Enzymes

Livestock

Identification

Chemical Names:
Various. For bromelain: ananase, bromelin, extranase, inflamen, traumanase.

Other Names:
Many other enzymes are used in livestock production. The model enzyme used for the purposes of this review is bromelain. Other plant derived enzymes include amylase, ficin, and papain.

CAS Numbers:
9001-00-7; 37189-34-7

Other Codes:
IUB #s 3.4.22.32; 3.4.22.33

Recommendation

Synthetic / Non-Synthetic:	National List:	Suggested Annotation:
Non-synthetic (consensus)	*Allowed for use in livestock feed* *Allowed for livestock health care (consensus)*	*Must be derived from non-pathogenic bacteria, non-pathogenic fungi, or edible, non-toxic plants that are not genetically engineered as defined by the NOSB. Co-factors must either be organically produced or appear on the National List of ingredients allowed for use in foods labeled as "Organic." This includes water and substances that are insoluble in feed but removed from the feed after processing. (2-1)*

Characterization

Composition:
Enzymes are proteins composed of up to 20 amino acids (Nielsen et al., 1991). Plant enzymes are generally complex mixtures of proteins. Given their complexity, they have been difficult to either synthesize or isolate. For example, bromelain has a molecular weight of over 33,000 (Budavari, 1996).

Properties:
Enzyme preparations may consist of whole cells, parts of cells, or cell-free extracts from the source used. Individual preparations are generally characterized by functionality and activity rather than the properties of the product. Enyzmes may be in liquid, semi-liquid, or dry (crystalline) form. The color of preparations may vary from virtually colorless to dark brown (National Academy of Sciences, 1981). Bromelain is a combination of proteolytic (protein-digesting) enzymes that convert proteins into peptides by cleavage of peptide bonds (Budavari, 1996).

How Made:
Enyzmes may be extracted from bacteria, fungi, plants, or animals by a number of different methods (Nielsen, et al., 1991). The NOSB has previously considered bacterial enzymes for processing of food for human consumption (NOSB, 1995). Fungal enzymes are discussed in the TAP review for processing enzymes. Animal enzymes are not considered in this review. For the purposes of illustration, a plant-derived enzyme, bromelain, is used as a model enzyme for use in livestock production.

Bromelain can be isolated and purified from pineapple by several different methods. The stem tissue contains a different bromelain from the fruit (Collins, 1960; Budavari, 1996). Fruit bromelain was first isolated in 1891 (Budavari, 1996). Some methods involve the use of sodium chloride and ammonium sulfate (Collins, 1960). Other methods might involve the use of sodium cyanide and acetone (Tauber, 1949). Solvent extraction has been declining for a number of years (Pariza and Foster, 1983; a continuing trend confirmed by reviewers).

Bromelain can be extracted from plant tissue (pineapple) by salting out with sodium chloride followed by physically separating out the salt by physical methods such as dialysis or ultrafiltration. Tauber describes a process where alcohol is used to precipitate the bromelain (all enzymes are protein) from the plant extract

(Tauber, 1949). The alcohol can be recovered and re-used. Organic bromelain could conceivably be manufactured from organically grown pineapple by at least three practical methods: the use of salt (sodium chloride), organic ethyl alcohol, or physical methods such as ultrafiltration or dialysis.

Because of the relative value of the fruit, the difficulty of processing the juice, and the improvements of methods to extract bromelain from stems, stem bromelain is the source of most bromelain. Because of the length of time it takes to grow plants relative to fermentation, there is interest in replacing plant enzymes with those from fermentation. Transgenic organisms are the likely source of such a fermentation product (Tucker and Woods, 1995).

Specific Uses:
Enzymes are used as feed components to improve digestibility, palatability and feed conversion; reduce mortaility; remove toxic substances from unconventional feed sources; and improve the consistency of manure or droppings and thus the ability to manage manure (Nielsen, et al., 1991). Bromelain in particular helps to make protein sources more digestible. Therapeutic uses of bromelain include as an anti-inflammatory, for obstetric manipulations, and to enhance the activity of drugs (Jayaran, Ahluwalia, and Cooney, 1991). Bromelain has been shown effective in reducing *E. coli* diarrhea in piglets (Mynott, Luke, and Chandler, 1996; Chandler and Mynott, 1998).

Action:
Enzymes act as catalysts. As such, they accelerate the rate at which various biochemical reactions achieve equilibrium, but are not themselves changed in the reaction. Protease enzymes such as bromelain remove various peptide bonds from proteins (Budaveri, 1996). Proteins that are in solution with protease enzymes like bromelain are more quickly broken down into proteins of lower molecular weight. In this way, bromelain makes protein from animal and vegetable sources more digestible by partially hydrolyzing protein into smaller peptides (Fennema, 1996).

Bromelain enhances serum fibrinolytic activity and inhibits fibrinogen synthesis (Lotz-Winter, 1990). Malignant cell growth appears to be inhibited by bromelain (Taussig and Batkin, 1988; Lotz-Winter, 1990). Application prevents or inhibits edema often associated with trauma (Lotz-Winter, 1990). When fed orally, bromelain inhibits the ability of enterotoxic diarrhea inducing *E. coli* to attach to the intestines of pigs (Mynott, Luke, and Chandler, 1996).

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 and stabilize and maintained the desired enzymatic activity (Pariza and Foster, 1983). Bromelain is often combined with other enzymes, particularly the plant derived protease papain extracted from papaya fruit. Commercial preparations of bromelain may also include ammonium sulfate and monopotassium phosphate (White and White, 1997).

Status

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

Regulatory
Enzymes are regulated as feed additives under 21 CFR 573 and by state feed labeling laws. The association that develops uniform and equitable state feed labeling laws and regulations, the Association of American Feed Control Officials (AAFCO) recognizes that not all applications of enzymes used to process food for human consumption are directly transferable to animal applications (AAFCO, 1998). However, with a few specific exceptions, AAFCO recommends that the guidelines and annotations used for enzymes in food processing should be applied to enzymes used in animal feed milling. Bromelain is regulated for human food use under 21 CFR 184.1024.

Status among Certifiers

Enzymes have historically been allowed by certifiers in feed labeled as organic.

<u>Historic Use</u>
Enzymes have been used by organic food processors. Because organic livestock has not developed as quickly as processing, enzymes appear to have been little used by organic livestock producers and feed mill operators.

<u>International</u>
In general, enzyme standards for international trade are set by the Joint FAO/WHO Expert Committee on Food Additives. Enzymes from non-GMO sources are allowed in both the current Canadian (Canadian General Standards Board, 1999) and European (European Union, 1999) organic standards. Enzymes are not specifically addressed in the most recent draft of the Codex Alimentarius organic standards. 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.

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.

(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.

This is discussed in the processing section below.

(3) The probability of environmental contamination during manufacture, use, misuse or disposal of such substance.

This is considered in the processing criteria below.

(4) The effect of the substance on human health.

This is considered in the context of the effect on nutrition and FDA GRAS status.

(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.

Bromelain appears to have little acute toxicity for animals according to studies done in rodents and rabbits and yet possesses many of the therapeutic properties it demonstrates in humans (Lotz-Winter, 1989). It also has a protective effect against enterotoxigenic *E.coli* in pigs. (Chandler, 1998)

(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 below in the context of organic handling in (6) below.

NOSB Processing Criteria Used Here for Livestock Feed Processing

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.

   Animals will secrete their enzymes as part of their digestive processes (Pond, Church, and Pond, 1995). Various physical, biological, and mechanical forms of processing can enhance the nutrient availability of feed, such as cooking (Sunde, 1973), fermentation (Oldfield, 1973), and pelleting (Slinger, 1973). Excessive processing can degrade feed quality, reduce nutrient content, and decrease nutrient availability (Morrison, 1951; Sunde, 1973). Higher quality organic feed may be a viable substitute for the use of enzymes to enhance inferior feed. Pineapple bran as a by-product of processing has historically been used as an animal feed, and was at one time shipped to the West Coast of the US for use as cattle feed (Morrison, 1951). Organic pineapple may at some point become a viable feed supplement.

   Other enzymes such as papain and rennet which are GRAS as feed additives may be considered alternatives from the viewpoint of a more acceptable regulatory status and perhaps equal status regarding sustainability. However, whether they are equivalent functionally as feed additives is debatable. Enzymes derived from bacteria can be used to substitute for plant-derived enzymes, with some limitations (Gallagher, et al., 1994).

   Bromelain's therapeutic uses may indicate it has a unique contribution to make to organic livestock production. For use as an anti-diarrheal in piglets, no acceptable non-chemical alternatives exist and conventional treatment is with antimicrobials / antibiotics (Aiello, 1998).

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

   In general, enzymes are totally biodegradable (Nielsen et al., 1994). To be safe for human consumption, it is important for plant-derived enzymes to be extracted from plants that are both edible and non-toxic (Pariza and Foster, 1983). Food-grade and pharmaceutical-grade enzymes need to meet chemical and biological purity standards established by Food Chemicals Codex (National Academy of Sciences, 1981). Materials used to extract, concentrate, and standardize bromelain itself is non-toxic, but the production of the raw material and processing into purified form both may involve the use of toxic chemicals.

   Bromelain is derived from the fruit and stem of pineapples. Pineapples are edible, non-toxic plants. However, the production of conventional pineapples raise several concerns for contamination of the environment. Pineapple is a fruit crop with a history of intensive pesticide use. An increasing amount is grown outside the U.S. in coutries like Mexico, Honduras, and the Phillipines. The best data on pesticide use is from Hawaii. Targeted monitoring by the FDA has been conducted on pineapples throughout the 1980s and 1990s for N-methylcarbamates, organochlorines, organophosphates, atrazine and simazine. In 1991, a FDA survey of processed foods found that 17% of pineapple samples had residues of the carbamate pesticide - benomyl. In 1992, the food contamination database maintained by FDA found that Hawaii had pesticide residues in 72% of food samples tested and was the #1 state for significant residues (14%) with a rate 2.5-fold greater than the next leading state (FDA, 1992).

   One source indicates that bromelain is manufactured by precipitation processes involving acetone and ammonium sulfate. Neither of these chemicals appear to have a significant toxicity profile. Ammonium sulfate is not considered a workplace hazard. Sodium chloride--table salt--is allowed in organic production. Acetone is moderately toxic (Sax, 1984). However, an earlier source indicates that sodium cyanide is involved in the precipitation process. (Tauber, 1949) Sodium cyanide has a toxicity rating of 6 (on a scale of 1 to 6 with 6 being highly toxic) and is one of the fastest poisons known to man (Gosselin, et al., 1984). Chronic exposure to low levels of cyanide is suspected to be responsible for various neuropathic and thyrotoxic conditions in humans (NTP, 1993).

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.

For all practical purposes, bromelain is non-toxic. Different studies have been unable to derive an LD_{50} that would induce death in rats or mice (Lotz-Winter, 1990). Most reported effects are beneficial, such as anti-carcinogenesis (Taussig and Batkin, 1988; Lotz-Winter, 1990). Bromelain improves digestibility of proteins, therefore it enhances nutritional quality (Chandrasiri, et al., 1990; Gallagher, Kanekanian, and Evans, 1994). Isolated soy protein treated with bromelain to remove bitterness peptides had over twice as much available lysine as acetylated soy protein (Yeom, Kim, and Rhee, 1994). By removing bitterness peptides, feed may be made more palatable.

Pigs given bromelain orally as an anti-diarrheal agent showed no adverse effects (Chandler and Mynott, 1998). Bromelains are used in human medicine in the treatment of soft tissue inflammation, edema from trauma and surgery, and as an aid to digestion. Adverse effects include nausea, vomiting, diarrhea, and menstrual flow abnormalities. However, the only precautions suggested were for cautious use in patients with impaired hepatic or renal function (Reynolds, 1996).

Like all proteins, enzymes carry with them the possibility of inducing allergies (Nielsen, et al., 1991). Its use as a food processing enzyme had not resulted in any reported cases of allergies in consumers as of the early 1980s, as might be expected due to its presence in foods at very low levels (Pariza and Foster, 1983). Allergies through other exposures may not be well-understood. Hypersensitivity reactions have occurred, including skin reactions and asthma, generally from occupational inhalation exposure or direct skin challenge allergy testing. There also, appears to be some cross-reactivity with papain manifested by skin reactions. Bromelain may also work synergistically in the presence of a given protein or free amino acid allergen, even when it is not an allergen *per se* (Pike, Bagarozzi, and Travis, 1997). Feed-mill workers who handle enzymes should wear proper clothing and respiratory equipment.

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 are not used for any of these purposes. However, enzymes may be used to improve the nutrient quality and digestibility of inferior feeds (Nielsen, et al., 1991). Bromelain is not used as a feed preservative.

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.

   At present, bromelain is not specifically GRAS as a feed additive. The only two such enzymes are papain and rennet (21 CFR 582). For an interim period, FDA will accept as GRAS for use as animal feed additives those enzymes that are GRAS for human food (AAFCO, 1998). Bromelain qualifies as GRAS for such use under this provision per 21 CFR 184.1024:

> "(a) Bromelain (CAS Reg. No. 9001-00-7) is an enzyme preparation derived from the pineapples *Ananas comosus* and *A. bracteatus* L. It is a white to light tan amorphous powder. Its characterizing enzyme activity is that of a peptide hydrolase (EC 3.4.22.32).
>
> "(b) The ingredient meets the general requirements and additional requirements for enzyme preparations in the Food Chemicals Codex, 3d ed. (1981) . . .
>
> "(c) In accordance with Sec. 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct food ingredient is based upon the following current good manufacturing practice conditions of use:
>
> "(1) The ingredient is used as an enzyme as defined in Sec. 170.3(o)(9) of this chapter to hydrolyze proteins or polypeptides.
> "(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.
>
> "[60 FR 32910, June 26, 1995]"

6. Is compatible with the principles of organic handling.

   Enzymes appear to be compatible given their natural origin and specific mode of action. The two main questions are (1) should enzymes from plants be required to be derived from organically raised plants? and (2) which, if any, extractants should be allowed to isolate and concentrate the enzyme? The reviewers addressed these questions in the discussion.

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.

   To assist in increasing the digestibility and palatability of certain foods and feeds, use of some enzymes may be beneficial. Enzyme concentrations in feed processing are effective at levels of less than 1% (Bedford, 1995). The minimum amount showing a positive effect in piglets was 250 mg/day. In hydrolyzing casein, the amount used is 1:500 bromelain:casein. Hydrolysis occurred with smaller amounts of enzymes derived from bacteria. However, bromelain has certain properties that are preferable to other bacterially derived protease enzymes screened (Gallagher, Kanekanian, and Evans, 1994).

Discussion

Condensed Reviewer Comments

None have a direct commercial or financial interest in enzymes in general or bromelain in particular. Reviewer 1 is a consultant in animal nutrition; reviewer 2 is a veterinarian and pharmacology researcher; reviewer 3 is a professor of food science.

Reviewer 1
(1) Should enzymes from plants be required to be derived from organically raised plants?
No strong feelings here but I would not think this would be necessary.

(2) Which, if any, extractants should be allowed to isolate and concentrate the enzyme?
The sodium cyanide and acetone method does not seem particularly compatible with organic standards and purposes, and therefore perhaps should not be allowed, but again no strong convictions on this question. However, [I] think it should be stated that production methods must be compatible with organic standards and purposes.

Enzymes derived from edible, non-toxic plants that have not been genetically engineered should be allowed as a non-organic ingredient, or if obtained in an organic manner from an organic plant as an organic ingredient, in food and as a feed additive for organic livestock production.

Reviewer 2
Based upon the literature, I would classify bromelain or any other plant derived enzyme as non-synthetic. However, the determinant is how it is isolated and manufactured. If the enzyme is manufactured using sodium chloride or organic ethyl alcohol followed by physical separation and concentration, then I believe that the enzyme should retain its non-synthetic status. Additionally, the source of all plant-derived enzymes must be from non-genetically engineered sources. Overall, I agree with the [information provided]. However, the official name for bromelain is spelled bromelain, not bromelin according to Fennema. I feel the most important issue outside the non-genetically engineered requirement, is how the enzyme is manufactured. Therefore the manufacturers of all commercial preparations of plant enzymes and microbial derived enzymes should be required to submit a process flow diagram showing use of all chemicals and process operations. This requirement is similar to the proposed annotation.

In summary, I agree with OFPA that bromelain as a plant derived enzyme is not synthetic and must meet the requirements of the proposed annotation to retain its use as an organic feed additive.

Reviewer 3

The first question is whether it is synthetic or not. This may be a close question. The processes described in the literature for its derivation from pineapples appear to be more physicochemical processes, i.e., filtration, washing, dissolution, and precipitation, designed to separate the substance from its biological matrix in the natural state rather than processes implemented for the purpose of inducing chemical change through reaction and conversion. Nonetheless, it is extracted "by a chemical process" and therefore is presumptively synthetic unless a particular petitioner wants to put forth proof to the contrary for his or her particular product.
The next question is what is its use in organic livestock production - is it a medicine or a routine feed additive. It could be considered either one as continued research will probably add to its value as a "homeopathic" (using the word in the broad sense) therapeutic agent perhaps giving it wider scope for inclusion in organic livestock production. In either case, its use appears to be compatible with a program of sustainable agriculture. I would, however, concur with the proposed annotation for these substances in the NOSB Materials Database and add in the case of bromelain that it should be derived from organically grown pineapples (because of the particular environmental and health implications associated with the pesticidal regimens typically used in pineapple production) unless that is currently a commercial impossibility.

Conclusion

While enzymes may not be necessary in every situation, they appear to be naturally occurring substances that are compatible with organic principles. Enzyme treatment of some feedstuffs may improve amino acid availability and as a result reduce nitrogen pollution (Tamminga and Verstegen, 1992). Some plant enzymes may also have some anthelmintic properties (Tauber,1949). By improving feed efficiency, animals can meet their nutritional needs with less feed and produce less manure (Bedford, 1995). Enzymes derived from edible, non-toxic plants, and non-pathogenic bacteria or fungi that have not been genetically engineered should be allowed as a non-organic ingredient in food and as a feed additive for organic livestock. The suppliers of enzymes should report any co-factors used in the formulation. Carriers, diluents, and processing aids used in enzyme preparations for animal production must be of feed grade and be allowed for use in organic food processing or organic livestock production.

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