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IMPORTANCE OF ENZYMES TO VALUE-ADDED QUALITY OF FOODS

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Abstract

New uses of enzymes are of major importance for value-added quality of foods. At the production and postharvest handling levels, enzymes associated with maturation, color, texture, flavor and nutritional changes are important. Recent examples are discussed which include longer storage prior to initiation of ripening, deletion or control of genes that result in softening, browning and other quality defects and the enhancement of genes that improve the nutritional quality of foods. At the processing level, new uses of enzymes are described to change the properties of proteins and lipids, to eliminate off-flavor in beers and sterilized milk, and to monitor adequate blanching of fruits and vegetables.

Key Words: Enzymes, value-added quality, food quality, sensory properties, nutritional quality, gene manipulation, protein modification, lipid modification, off-flavor elimination, beer, milk, vegetables, blanching.

Introduction

Future major increases in jobs in the United States will be primarily in the service sector, economists and planners agree. Much of the emphasis will be on the high tech industries, i.e. computers, electronics, fine chemicals, etc. But we must not overlook the important opportunities that food materials produced by the United States will play in helping feed the world. Our past and, to some extent, our current role is based primarily on the quality and quantity of raw food materials produced. However, several other countries now produce and export raw food materials of equivalent quality to products from the United States, at highly competitive, or in some cases lower, prices. This competitive environment has led to emphasis from the United States on "value added products" for export. Value added products usually imply post harvest modifications (formulations for example) to increase the net value of the product. In pursuing this approach, we must remember that it is difficult, if not impossible, to make a high quality food from a low quality raw material.

This article explores several applications of enzymology that significantly enhance the quality of foods or raw materials. The discussion emphasizes several practical applications to be considered in applying enzymes to: a) improve the quality of raw food materials; b) control and/or monitor food quality attributes; c) stabilize foods; d) enhance nutritional, safety, and functional qualities of processed foods; and e) produce food ingredients via cell cultures.

Improve the Quality of Raw Food Materials

Enzymes are very important in the growth, maturation and post harvest stability of raw food materials. They are also very important in the quality (color, flavor, aroma, texture and nutritional quality) of foods. For many years, plant and animal breeders have diligently developed crops and animals which provide greater yield. More recently, attention has been given to developing plants and animals with improved disease and drought resistance and animals with improved yields based on feed consumption and improved lean to fat ratios. Less attention has been given to improving the

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Table 1. Problems and solutions.*

Problems	Solutions
1. Deficit of casein clotting enzymes	1. Produce chymosin in microorganisms
2. Disposal of lactose containing whey	2. Produce lactase in yeast
3. Enzyme instability/stability	3. Change critical amino acids via recombinant DNA
4. Low yields of needed enzymes and proteins	4. Amplify number of copies of genes
5. Frost damage to plants	5. Delete ice nucleation protein from <i>Pseudomonas syringae</i>
6. Chilling damage to plants	6. Insert gene for antifreeze protein
7. Softening of fruits and vegetables	7. Regulate expression or level of pectic enzymes
8. Lipoygenase caused off flavors	8. Limit expression of gene
9. Polyphenol oxidase caused browning	9. Limit expression of gene
10. Low nutritional value of proteins	10. Increase digestibility by enhancing instability; amplifying genes for proteins with higher level of essential amino acids
11. Control ripening	11. Repress gene for ethylene producing enzyme
12. Insect resistance	12. Incorporate genes for insect inhibitors
13. Selective resistance to herbicides	13. Insert gene for enzyme to catalyze herbicide

*Adapted in part from Wasserman *et al.*, 1988.

nutritional quality, safety and sensory properties of raw food materials. Post harvest storage under controlled atmospheric conditions has significantly increased the storage life of products by maintaining the color, flavor, and texture of some foods. Particularly important has been controlled atmosphere storage of apples, and more recently fish and salads. Results with apples now allow delivery of high quality apples year-round.

What opportunities exist for further improvements? Recent research has suggested several intriguing and potentially major opportunities which are worth considering (Table 1). These opportunities include: a) control, reduction or elimination of enzymes that adversely affect food quality; b) elimination of toxic and/or anti-nutritional components; and c) quantitative increases in enzymes that lead to more desirable qualities. Let us now consider some specific examples of enzyme control or application that can significantly impact food quality.

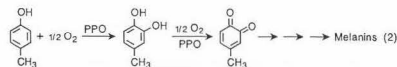
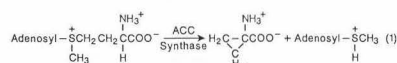
Color

Changes from green to red or yellow during the ripening of tomatoes, strawberries, red delicious apples, bananas, etc., are essential, important positive changes, while the browning of bruised or sliced potatoes, apples, peaches, and leafy salads, and the bleaching of the green color of green beans, English peas, and leafy vegetables are usually considered undesirable changes.

Color develops during ripening. It results from the maturation (senescence) process that leads to rapid increase in cell size, flavor enhancement, texture decrease, and other changes, all catalyzed by various enzymes. Senescence also signals the near completion of the life cycle of the fruit at which point continued changes lead to deterioration.

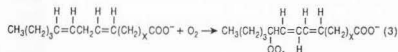
Ideally, we want to stop or delay the process at this point through processing. Currently, delivery of quality products such as for climacteric fruits requires harvesting at the mature but green stage to allow for storage and transportation time. The quality of such

fruits never seems to be the same as a fruit ripened on the vine or tree. Very recently, Oeller *et al.* (1991) demonstrated that tomato fruit senescence can be reversibly inhibited by antisense RNA. They engineered into the tomato the expression of antisense RNA to the biosynthesis of 1-aminocyclopropane-1-carboxylate synthase (ACC synthase), the rate-limiting enzyme step in the production of ethylene required for ripening (Eqn. 1; Yang and Hoffman, 1984). The inhibition of ethylene production allows storage of tomato in a suspended maturation state, until exogenous ethylene or propylene is administered to initiate continuation of the ripening steps. The authors discuss the prospect that the life-span of plant tissues can be extended, thereby increasing and controlling the storage time. An example of a current practice is the maintenance of bananas at the green mature stage in storage, and ripening by ethylene administration; this enhanced storage time has been practiced for several years.



Enzymatic browning of fruits and vegetables is usually considered an undesirable change. This browning of fruits and vegetables is primarily due to the action of the enzyme polyphenol oxidase (PPO). PPO catalyzes the oxidation of phenols to *o*-benzoquinones which then polymerize nonenzymatically to melanins (Eqn. 2) of various colors. Up to 50% of some tropical fruit crops are lost commercially due to browning. pH adjustment, or addition of sodium bisulfite, sulfur dioxide, ascorbic

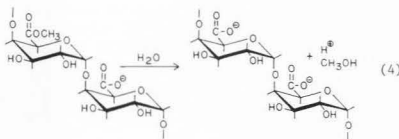
acid or thiol compounds are effective methods to control enzymatic browning in juices, purees and slices but cannot be used in intact fruits. Conventional plant breeding has reduced the amounts of PPO in some fruits, such as peaches. Further reduction should be possible by recombinant DNA technology through repression of the expression of the PPO gene (antisense RNA) or by removal of the genes that express PPO. The only known physiological function of PPO is in wound healing of plant tissue.



Another undesirable color change is the loss of green color in certain vegetables. Bleaching of the green color, due to chlorophyll, of green beans, English peas and some leafy vegetables is due primarily to hydroperoxy-free radicals produced by lipoygenase acting on polyunsaturated lipids and/or polyunsaturated fatty acids (Eqn. 3). The lipoygenase level of soybean seeds has been substantially reduced without adverse physiological effects (Kitamura, 1984; Wang *et al.*, 1990). There is no apparent reason why lipoygenase levels of other plants cannot be reduced by recombinant DNA techniques, to minimize the effect of this enzyme on quality of raw foods.

Texture

Ripening of fruits and some vegetables leads to softening. Some softening is essential, but too much softening is undesirable. Softening is primarily the result of pectic enzymes acting on the pectic substances in the middle lamella between cells. In some cases, cellulases and hemicellulases may also play a role. In tomatoes, control of softening has been attempted through two approaches which have been used to try to decrease the level of one or more of the polygalacturonases. These approaches included manipulation of the gene expressing one of the polygalacturonases (Bennett *et al.*, 1989; DellaPenna *et al.*, 1990); the other method used antisense RNA to inhibit expression of polygalacturonase (Smith *et al.*, 1988). As demonstrated by Oeller *et al.* (1991), expression of the polygalacturonase gene during ripening appears to be ethylene independent, raising the provocative question of what is the control mechanism for polygalacturonase production in the senescencing tomato.



Pectin methyl esterase (PME), a pectic enzyme, is responsible for cloud separation in citrus juice. The

cloud, due to suspended pectic substances, is highly desirable in citrus juices. When PME removes the methoxy group by hydrolysis, forming pectic acid (Eqn. 4), Ca^{2+} cross-links pectic acids, leading to precipitation. Possible solutions, all at the juice level, include heat inactivation of PME (leading to some loss of fresh flavor), removal of Ca^{2+} (not very practical) or addition of exogenous polygalacturonase which hydrolyzes the pectic acid formed to smaller fragments that still give a cloud, but do not precipitate with Ca^{2+} (Baker and Bruemmer, 1972). Two possible solutions at the production side would be to reduce the PME level or enhance the polygalacturonase level by recombinant DNA techniques. The author is not aware of any research along these lines.

Flavor

Citrus juices, especially grapefruit, can be quite bitter due to naringin and limonin. Naringinase has been used, in batch and immobilized column formats, to hydrolyze the bitter compounds to non-bitter compounds (Maier *et al.*, 1973). There has also been substantial research on inhibition of the biosynthesis of naringin and limonin, since the pathway of their biosynthesis via the mevalonic pathway is well known (Hasegama and Herman, 1986; Ou *et al.*, 1988) and intervention can take place at several points to effectively inhibit formation of naringin or limonin.

Nutritional Quality

Relatively little attention has been given to improving the nutritional quality of raw food materials. Nutritional quality of products might be enhanced in one of two ways, either by decreasing the levels of toxic or antinutritional factors naturally present or by increasing the nutritional quality of the product. Some antinutritional compounds in foods include erucic acid, raffinose, phytic acid, cyanogenic glycosides, thioglycosides, solanine, protease inhibitors, and α -amylase inhibitors. There is considerable opportunity to reduce the levels of these antinutritional compounds in foods by traditional plant breeding or by recombinant DNA techniques. They can also be reduced or removed via added enzymes during processing (Whitaker, 1990).

Many plants, especially the cereals and legumes, produce high levels of protein in their seeds. However, these proteins are frequently deficient in one or more essential amino acids such as methionine (legumes) and lysine (cereals). Recently, de Lumen and his co-workers, and others (Gepts and Bliss, 1984), have begun to address this. As shown by de Lumen and coworkers, the soybean contains a small amount of a protein high in methionine (~12%). The protein has been isolated, sequenced and there is research designed to insert the isolated gene for this protein into common beans (*Phaseolus vulgaris*) genome and amplify its expression in the seed (A.A. George and B.O. de Lumen, personal communication, 1992).

Two other methods are important in enhancing the nutritional levels of methionine available from beans.

Sgarbieri and his colleagues in Brazil have developed a new cultivar of Carioca (Carioca 80) in which greater than 80% of the methionine is biologically available, whereas less than 50% of the methionine is biologically available in Carioca and other common beans (Tezoto and Sgarbieri, 1990). George *et al.* (1992) have suggested explanations for this difference in biological availability of methionine in Carioca 80 seeds versus other common bean seeds, based primarily on differences in digestibility of the proteins. In a second method, substantial improvement in nutritional quality could be achieved by preventing the rapid loss of methionine, the limiting essential amino acid, during storage. The loss is probably a result of lipid peroxidation, which might be eliminated by preventing lipid peroxide formation by enhanced antioxidant levels in the bean, changing lipid composition of the bean or removing lipoxygenase from the bean, all by recombinant DNA biotechnology.

Other

Space does not permit discussion of other opportunities to improve the quality and quantity of raw food materials by controlling: a) chilling damage by incorporation of genes for antifreeze proteins (Feeney *et al.*, 1986); b) deleting ice nucleation protein from *Pseudomonas syringae* (Lindow and Connell, 1984; Orser *et al.*, 1985); c) decrease insect damage by incorporation of genes for inhibitors of insects; and d) selective resistance to herbicides by amplification of key enzymes (Steinrücken and Amrhein, 1984; see Table 1).

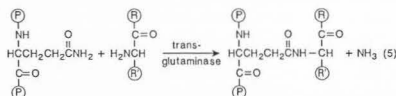
Improve the Quality of Processed Foods

Whitaker (1990) has recently described many new and future uses of enzymes in food processing (Table 2). Discussed are enzymes used to: a) report on the quality of selected foods; b) produce wanted compounds; c) remove unwanted compounds; d) control microorganisms; e) modify/change pectic substances; f) improve specificity by purification; g) modify lipids; and h) modify selected food products. He made a special case for use of more highly purified enzymes, so that the specificity and selectivity of enzymes would be paramount. A few selected examples are detailed here to illustrate the diversity of use of enzymes in food processing.

Change in Protein Properties

Proteins are responsible for many functional properties of foods. Sometimes, the source of the protein, as well as the proteases, pentosanases and other enzymes, can make a difference in properties, as in bread, crackers and cakes. These properties can be further modified by limited (2-5%) specific hydrolysis of proteins, with resulting changes in solubility, emulsifying, foaming and whipping properties (Chobert *et al.*, 1987; F. Vojdani and J.R. Whitaker, 1992, unpublished data). Major changes in functionality can also be achieved by cross-linking of proteins, either molecules of the same protein or different proteins (Neidle *et al.*, 1958; Cooke and Holbrook, 1974; Soria *et al.*, 1975; Ikura *et al.*, 1980a,

1980b; Motoki and Nio, 1983). The enzyme, transglutaminase, catalyzes the nucleophilic addition of an amine group (on a protein, amino acid or other compound) to the side chain of a glutamyl group of a protein (Eqn. 5). Therefore, one can: a) cross-link molecules of the same protein, doubling, tripling, etc., the molecular weight and changing the functional properties; b) cross-link molecules of two or more proteins, increasing the molecular weight and substantially changing the functional properties; c) covalently attach amino acids (such as essential amino acids); or d) covalently attach homogeneous or heterogeneous synthetic polymers of amino acids. The covalent attachment of essential amino acid-containing polymers can alter substantially the nutritional quality of a protein, as well as its functional properties. Based on research of Puigserver *et al.* (1979), the peptide bonds of the polymer are hydrolyzed by intestinal proteases.



Change in Lipid Properties

The fatty acid composition and location of a fatty acid on glycerol markedly affect the physical states of triglycerides. The higher the molecular weight and the more saturated the fatty acid, the higher the melting point of a triglyceride. Tributyrin is a liquid at room temperature (melting point, m.p. -75°C) while trimyristin is a solid (m.p. $56-57^\circ\text{C}$). Tristearin melts at 55°C , while triolein melts at -4 to -5°C . Homogeneous triglycerides have sharp melting points, while heterogeneous triglycerides or mixtures of several homogeneous triglycerides melt at a lower temperature and over a broader range. Synthetic lipids can now be tailored to have properties approximating those of high value natural lipids such as found in cocoa butter. Required is knowledge not only of the fatty acid composition but also percentage of each fatty acid in the α and β positions. Then, by the use of 1,3- and 2- specific lipases the fatty acids can be correctly attached to glycerol to give a mixture of triglycerides with properties similar to that found in nature, such as cocoa butter (Sawamura, 1988). The reactions involved with specific lipases are shown in Equations 6 and 7, in contrast to that with a non-specific lipase (Eqn. 8).

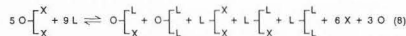
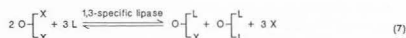
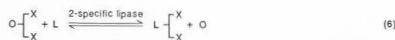


Table 2. Improve quality of processed foods.

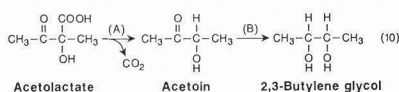
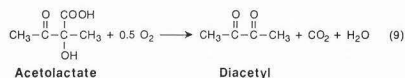
Problems	Solutions
1. Resolve DL-amino acids	1. Aminoacylases
2. 5'-Nucleotides for flavor	2. 5' Phosphodiesterases; 5' Adenylic deaminase
3. Cheese/butter flavors	3. Lipases (pregastic)
4. Decrease cheese ripening times	4. Proteases, lipases
5. Cyclodextrins for separations	5. Cyclomalto-dextrin glucanotransferase
6. Modified food gums	6. α -Galactosidase
7. a. Remove raffinose b. Remove phytic acid c. Remove oxidized flavor of milk d. Remove carbamates e. Remove bitter compounds in citrus f. Remove cyanogenic glycosides	7. a. α -Galactosidases b. phytase c. sulphydryl oxidase d. urease e. naringinase
8. Specialty triglycerides, others	8. Specific lipases
9. Soybean milk coagulation	9. Microbiol proteases
10. Increase dough moistness	10. Pentosanases
11. Light beer	11. Amyloglucosidases
12. Avoid diacetyl in beer	12. Acetolactate decarboxylase

Waxes and specific di- and monoglycerides can also be produced by transesterification reactions (Heldt-Hansen *et al.*, 1989).

Brewing

There are at least three major changes in uses of enzymes in brewing during the last five to six years. These include the addition of α -amylglucosidases in the mashing phase to hydrolyze the α -1,6 glucose linkages of amylopectin, thereby permitting complete conversion of starch to glucose and its fermentation to CO₂ and ethanol (light beer; Scott, 1989). β -Glucanases are also added during the mashing phase to hydrolyze the glucans present from plant cell walls, thereby reducing the viscosity of the mash, and facilitating the filtration step (Enari *et al.*, 1987).

The most remarkable enzyme advance in brewing has been the use of acetolactate decarboxylase (Olsen and Aunstrup, 1986; Rostgaard *et al.*, 1987; Sone *et al.*, 1987). Addition of acetolactate decarboxylase is designed to prevent formation of diacetyl, in order to avoid an off-taste and to cut the fermentation time. The reaction involved in diacetyl formation is shown in Equation 9.

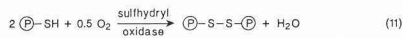


During fermentation, acetolactate is produced by the yeast as an intermediate in the biosynthesis of isoleucine and valine. The acetolactate left at the end of the primary fermentation is oxidized nonenzymatically to diacetyl (Eqn. 9). The level of diacetyl at the end of primary fermentation is around 0.10-0.15 mg/liter, while the taste threshold is 0.05 mg/liter. Therefore, beer is subjected to a secondary fermentation, lasting several weeks, during which the acetolactate diffuses back into the yeast cells where it is reduced to acetoin. By adding acetolactate decarboxylase, the acetolactate is decarboxylated to acetoin and CO₂ (Step A, Eqn. 10) and the acetoin is then reduced by a dehydrogenase to 2,3-butylene glycol (Step B, Eqn. 10), which contributes no flavor.

The gene for acetolactate decarboxylase has been inserted into brewing yeast, so that the acetolactate is removed as rapidly as formed during the primary fermentation step (Sone *et al.*, 1987).

Elimination of Cooked Flavor in Milk

Ultra high temperature (UHT) treatment of milk, sterilization, permits storage of milk for several weeks at room temperature. This process is widely used in some countries, such as Mexico and Brazil, because of lack of refrigerators in many homes. The process is not used in the United States because of the cooked off-flavor. It has been shown that the off-flavor results from the thermal reduction of protein disulfides, forming thiol compounds (Swaigood and Horton, 1989; Swaigood *et al.*, 1982). Sulphydryl oxidase-treated UHT milk does not develop the typical oxidized-type flavors during long storage, since the sulphydryl groups are oxidized to disulfides (Eqn. 11; Swaigood *et al.*, 1982; Swaigood and Horton, 1989). Sulphydryl oxidase has also been used to strengthen weak wheat doughs by catalyzing disulfide formation (Scott, 1989).



Blanching of Fruits and Vegetables

Fruits and vegetables heat treated to kill microorganisms and inactivate enzymes can be stored for several months in the frozen state, as long as they are protected from recontamination with microorganisms as shown first by Kohman (1928). In order to preserve as much of the fresh qualities (flavor, aroma, color, texture and nutrition) as possible, at the same time killing microorganisms and inactivating enzymes, the minimum heat possible should be applied. Almost from the beginning of the frozen food industry in the 1930's, there was disagreement as to the indicator enzyme to use to determine adequate heat treatment. Catalase was used in some

cases, peroxidase in others. Eventually, peroxidase was almost universally the enzyme of choice, because its inactivation led to the best keeping quality of the frozen foods. This is not surprising since peroxidase is usually the most heat-stable enzyme found in vegetables and fruits, so by the time it is inactivated no other enzymes or microorganisms remain. But there is no evidence that peroxidase is involved in deteriorative reactions in the food.

Williams *et al.* (1986) discussed the question of which indicator enzyme to use. It would appear obvious the best indicator would be the enzyme causing the problem. Enzymatic browning is caused by polyphenol oxidase; bleaching of carotenoids and chlorophyll is generally caused by lipoxygenase. Softening is caused by the pectic enzymes and flavor loss or off-flavor is caused by a number of enzymes. Williams *et al.* (1986) developed a protocol, combining protein chemistry, enzymology and sensory testing, to determine the key enzyme(s) involved in quality deterioration. Their research has led to the conclusion that lipoxygenase is responsible for off-flavor development in green beans, English peas and corn (Lim *et al.*, 1989; Velasco *et al.*, 1989) while cystine lyase is the key enzyme in development of off-flavor in broccoli and cauliflower (Velasco *et al.*, 1989). Off-flavor development by lipoxygenase is due to polyunsaturated fatty acid peroxidation, regardless of whether the fatty acids are free or in triglyceride form. Cystine lyase converts cystine to pyruvate, hydrogen sulfide and ammonia. The off-flavor and off-odor results from the last two compounds. Pilot plant studies, including storage, show that a superior product results when these enzymes are used as indicators of adequate blanching.

Tailor Enzyme Preparations for Value-Added Foods

To date, most enzyme preparations used in food processing are crude extracts. For example, pectic enzyme preparations produced by *Aspergillus niger* have been used for a long time to increase juice yield and to clarify juices. But, as shown in Table 3, there are numerous other hydrolytic enzymes present that also break down polymers in plants. The overall results - juice yield, clarity, color, flavor, taste - occur from the composite effect of these enzymes. Would better food products be achieved if only the pectic enzymes were used? Perhaps only one of the three groups of pectic enzymes should be used? Could industry afford to use more highly purified enzymes? No one probably knows the answers to these questions at the moment. But if we are to be competitive with the European Common Market, Japan and other developed, and developing countries, we need answers to these questions. New, large scale purification techniques, production of desired enzymes by recombinant DNA techniques and increasing knowledge of enzymology permit us to make better food products, if we are willing to ask and answer the right questions of what needs to be done and how we can do it.

Food Ingredients By Cell Culture

One can clearly envision the time, not too distant, when many food ingredients will be produced in factories using plant and animal cell culture, just as many of our ingredients for food and medicine are made by bacterial fermentations. Plant and animal cells are more fragile than microbial cells, so that must be researched and solved. Removal of heat and transport of nutrients in and products out through the cell wall and how to turn on the desired genes and turn off others, including those for proteases, need to be solved. Minimization or elimination of toxic substances is needed. But colors and flavor compounds, vitamins and enzymes have been produced in the laboratory. Pilot scale-up and commercialization is not too far away, especially where the economics are favorable.

Summary

Several enzymatic methods have been described for value added enhancement of raw and processed foods. These methods include strategies for color, flavor, texture and nutritional quality improvement of the products. In some cases, the strategy calls for elimination of one or more deteriorative enzymes by use of selective heat treatment or recombinant DNA technology. In other cases, the genes for an undesirable enzyme may be removed by recombinant DNA technology or a desired enzyme may be inserted or increased in level, by recombinant DNA technology. Exogenous enzymes can be added during processing to change the properties of proteins, lipids and carbohydrates in foods, thereby improving the water-holding properties, the foaming properties, the emulsifying properties, and/or the solubilities of key food ingredients.

Crude enzyme preparations used in food processing will soon be replaced by more highly purified enzyme preparations, leading to more specificity and control of the desired changes. Extraction of food ingredients, especially flavors and colors will shortly be replaced with use of plant, animal and microbial cell cultures that synthesize predominantly one ingredient; this will greatly improve the efficiency of production, and the economy and quality of product by minimizing extraction and purification steps required.

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Table 3. Enzymes produced by *Aspergillus niger* which hydrolyze polymers.*

Enzyme	Substrate
α -L-Arabinofuranosidase	Arabinans
Cellulases, cellobiohydrolases, β -glucosidases	Cellulose
Dextranase	Dextran
Deoxyribonuclease, ribonuclease	DNA, RNA
β -Glucanase	β -Glucans
Hemicellulases	Hemicellulose
Inulinase	Inulin
β -Mannanase	Mannans
Pectin methylsterase, pectate lyase, polygalacturonase	Pectic substances
Proteases	Proteins
α -Amylase, glucoamylase	Starch
Xylanase	Xylans

*Enzymes referenced in Chemical Abstracts 1977-1982 alone.

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Discussion with Reviewers

R.F. McFeeters: Has it been demonstrated that reduction of polygalacturonase activity by manipulation of gene expression in the tomato has actually affected softening of the tomato tissue during ripening?

Author: This is an important question since different results are reported in the literature. The Flavr Savr tomato, developed by Calgene using an antisense RNA method against a polygalacturonase, does have a substantially reduced rate of pectin breakdown, effectively extending the storage time. Other workers (Bennett *et al.*, 1989; DellaPenna *et al.*, 1990) do not find similar results in transgenic *rin* tomato fruits. The *rin* tomatoes are mutants with other properties also different from wild type tomatoes.

J.E. Spradlin: Do you think it feasible to expand the delayed ripening of tomatoes via antisense RNA concept to a wide variety of other fruits and vegetables such that in the future produce can be ripened on demand either by the grocer or in the home by the consumer?

Author: The antisense method of inhibiting expression of a gene is a general approach. The method requires isolating the gene for a particular enzyme or protein, sequencing a portion of the gene and synthesizing a complementary antisense (reverse) RNA segment. All of these requirements are met using present technologies. Therefore, there is every reason to expect this method can be extended to other fruits and vegetables, and to animals.

J.E. Spradlin: You referred to antisense RNA and recombinant DNA technologies several times. Do you think that commercial quantities of produce will be produced from plants resulting from these technologies within the next five to ten years?

Author: Commercial quantities of produce from plants produced by these technologies definitely will be available within five to ten years, and likely as early as 1993. This results from the decision of the U.S. Department of Agriculture in May 1992 to not subject transgenic plants to more stringent review than plants produced by standard breeding practices. Calgene has received approval of the U.S. Department of Agriculture to proceed with commercialization of its Flavr Savr tomato, which it expects to do in 1993. Some 70 other transgenic plants are in various stages of development.