vitulinus	B	Dairy and meat products
Staphylococcus warneri	B	Meat
Staphylococcus xylosus	B	Dairy and meat products
Streptococcus gallolyticus	B	Dairy products
Streptococcus salivarius	B	Meat
Streptococcus thermophilus	B	Yogurt and cheese
Streptomyces griseus	B	Meat
Streptomyces mobaraensis	B	Meat, fish
Tetragenococcus halophilus	B	Soy products
Tetragenococcus koreensis	B	Pickled vegetables (kimchi)
Torulaspora delbrueckii	F	Cheese
Weissella beninensis	B	Alcoholic beverages
Weissella cibaria	B	vegetable kimchi
Weissella fabaria	B	chocolate
Weissella ghanesis	B	chocolate
Weissella koreensis	B	Pickled vegetables (kimchi)
Weissella paramesenteroides	B	Meat sausage
Weissella thailandensis	B	Fish
Yarrowia lipolytica	F	Dairy products
Zygotorulaspora florentina	F	Dairy kefir
Zymomonas mobilis	B	Wine and liquor

Source: (Hutkins, 2006; IDF, 2018; Steinkraus, 1983; US FDA, 2023b, 2023c, 2023b).

Glossary

Active Site – The part of the enzyme molecule that interacts with the substrate where catalysis takes place.

Bacterium – (*Pl. bacteria*) A single-celled prokaryotic microorganism that does not have chlorophyll.

Catalase – An enzyme that catalyzes oxidation by converting hydrogen peroxide to water.

Catalysis – The change in the rate of a reaction by a substance that undergoes no chemical change, or that can be recovered in its original state after the reaction is completed.

Catalyst – A substance that changes the rate of reaction without being changed by the reaction.

Cell Fusion – The merging of cells by the fusion of their plasma membranes in a way that results in a bi- or multi-nucleate complex.

Coenzyme – A substance that facilitates the action of an enzyme.

Conjugation – The temporary union of two bacteria for the exchange of genetic material.

CRISPR (Clustered Regularly InterSpaced Palindromic Repeats) – A gene editing technique that involves 1) a guide RNA to match a desired target gene and 2) an endonuclease (e.g., Cas9) that causes a double-stranded DNA break that allows modifications to the genome.

Culture – A microorganism or collection of specific microorganisms, their tissue, or an organ growing in or on media used to support their reproduction.

Current Good Manufacturing Practices – Systems that assure proper design, monitoring, and control of manufacturing processes and facilities.

Endogenous enzyme – An enzyme that is present in a food ingredient or fermentation culture used to prepare a food.

Enzyme – A protein that acts as a catalyst for biochemical reactions.

Eukaryote – An organism that has cell nuclei. Includes protozoa, fungi, and most multicellular organisms.

Exogenous enzyme – An isolated enzyme preparation that is added with other ingredients to prepare a food.

Feedstock – The raw base material used for fermentation.

Fermentation – An intentional biological process used to convert specific raw biomass ingredients to make a product through the introduction of one or more specific microorganisms.

Functional food – A food that contains benefits to health in addition to nutrients.

Fungus – (*Pl. fungi*) A heterotrophic, eukaryotic, non-motile organism lacking chlorophyll that reproduces sexually through spores.

Homologous recombination-mediated gene targeting – A genetic modification technique that exchanges nucleotide sequences for two similar or identical DNA molecules on defined genes of interest.

Hydrolase – An enzyme that catalyzes hydrolysis reactions.

Inhibitor – A substance that slows or prevents a reaction.

Isomerase – An enzyme that catalyzes change within a single molecule.

Ligase – An enzyme that catalyzes the joining of two molecules or two parts of a molecule with the hydrolysis of a diphosphate bond in a triphosphate. Such enzymes are sometimes referred to as "synthase," "carboxylase," or "synthetase."

Liquid fermentation *(Abbrev. LF)* **–** An intentional biological process that uses liquid, free-flowing substrates and microorganisms in a high moisture broth enclosed in a container.

Lyase – An enzyme that cleaves chemical bonds by means other than hydrolysis or oxidation.

Malt – 1. (*v.*) To prepare cereal grains by sprouting, withering, and kilning. 2. (*n.*) Malted cereal grain.

Mash – (*n.*) Powdered malt steeped in hot water.

Macroencapsulation – Filling a hollow semipermeable membrane with multiple cells in a polymeric matrix.

Microencapsulation – Immobilization of cells within a polymeric semi-permeable membrane.

Must – (*n.*) Freshly crushed fruit juice prepared for fermentation.

Nanozyme – An enzyme synthetically manufactured through nanotechnology.

Oxidoreductase – An enzyme that catalyzes oxidation / reduction reactions.

Prokaryote – An organism that lacks cell nuclei. Includes bacteria and blue-green algae.

Recombination – The process of creating a new assortment or combination of genes in progeny that did not occur in either parent.

Solid-State Fermentation (*Abbrev. SSF, also called "Solid-Phase Fermentation" or SPF*) – An intentional biological process that cultures microorganisms on substrates in solid form.

Submerged Fermentation (*Abbrev. SF or SmF*) – See Liquid fermentation (LF).

Transferase – An enzyme that transfers an atom or group—such as a methyl group—between from one molecule known as the "donor" to another molecule known as the "acceptor".

Translocase – An enzyme that catalyzes the movement of a molecule, usually across a cell membrane.

Wort – An infusion drained from the mashed grains prepared for fermentation.

Yeast – A single-celled fungus that reproduces asexually by budding, and sexually reproduces through spores and conjugation.