

Enzymes, Plant and Fungal

Processing

Identification

Chemical Names:

There are many different plant and fungal enzymes used in processing. Among the chemical names for pectinase are poly(1,4- α -D-galacturonide)glycanohydrolase, poly(1,4- α -D-galacturonide)lyase, and pectin pectylhydrolase.

Other Names:

The model enzyme is pectinase. Among the other names for pectinase are pectin lyase, pectin methylesterase, pectinesterase, and polygalacturonase. See the attached table of other enzymes commonly used in food processing.

CAS Numbers:

Pectinase: 9032-75-1

Other Codes:

Enzyme Commission numbers for the major components of pectinase: Pectin methylesterase--3.1.1.11; Pectin lyase: 4.2.2.10; polygalacturonase: 3.2.1.15

Recommendation

Synthetic / Non-Synthetic:	National List:	Suggested Annotation:
<i>Non-synthetic (consensus)</i>	<i>Allowed 95%+ Allowed 50%+ (consensus)</i>	<i>Enzymes derived from edible, non-toxic plants or non-pathogenic fungi that are not genetically engineered as defined by the NOSB may be used in processed foods labeled as "Organic." Incidental ingredients used in the production of enzyme preparations must be non-synthetic as defined by OFPA and the NOSB, or be substances that appear on the National List of ingredients allowed for use in foods labeled as "Organic." This includes water and substances that are insoluble in food but removed from the foods after processing. (2-1-1; see reviewer 1 for discussion)</i>

Characterization

Composition:

Enzymes are proteins composed of up to 20 amino acids (Nielsen et al., 1991). The active components of enzymes consist of the biologically active proteins. These proteins have highly complex structures and may be conjugated with metals, carbohydrates and / or lipids. The model enzyme for this review, pectinase, actually refers to a combination of at least six different enzymes (Wingard, Katchalski-Katzin, and Goldstein, 1979). The principle enzymes in pectin are pectin methylesterase, pectin lyase, and polygalacturonase (Food Chemicals Codex, 1981). Pectinase is marketed in powder or liquid form (White and White, 1997).

Properties:

Enzyme preparations may consist of whole cells, parts of cells, or cell-free extracts from the source used. Active components have known molecular weights that range from 12,000 to several hundred thousand (Food Chemicals Codex, 1981). Enzymes may be in liquid, semi-liquid, or dry form. Enzymes in general and pectinase in particular is readily soluble in water. Enzymes are practically insoluble in alcohol, in chloroform, and in ether. The liquids are generally in aqueous solution, having many of the same properties of water, with the liquid form boiling point slightly above 100° C (212° F). Dry preparations are off-white to tan amorphous, finely divided powders. Liquids usually range in color from tan to dark-brown.

Individual preparations are generally characterized by functionality and activity rather than the properties of the product. The color of preparations may vary from virtually colorless to dark brown (National Academy of Sciences, 1981). For example, pectinase hydrolyzes the pectin molecule (Reed, 1975).

How Made:

Enzymes are produced by cellular anabolism, the naturally occurring biological process of making more complex molecules from simpler ones. Source organisms for food processing include bacteria, fungi, higher plants, and animals (White and White, 1997). Enzymes may be extracted from a given source organism by a number of different methods (Nielsen, et al., 1991). Most of the organisms that produce commercial enzymes are considered fungi of some sort. These organisms include the molds *Aspergillus Niger*, *Rhizopus oryzae*, *Rhizomucor meibei*, blights such as *Endothia parasitica* and yeasts such as *Candida* spp and *Saccharomyces* spp. A considerable amount of research has been conducted on genetically modifying fungi and other organisms to increase the yields and consistencies of enzymes. Many of the prospective donor organisms are pathogenic and are being screened for genetic sequences to be inserted into non-pathogenic hosts (see, for example, Surgey, Robert-Budouy, and Condemine, 1996). Continuous improvement of production methods is possible without the use of recombinant DNA techniques. For example, classical methods of hybridization can also be used to improve enzyme-producing organisms (see Solis, Flores, and Huitron, 1997).

The model enzyme used for this TAP review, pectinase, is generally produced by a fungal source organism. Enzymes derived from higher plants are discussed more fully in the review of enzymes used for livestock production. Animal derived enzymes are not considered for the purpose of this review. The NOSB has previously considered bacterial enzymes for processing of food for human consumption (NOSB, 1995).

Until recently, all enzymes produced and used for food were from these naturally-sourced biological products. Pectinase and other enzymes can be produced by a wide number of methods. One source of commercial pectinase is the mold *Aspergillus niger* grown by controlled fermentation (Aunstrup, 1979). The substrate often contains various grains and synthetic nutrients.

Isolation of the enzymes from their intracellular sources generally begins with separation from the media, usually by physical means such as centrifuging and sorting by specific gravity. The cell walls of the organisms are then burst through a mechanical process of homogenization, similar to that used on milk. Extracellular production--where the fermentation organism excretes the enzymes in a form that can be safely isolated--does not necessarily involve breaking the cell walls of an organism to recover the enzyme. However, techniques such as ion-exchange may be used to remove impurities in extracellular production (Lilly, 1979).

Further extraction, purification, and standardization from this point generally involves use of synthetic substances. Because extraction is pH dependent, the pH may be adjusted through the use of various strong acids, such as sulfuric acid, and bases such as sodium hydroxide. Other chemical extractants may be organic solvents, such as acetone; polymers, such as methylcellulose or polyvinyl alcohol; glycol ethers such as polyethylene glycol (PEG); or salts, such as sodium phosphate (White and White, 1997). Organic solvent extraction has been declining for a number of years (Pariza and Foster, 1983; a continuing trend confirmed by reviewers). Specific enzymes are then precipitated or absorbed by the use of a variety of chemical constituents and / or ion exchange columns. Final purification removes the extractants by further centrifugation, adsorption to a suitable adsorbent, and subsequent elution (Albertsson, 1971; Kula, 1979).

The isolated material is molecularly and functionally the same as produced by the functioning cell, thus non-synthetic. Enzymes that are molecularly the same, but not functionally the same are called 'denatured.' Recent technological advances in genetic engineering have made it possible to alter cellular genetic content, resulting in new production capabilities of the cell. The NOSB has considered other such alterations to be synthetic.

Specific Uses:

Enzymes have a wide variety of uses (ETA, 1999). Specific applications of pectin lyase is in juice clarification, extraction, wine clarification and production, cloud stabilization of citrus juices, extraction of citrus juices, and use in production of vegetable and fruit purees. In particular, pectinase is used primarily to depolymerize and esterify plant pectins in fruits such as apples, lemons, cranberries, oranges, cherries, grapes, and tomatoes, to name a few. The application of pectinase enables the entire fruit to be liquified. This has the effect of improving saccharification and thus sweetness, reducing waste and energy use per unit of juice produced, improving aroma and color; enhancing clarity, removing haze, preventing gel formation, and increasing fruit juice yield (Nielsen et al., 1991; White and White, 1995).

Action:

Enzymes increase the rate of biochemical reactions and decrease the time for those reactions to reach equilibrium. They are not consumed in the chemical reactions and, as such, their action is catalytic. For example, two constituents of pectinase are pectin methylesterase and polygalacturonase. Pectin methylesterase demethylates pectin; polygalacturonase hydrolyzes the α -1,4-galacturonide bonds in pectin (Food Chemicals Codex, 1981). A large variety of pectic enzymes are available both in liquid or solid forms and in various strengths, as measured by the level of activity. In the case of pectin, this is measured by the ability of the enzyme to hydrolyze the glycosidic bond between the biopolymer pectin of repeating chains of the sugar galactose or galacturonic acid. The amount of pectin in fruits depends on the maturity, degree of ripeness, variety, and subsequent storage conditions of harvested fruit (Reed, 1975).

Combinations:

Enzymes often are included in whole cells or parts of the cells of the source (National Academy of Sciences, 1981). They are often packaged with various carriers that do not have catalytic activity that may or may not be synthetically derived (White and White, 1997). Synthetic preservatives are almost always added during processing, and may be present in the final preparation to prevent microbial growth, stabilize the preparation, and maintain the desired enzymatic activity (Pariza and Foster, 1983). Other incidental ingredients in enzyme preparations function as carriers, stabilizers, humectants, and diluents.

Enzymes are usually used in combination with other enzymes. For example, pectinase is often used with cellulases, hemicellulases, and proteases. Several of these are also produced by *A. niger* (White and White, 1997). Some of these materials are on the recommended National List.

Status**OFPA**

The substance is used in handling and is non-synthetic but is not organically produced (7 USC 6517(b)(1)(C)(iii)).

Regulatory

Enzymes are considered food additives under the Food, Drug, and Cosmetic Act. See 21CFR 184 for various specific GRAS listings. Pectinase has been self-declared GRAS by the Enzyme Technical Association (ETA, 1999).

Status among Certifiers

Most US certifiers have allowed the use of fungally derived enzymes documented to not be from genetically engineered sources. Specific conditions for extractions and incidental additives does not appear to be uniform among US certifiers at this point.

Historic Use

Enzymes contained in various ingredients have been used to prepare foods since before recorded history. However, production and application of pure enzymes has become increasingly sophisticated over the past century. The first use of pectinase in fruit juice processing dates back to the 1930s (Nielsen et al., 1991). Steady supplies of purified, standardized pectic enzymes have been commercially available for about fifty years. Enzymes have been used in a broad number of applications by organic food processors for as long as organic processed food has been on the market.

International

In general, enzyme standards for international trade are set by the Joint FAO/WHO Expert Committee on Food Additives (1990). The Codex Alimentarius Commission organic food guidelines allow “[a]ny preparations of microorganisms and enzymes normally used in food processing, with the exception of microorganisms genetically engineered/ modified or enzymes derived from genetic engineering” (Joint FAO/WHO Food Standards Programme, 1999). The most recent edition of the IFOAM *Basic Standards* considers enzymes acceptable for use in organic food processing provided they are based on the established Procedure to Evaluate Additives and Processing Aids for Organic Food Products (IFOAM, 1998). These standards are parallel to, but not exhaustively covered by the OFPA criteria.

OFPA 2119(m) Criteria

- (1) The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems.
As this is a processing material, the substance is not used in organic farming systems.
- (2) The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment.
See processor criteria (3) below.
- (3) The probability of environmental contamination during manufacture, use, misuse or disposal of such substance.
This is considered below under processor criteria (2).
- (4) The effect of the substance on human health.
This is considered in the context of the effect on nutrition (3) below as well as consideration of GRAS and residues (5) below.
- (5) The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock.
As this is not released into the agroecosystem, there is no direct effect.
- (6) The alternatives to using the substance in terms of practices or other available materials.
See discussion of alternatives in (1) below.
- (7) Its compatibility with a system of sustainable agriculture.
This is considered more specifically in the context of organic handling in (6) below.

NOSB Processing Criteria

A SYNTHETIC PROCESSING AID OR ADJUVANT may be used if;

1. An equivalent substance cannot be produced from a natural source and has no substitutes that are organic ingredients.

Enzymes frequently offer the only way to achieve a desired technical effect. Nearly all commercially prepared foods contain at least one ingredient that has been made with enzymes. In a number of cases, the alternatives would be prohibited for use in organic production (e.g. sulfuric acid); in other cases, the alternative would be chemical modification (e.g. sodium hydroxide used to produce starch). Some enzymes are essential for the production of certain foods, for example α -amylase to produce barley malt or rice syrup; or various coagulants used to produce cheeses. In the case of pectinase, different fruits can be processed into juice, wine, oil, or preserves with lower yields (Faigh, 1995), longer processing times (Gist-Brocades, 1993), and subjectively lower quality (Chang, et al., 1994).

2. Its manufacture, use and disposal does not contaminate the environment.

Production of enzymes is generally conducted in controlled, closed environments. Materials necessary for their manufacture generally do not in and of themselves constitute an environmental hazard. Good manufacturing and handling practices are sufficient to protect workers from any negative effects of exposure, although inhalation or other ingestion of enzymes can have irritating or allergenic effects on some people.

The fermentation process is relatively efficient and closed. Because of their catalytic nature, enzymes theoretically can react indefinitely, and relatively small amounts are effective in performing their functions.

Enzymes need to be replaced when they are degraded by physical conditions (e.g. heat) or removed with the processed food.

Release of enzymes into the environment is generally not a concern. They are active in very low concentrations, and each enzyme's action is specific to a very narrow range of substrate(s). They can be relatively stable molecules, but are generally degradable by heat or other environmental factors. Enzymes in the environment may accelerate the rate that pollutants are metabolized (Tinsley, 1979). This may be detrimental, beneficial, or have no net effect, depending on the substrate and metabolite.

Escape of enzyme-producing organism into the environment is not considered an environmental concern (Nielsen, et al., 1991). Genetically-engineered organisms, particularly microorganisms--may change the nature of this concern. Wild-type producing strains have shown a fair ability to be controlled in open ecosystems by natural competition. Genetically-engineered strains, on the other hand, may have far-reaching consequences if released into the environment. At present, there is insufficient data and experience with such strains to regard their potential interactions as safe, in anything but a very controlled environment, and even then this may not be a certainty.

3. If the nutritional quality of the food is maintained and the material itself or its breakdown products do not have any adverse effect on human health.

Enzymatic activity on foods is specific and transformational, usually resulting in a significant change in the characteristics of the substrate. The new food product may have a significantly different effect on the human system when ingested. For example, consider the difference between corn meal and corn syrup, or milk and cheese. That a transformation occurs is not by itself enough to say whether the ultimate effect on human health is positive or negative. Most studies show that nutritional quality as measured by vitamin and mineral content, as well as other parameters, is maintained (Braddock, 1981). In some cases, because of the enzyme's role in the removal of the non-nutritional part of the food and making the nutrients of the food more digestible, enzymes can measurably improve the nutritional quality of food. Other indicators of quality are arguably improved (Chang et al., 1994).

There is an on-going debate in human nutrition as to the advantages of whole over processed foods. This is discussed further in the reviewer comment section. A recent study based on an analysis of 46 supplements for the quality of their antioxidants composition demonstrated that natural intact food sources were better (Tufts, 1999). By implication therefore, the pectin in an apple, and the overall nutritional value of an apple, is much greater to the consumer than is depectinized, filtered apple juice.

There is the potential for enzymes to pose a threat to human health and safety. As proteins, enzymes can cause allergic reactions in sensitive individuals (Tucker and Woods, 1995). Enzymes can remain active after digested and there is concern that novel enzymes--particularly some of the more potent ones being developed by genetic engineering--will attack human tissues in some instances (Tucker and Woods, 1995). Perhaps the greatest concern with fungal enzymes is the presence of mycotoxins from either the source organism or a competing organism that invades the fermentation media. Many of these organisms are capable of producing antibiotics. While Good Manufacturing Practices require that non-pathogenic strains be used, quality control and Hazard Analysis Critical Control Point (HACCP) need to be sufficient to ensure that both the strains and the media avoid contamination with pathogens and toxins. The organism used as a case study for this review, *A. niger*, provides a good example. *A. niger* is capable of producing low levels of toxins, but most strains are considered non-toxicogenic because the levels of toxins are so low (Pariza and Foster, 1983).

Enzymes are widely used for therapeutic purposes (Jayaram, Ahluwalia, and Cooney, 1991; Cichoke, 1999). While there are a number of contraindications that need to be considered in a number of cases, and a recognition that not all uses of enzymes are beneficial or desirable, they are generally not a threat to human health when properly handled and used.

Finally, after processing and packaging, the enzyme may be prone to spoilage by a microbial contaminant. For this reason, preservatives are almost always added during processing and after final preparation (Pariza and Foster, 1983).

4. Is not a preservative or used only to recreate/improve flavors, colors, textures, or nutritive value lost during processing except in the latter case as required by law.

Enzymes in and of themselves generally would not be considered preservative materials. The products of enzyme activity could conceivably act as preservatives, but these would be from the breakdown of the food material itself, not from an outside source. Food qualities are changed by enzymatic activity, but this change should not necessarily be construed as a means of re-creating qualities of the original product lost in processing. The product is substantially different from the raw ingredient(s). While enzymes can be used to transform food into a more stable product, these processed foods are generally identified as different from their raw ingredients. For example, raspberry jelly is considered to be different from raspberries. The use of pectinase neither increases nor decreases the shelf life of a raw product. In a natural situation, various enzymes are produced by either the plant itself (Kays, 1991) or various organisms to accelerate decay, decompose cell walls, increase sugar content, and release the nutrients contained in the fruit and other plant organs in the senescence process.

5. Is Generally Recognized as Safe (GRAS) by FDA when used in accordance with Good Manufacturing Practices (GMP), and contains no residues of heavy metals or other contaminants in excess of the tolerances established by FDA.

Enzymes are unchanged by their action on their substrates; they remain as they are, and active, until denatured by heat or other factors, or until the substrate is exhausted. Depending on the process, enzymes may be removed from the final product, or denatured and left in, or may even be potentially active. How they are labeled in final product formulations should be dependent on the specific outcome for the product in question. As was mentioned above, carriers, preservatives, or other commercial enzyme formulation components are also potential residues in finished foods.

Many enzymes are classified as GRAS, although such determination has not been universally made. GMPs, quality control measures, and analytical protocols can reduce the risk of mycotoxins being included in fungal enzyme formulations as by-products of the manufacturing process (Pariza and Foster, 1983). Implementation of HACCP plans can take further steps to reduce risk to food safety posed by enzymes.

A number of fungal enzymes are generally and specifically considered GRAS. The Enzyme Technical Association has made a self-declaration of GRAS for a number of enzymes (ETA, 1999).

The Food Chemicals Codex places the following limits on residues:

- Arsenic (as As) not more than 3 ppm.
- Coliforms: not more than 30 per g.
- Heavy metals as lead: not more than 0.004%.
- Lead (Pb): not more than 10 ppm.
- Salmonella spp: Negative by test.

The Food Chemicals Codex also states that “[a]lthough tolerances have not been established for mycotoxins, appropriate measures should be taken to ensure that the products do not contain such contaminants.”

6. Is compatible with the principles of organic handling.

Enzymes have been used in organic processing for as long as organic processed food has been marketed, and are currently being used by certified organic processors. An industry survey of organic food processors regarding the compatibility of various processes found that enzymes were rated between 2.5 and 2.7 on a scale of 1 to 5, or approximately mid-range, as compatible with organic processing (Raj, 1991).

In certain food products, enzymes are the only way to produce the desired product, such as barley malt or rice syrup, or for certain cheeses. In others, such as production of certain invert starches, the alternatives--sulfuric or phosphoric acid--would not be compatible with an organic handling system, and may result in

products of lower quality. There are some cases in which microbial fermentation can offer a more holistic approach to processing than the use of isolated enzymes. One such example is the use of *Mucor mehei* to produce certain kinds of cheeses.

7. There is no other way to produce a similar product without its use and it is used in the minimum quantity required to achieve the process.

For a number of foods, enzymes are essential to the identity of the food. Even where they are not required to be used to make an identifiable food, they are needed to produce a food of the quality consumers expect. In determining the standard of identity of natural juice, juice extracted using pectinase is usually considered minimally processed (Haight and Gump, 1995). Because they are effective in small amounts, very little of any enzyme is needed to process a given food. An industry survey found the amount of enzymes used in processing is in all cases less than 0.06%. The maximum amount of pectinase used in baked goods was found to be 0.000002% (Pariza and Foster, 1983).

Discussion

Condensed Reviewer Comments

None of the reviewers have a commercial or financial interest in pectinase in particular or enzymes in general.

Reviewer 1

NOTE: The following review covers enzymes as a general class of materials, using pectin [enzymes] as an example in parts of the discussion.

Research included by OMRI for this review suggests that processing of foods with enzymes can enhance the nutritive value of foods by breaking down “indigestible” food components, thereby making certain nutrients more available in the final product. One example given is that pectinase activity on plums in juice manufacture can result in greater availability in the final juice product of antioxidant components, which otherwise might not have been yielded during processing. How such laboratory trials correlate to human nutrition is not clear from the information presented, and not completely known - that is, whether such enzymatic treatments would be necessary to make said antioxidants as available in the human gut, or whether the altered food overall is definitely better than the original whole food. (This question/example could be extended to other fruits and nutritional components thereof, which are processed similarly with pectinases.) Generally speaking, processing yields of total juice from various fruits is increased when pectinases are used, as more of the fruit can be liquefied and separated from the seed and fiber; this is the primary reason enzymes are used in fruit processing.

There are some potential drawbacks to considering the advantages in exclusion to other effects of use of these enzymes, both from a nutritional standpoint and from an organic foods perspective. The assumption given in the first paragraph in this section of the NOSB database for this part of the criteria, i.e. that so-called “non-nutritive” or “indigestible” food components serve no positive function as part of a human diet and can therefore be removed, is based on incomplete knowledge at best. For example, the pectin in an apple, and the overall nutritional value of an apple, is much greater to the consumer than is pectinized, filtered apple juice. In which instances an isolated food component is desirable or valuable and which cases it is not is subject to variance from one commodity to another. We do not fully understand the complex balance of nutrients and how they interact on human nutrition for any agricultural product, and we should therefore be careful in choosing which components we deem appropriate to keep in the product and which to discard. Research continually shows how previously unidentified or poorly-understood food components can play significant roles in human health and nutrition. The value of food fiber is a good case in point.

A purely materials-based approach to processing of organic foods is flawed, in that the effects of individual processing steps and their associated materials can be cumulative. Analysis of each component step in the process does not necessarily reveal the total effect of all processes combined to make the final product. For this reason, although use of enzymes may not in and of themselves be seen as negative, use may be an integral part of a negative outcome as regards one of the goals of organic foods production, namely wholesome foods of high nutritional value. Fruit and juice processing is sometimes a good example of this; although initial yield of juice from the fruit may be higher than without use of the enzyme(s), the final products often are only a shadow of the original material. The nutritive values of corn meal and corn syrup are starkly different from each other; this is partly due to the action of enzyme, and partly due to the subsequent isolation of the product of the enzyme's activity. In such cases, it could be said that the nutritional value of the organic agricultural commodity has largely been lost.

Allowance of use of enzymes on organic foods therefore poses a potential danger as regards the nutritional value of the finished product. Non-specific allowance of all enzymes (or allowance of a specific class of enzymes used non-specifically, i.e. on any commodity), can lead ultimately to production of organic food products which lack much of the nutritive value of the original agricultural component(s).

What is needed, therefore, is a broader principle on which to base decisions as to whether or not materials such as this are appropriate for particular foods. In this discussion, nutritive value is the determining criterion. The annotation as proposed in the NOSB database file should therefore be amended with a statement similar to the following: "Use of enzymes in any given process is subject to overall evaluation of the final nutritional value of the finished product compared to its initial ingredient(s). Such evaluation shall take into account all processing steps involved, not just those involved with the use of the enzyme(s). In cases where the nutritional profile of the raw ingredient(s) is deemed to have been substantially weakened, such finished food products may only be labeled as 'made with organic ingredients,' but not as 'fully organic.'" (Such "made with" products, if they are further used as ingredients in other organic product formulations, will themselves have to have classifications as to whether they can be ingredients in "organic" product formulations, or only in "made with organic" products. An illustrative example of this might be corn syrup, or commonly-produced white grape juice concentrate.)

There are some potential drawbacks to considering the advantages in exclusion to other effects of use of these enzymes, both from a nutritional standpoint and from an organic foods perspective. The assumption given in the first paragraph in this section of the NOSB database for this part of the criteria, i.e. that so-called "non-nutritive" or "indigestible" food components serve no positive function as part of a human diet and can therefore be removed, is based on incomplete knowledge at best.

An alternative which also might serve organic principles is an itemization of enzyme use by food type (either substrate or final product), the allowances or restrictions for enzyme use being specific to each; such listing is more arduous to generate, but allows for more consistent application.

As some other points to consider, human safety can potentially be threatened by enzymes, either due to allergenic interactions or toxic by-products of microbial production of enzymes. Selection of appropriate strains, along with GMP's and HACCP plans can be used to minimize these dangers, usually with good results. Far less certainty on this point applies to those enzymes and microbes which are products of genetic engineering.

Finally, enzymes are often packed for industrial use with a number of carriers and preservatives, for convenience of both the enzyme manufacturer and the product user. All formulations, if they are to be used in organic systems at all, must have full disclosure as to all components in the formulation used, and only include components which are deemed acceptable materials on the National List for foods labeled as "organic." Processed products made with enzyme formulations which do not meet this requirement may or may not be labeled as "made with organic ingredients," depending on the formulation's component(s) in question. Carriers, standardization materials, and other commercial enzyme formulation ingredients should be listed on all product labels as ingredients, if they indeed end up in the final food product.

It is possible to produce fruit juice without use of enzymes. Conventional food products on the market do not necessarily have to have an identical organic version, and in some cases, should certainly not, if we are to yield to the higher principle, which in this case is human nutrition. Enzymes should only be allowed in organic production if they serve the principle of maintenance of nutritional quality and truly are essential to the formation of the product. Being essential to achieving a desired technical effect which results in a product of degraded nutritive value should not be considered an essential need.

... [E]nzymes are compatible with principles of organic production, but only if they are placed in a larger perspective, not in all cases. It must be ensured that the ultimate nutritive value of foods is not robbed due to successive processing steps, where enzymes are an integral part in said processes, even if the enzymatic steps themselves do not result in the loss of nutritive value.

The discussion of enzymes for use in organic foods processing is complex, and several of the criteria discussed above overlap. Enzymes should be classified as a natural material, listed on the National List as being REGULATED, with the annotation being as proposed in the NOSB database, amended as discussed in this review (refer to section 2119(m)4 and NOSB processing criterion #3, above). Otherwise, itemized decisions on individual enzymes (or types of enzymes) would be appropriate. Blanket acceptance of enzymes as processing materials is strongly discouraged.

Enzymes which are products of genetic engineering as defined by the NOSB should be classified as synthetic materials, and PROHIBITED for use in organic production systems.

Reviewer 2

Since pectin lyase is biosynthesized from *Aspergillus* or other fungal sources and is not chemically derived, I would classify this enzyme class as non-synthetic. This classification is predicated on the following criteria:

- (i) Fungal organisms can not be derived from genetic engineered species and must be naturally occurring.
- (ii) Extraction and manufacturing operations can not chemically modify or change the enzyme preparation.
- (iii) All carriers, diluents and preservatives used in the final enzyme preparation shall be substances that appear on the National List of Ingredients allowed for use in foods labelled as organic.

I therefore agree with the OFPA status that this enzyme preparation if prepared from a non-GMO fungal source is non-synthetic. The risk to organic integrity depends on the isolation, purification and packaging (i.e., inclusion of stabilizers or preservatives).

Overall I found the NOSB materials database to be technically accurate. I agree with the proposed annotation inclusive of the three proposed criteria that qualify my recommendation that the NOSB consider that fungal enzymes (in this case, pectin lyase) are naturally derived enzymes but must be handled in a manner consistent with organic food processing criteria.

Reviewer 3

Fungal enzymes appear to be necessary for many types of food processing operations and the alternatives are either synthetic or less desirable. The use of fungal enzymes as described is compatible with organic production. Fungal enzymes, not produced through means of genetic modification should be added to the NOSB List of Allowed Non-organic ingredients.

Reviewer 4

Pectinase

This is a naturally occurring enzyme (or actually a class of them) and should not be considered synthetic. One note is that pectinase activity generally results in a loss of textural integrity; it's used to break down pectin for example in the juice industry. I don't think that there should be any question about the means by which pectinases are extracted or obtained from fungal cells. The enzyme database seems more straightforward to me. I don't think there have been any oversights in terms of information provided. There is nothing I disagree with here. I don't believe that enzymes could be considered "preservatives" (I always think microbial here!) but rather processing aids. The write-up on genetic engineering is well done; I agree that NOSB will probably not want to open that door again!

Conclusion

Enzymes are naturally occurring, widely used in food processing, and are currently used to process foods that are sold as organic without much controversy. The consensus is that enzymes from fungal and plant sources should be added to the National List of ingredients allowed for use in foods labeled as organic. However, there are some concerns that require annotations of what enzymes can be accepted. The reviewers appeared to all agree that not all enzymes are compatible with organic standards. The primary concern at present appears to be the degree that genetic engineering and recombinant DNA techniques are used and whether certain specific enzymes will be available in a non-genetically engineered form. Other concerns include extractants, preservatives, and incidental additives. While natural enzymes may be added to the National List, this does not imply to the TAP that all preparations that use natural enzymes will be formulated in a way that meets organic standards. Certifiers, processors, and suppliers are seeking clear, consistent industry guidelines on acceptable sources of enzymes. Finally, animal produced enzymes were not considered in this review and the NOSB may want to refer those to the TAP as well, or at least explicitly demur.

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