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Module
on
Role Of Microbial Enzymes In Foods

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TEXT

Introduction:

Enzymes are produced by all living cells as catalysts for specific chemical reactions. Not surprisingly enzymes are present in all foods at some time, and play an increasingly important role in food processing techniques. Enzymes, although not recognized as such, have played an essential part in some food processes, notably the making of cheese, leavened bread, wine and beer, for thousands of years. In the case of food industry, enzymes are used to improve characteristics in the final product such as texture, shelf life, color, flavor, odour and more. Although plants, fungi, bacteria and yeasts produce most enzymes, microbial source produced enzymes are more advantageous than their equivalents from animal or vegetable sources. The advantage assets comprise lower production costs, possibility of large-scale production in industrial fermenters, wide range of physical and chemical characteristics, possibility of genetic manipulation, absence of effects brought about by seasonality, rapid culture development and the use of non-burdensome methods. The above characteristics make microbial enzymes suitable biocatalysts for various industrial applications. Food processing industry uses around 29% of total enzymes produced and 58% of these enzymes are obtained from fungi, 28 and 5% from bacteria and yeast respectively. Enzymes of microbial origin have been employed since the decades of 80's due to their easiness in production, availability and cost-efficiency.

Modern Production of Food Enzymes

In the twentieth century, enzymes began to be isolated from living cells, which led to their large-scale commercial production and wider application in the food industry. Today, microorganisms are the most important source of commercial enzymes. Although microorganisms do not contain the same enzymes as plants or animals, a microorganism can usually be found that produces a related enzyme that will catalyse the desired reaction. Enzyme manufacturers have optimized microorganisms for the production of enzymes through natural selection and classical breeding techniques. Direct genetic modification (biotechnology) encompasses the most precise methods for optimizing microorganisms for the production of enzymes. These methods are used to obtain high-yielding production organisms. Biotechnology also provides the tools to have a genetic sequence from a plant, animal, or a microorganism, from which commercial scale enzyme production is not adequate, to be transferred to a microorganism that has a safe history of enzyme production for food use. Although the production organism is genetically modified the enzyme it produces is not. Enzymes produced through biotechnology are identical to those found in nature. Additionally, enzymes produced by microorganisms are extracted and purified before they are used in food manufacturing. Genetically modified microorganisms are useful from a commercial standpoint but would not survive in nature.

Major Enzyme Applications in Foods

In food industry, enzyme has been used to produce and to increase the quality and the diversity of food. Some examples of products that use enzyme are cheese, yoghurt, bread syrup etc. Ancient traditional arts such as brewing, cheese making, meat tenderization with papaya leaves and condiment preparation (e.g., soy sauce and fish sauce) rely on proteolysis, albeit the methods were developed prior to our knowledge of enzymes. Early food processes involving proteolysis were normally the inadvertent consequence of endogenous or microbial enzyme activity in the foodstuff. Some major applications by the types of microbial enzymes are:

Pectinase enzyme:

Plants, filamentous fungi, bacteria and yeasts produce the pectinase enzymes group with wide use in the food and beverages industries. The enzyme is employed in the food industries for fruit ripening, viscosity clarification and reduction of fruit juices. The main application of the above mentioned enzyme group lies within the juice processing industry during the extraction, clarification and concentration stages. The enzymes are also used to reduce excessive bitterness in citrus peel, restore flavor lost during drying and improve the stableness of processed peaches and pickles. Pectinase and β-glucosidase infusion enhances the scent and volatile substances of fruits and vegetables, increases the amount of antioxidants in extra virgin olive oil and reduces rancidity. The advantages of pectinase in juices include, for example, the clarification of juices, concentrated products, pulps and purees; a decrease in total time in their extraction; improvement in the production of juices and stable concentrated products and reduction in waste pulp; decrease of production costs; and the possibility of processing different types of fruit. For instance, in the production of passion fruit juice, the enzymes are added prior to filtration when the plant structure's enzymatic hydrolysis occurs. This results in the degradation of suspended solids and in viscosity decrease, speeding up the entire process. Several species of microorganisms such as Bacillus, Erwinia, Kluyveromyces, Aspergillus, Rhizopus, Trichoderma, Pseudomonas, Penicillium and Fusarium are good producers of pectinases. Among the microorganisms which synthesize pectinolytic enzymes, are fungi, especially filamentous fungi, such as Aspergillus niger and Aspergillus carbonarius, which are preferred in industries since approximately 90% of produced enzymes may be secreted into the culture medium. In fact, several studies have been undertaken to isolate, select, produce and characterize these specific enzymes so that pectinolytic enzymes could be employed not only in food processing but also in industrial ones. High resolution techniques such as crystallography and nuclear resonance have been used for a better understanding of regulatory secretion mechanisms of these enzymes and their catalytic activity.

Lipases:

Lipolytic enzymes such as lipases and esterases are an important group of enzyme associated with the metabolism of lipid degradation. Lipase-producing microorganisms such as *Penicilliumrestrictum* may be found in soil and various oil residues. Several microorganisms, such as *Candidarugosa, Candida antarctica, Pseudomonasalcaligenes, Pseudomonas mendocina* and *Burkholderia cepacia*, are lipase producers. Other research works have also included *Geotrichum* spp., *Geotrichum candidum*, *Pseudomonas cepacia, Bacillus tearothermophilus, Burkholderia cepacia*, *Candida lipolytica, Bacillus coagulans*, *Pseudomonas aeruginosa*, *Clostridium thermocellum and Yarrowialipolytica*.

The fungi of the genera *Rhizopus*, *Geotrichum*, *Rhizomucor*, *Aspergillus*, *Candida* and *Penicillium* have been reported to be producers of several commercially used lipases. Lipases are used for development of characteristic flavors, these enzymes hydrolyse short chain fatty acids, thus decreasing the formation of trans-fatty acids and obtaining about 15-30 times more flavor than traditional process. Types of cheese is dependent upon the process of ripening, which gives characteristic textures and aromas. In ripening lipases play a major role in hydrolysis of triglycerides, diglycerides, monoglycerides, fatty acids and glycerol to free fatty acids, which are responsible for characteristic flavor development. Ripening process with lipases is about 2 to 5 times faster than without it. However, it is important optimize the amount of enzyme added and its enzymatic activity, as high levels of lipases could lead to rancidity and reduction in yield.

Lactase:

Popularly known as lactase, β -galactosidases are enzymes classified as hydrolases. They catalyze the terminal residue of β -lactose and produce glucose and galactose. Lactase's production sources are peaches, almonds and certain species of wild roses; animal organisms, such as the intestine, the brain and skin tissues; yeasts, such as *Kluyveromyces lactis*, *K. fragilis* and *Candida pseudotropicalis*; bacteria, such as *Escherichia coli*, *Lactobacillus bulgaricus*, *Streptococcus lactis* and *Bacillus* sp; and fungi, such as *Aspergillus foetidus*, *Aspergillus niger*, *Aspergillus oryzae* and *Aspergillus Phoenecia.*

Lactase is highly important in the dairy industry, in the hydrolysis of lactose into glucose and galactose. Dairy products represent a wide area in the food industry, which produces a large variety of products such as milk, cream, cheese, butter, ice cream and yogurt. The principal function of microbial enzymes is to improve the process or characteristic of final products, such as flavor, colour, consistency, composition, appearance and structure. One of the principal focus of dairy industry is the development of lactose-free products. Because of intestinal enzyme insufficiency, some individuals, and even a population, show lactose intolerance and difficulty in consuming milk and dairy products. Hence, low-lactose or lactose-free food aid programme is essential for lactose-intolerant people to prevent severe tissue dehydration, diarrhea, and, at times, even death. Another advantage of lactase-treated milk is the increased sweetness of the resultant milk, thereby avoiding the requirement for addition of sugars in the manufacture of flavored milk drinks. Lactose is removed either by partial hydrolysis by β-amylase, or complete hydrolysis by lactase. Lactase hydrolyses the glycosidic bond resulting in the union of hydrogen in the extreme oxygen and union of OH- to free carbon, obtaining glucose and galactose. Lactase preparations from *Aspergillus niger*, *Aspergillus oryzae*, and *Kluyveromyces lactis* are considered safe because these sources already have a history of safe use and have been subjected to numerous safety tests. The most investigated *E.coli* lactase is not used in food processing because of its cost and toxicity problems.

Cellulases:

Cellulases are enzymes that break the glucosidic bonds of cellulose microfibrils, releasing oligosaccharides, cellobiose and glucose. These hydrolytic enzymes are not only used in food, drug cosmetics, detergent and textile industries, but also in wood pulp and paper industry, in waste management and in the medical-pharmaceutical industry. In the food industry, cellulases are employed in the extraction of components from green tea, soy protein, essential oils, aromatic products and sweet potato starch. Coupled to hemicellulases and pectinases they are used in the extraction and clarification of fruit juices. After fruit crushing, the enzymes are used to increase liquefaction through the degradation of the solid phase. The above enzymes are also employed in the production process of orange vinegar and agar and in the extraction and clarification of citrus fruit juices. Cellulases supplement pectinases in juice and wine industries as extraction, clarification and filtration aids, with an increase in yield, flavor and the durability of filters and finishers.

Cellulase is produced by a vast and diverse fungus population, such as the genera *Trichoderma*, *Chaetomium*, *Penicillium*, *Aspergillus*, *Fusarium* and *Phoma*; aerobic bacteria, such as *Acidothermus*, *Bacillus*, *Celvibrio*, *pseudonomas*, *Staphylococcus*, *Streptomyces* and *Xanthomonas*; and anaerobic bacteria, such as *Acetivibrio*, *Bacteroides*, *Butyrivibrio*, *Clostridium*, *Erwinia*, *Eubacterium*, *Pseudonocardia*, *Ruminococcus* and *Thermoanaerobacter*.

Amylases:

Amylases started to be produced during the last century due to their great industrial importance. In fact, they are the most important industrial enzymes with high biotechnological relevance. Their use ranges from textiles, beer, liquor, bakery, infant feeding cereals, starch liquefaction-saccharification and animal feed industries to the chemical and pharmaceutical ones. Currently, large quantities of microbial amylases are commercially available and are almost entirely applied in starch hydrolysis in the starch-processing industries. The species *Aspergillus* and *Rhizopus* are highly important among the filamentous fungus for the production of amylases. In fact, filamentous fungi and the enzymes produced thereby have been used in food and in the food-processing industries for decades. In fact, their GRAS (Generally Recognized as Safe) status is acknowledged by the U.S. Food and Drug Administration in the case of some species such as *Aspergillus niger* and *Aspergillus oryzae*.

The food industry use amylases for the conversion of starch into dextrins. The latter are employed in clinical formulas as stabilizers and thickeners; in the conversion of starch into maltose, in confectioneries and in the manufacture of soft drinks, beer, jellies and ice cream; in the conversion of starch into glucose with applications in the soft-drink industry, bakery, brewery and as a subsidy for ethanol production and other bio products. Amylases provide better bread color, volume and texture in the baking industry. The use of these enzymes in bread production retards its aging process and maintains fresh bread for a longer period. Whereas fungal a-amylase provides greater fermentation potential, amyloglucosidase improves flavor and taste and a better bread crust color. Amylases have an important role in carbon cycling contained in starch by hydrolyzing the starch molecule in several products such as dextrins and glucose. Dextrins are mainly applied in clinical formulas and in material for enzymatic saccharification. Whereas maltose is used in confectioneries and in soft drinks, beer, jam and ice cream industries, glucose is employed as a sweetener in fermentations for the production of ethanol and other bio products.

Proteases:

Proteases are enzymes produced by several microorganisms, namely, *Aspergillus niger, Aspergillus oryzae, Bacillus amyloliquefaciens*, *Bacillus stearotermophilus*, *Mucor miehei*, and *Mucor pusillus*. Proteases have important roles in baking, brewing and in the production of various oriental foods such as soy sauce, miso, meat tenderization and cheese manufacture.

Man's first contact with proteases activities occurred when he started producing milk curd. Desert nomads from the East used to carry milk in bags made of the goat's stomach. After long journeys, they realized that the milk became denser and sour, without understanding the process's cause. Curds became thus a food source and a delicacy. Renin, an animal produced enzyme, is the protease, which caused the hydrolysis of milk protein. Recently proteases represent 60% of industrial enzymes on the market, whereas microbial proteases, particularly fungal proteases, are advantageous because they are easy to obtain and to recover.

An enzyme extract, which coagulates milk and which is derived from the fungus *Aspergillus niger*, is already produced industrially. Although the bovine-derived protease called renin has been widely used in the manufacture of different types of cheese, the microbial-originated proteases are better for coagulant and proteolytic activities. The microbial proteases have also been important in brewery. Beer contains poorly solubleprotein complexes at lower temperature, causing turbidity when cold. The use of proteolytic enzymes to hydrolyze proteins involved in turbidity is an alternative for solving thisproblem.

Most commercial serine proteases, mainly neutral and alkaline, are produced by organisms belonging to the genus *Bacillus*. Whereas subtilisin enzymes are representatives of this group, similar enzymes are also produced by other bacteria such as *Desulfurococcusmucosus* and *Streptomyces* and by the genera *Aeromonas* and *Escherichia coli*.

Glucose oxidase

Glucose oxidase is an enzyme that catalyzes the oxidation of beta-D-glucose with the formation of D-gluconolactone. The enzyme contains the prosthetic group flavin adenine dinucleotide (FAD) which enables the protein to catalyze oxidation-reduction reactions.

Guimarães et al. in 2006 performed a screening of filamentous fungi, which could potentially produce glucose-oxidase. Their results showed high levels of Glucose oxidase in *Aspergillus versicolor* and *Rhizopusstolonifer*. The literature already suggests that the genus *Aspergillus* is a major Glucose oxidase producer.

This enzyme is used in the food industry for the removing of harmful oxygen. Packaging materials and storage conditions are vital for the quality of products containing probiotic microorganisms since the microbial group's metabolism is essentially anaerobic or micro aerophilic. Oxygen level during storage should be consequently minimal to avoid toxicity, the organism's death and the consequent loss of the product's functionality. Glucose oxidase may be a biotechnological asset to increase stability of probiotic bacteria in yoghurt without chemical additives.

Glucose isomerase:

Glucose isomerase catalyzes the reversible isomerization from D-glucose and D-xylose into D-fructose and D-xylulose, respectively. The enzyme is highly important in the food industry due to its application in the production of fructose-rich corn syrup. Inter conversion of xylose into xylulose by Glucose isomeraseis a nutritional requirement of saprophytic bacteria and has a potential application in the bioconversion of hemicellulose into ethanol. The enzyme is widely distributed among prokaryotes and several studies have been undertaken to enhance its industrial application. The isolation of Glucose isomerase in *Arthrobacter* strains was performed by Smith et al. in 1991, where as Walfridsson et al. cloned gene xylA of *Thermus themophilus* and introduced it into *Saccharomyces cerevisiae* to be expressed under the control of the yeast PGK1 promoter. These arch for Glucose isomerase their most able enzymes has been the target of protein engineering.

Invertase:

Invertase is obtained from *Saccharomyces cerevisiae* and other microorganisms. The enzyme catalyzes the hydrolysis from sucrose to fructose and glucose. The manufacture of inverted sugar is one of invertase's several applications. Owing to its sweetening effects which are higher than sucrose's, it has high industrial importance and there are good prospects for its use in biotechnology. Invertase is more active at temperatures between 40^{o}C and 60^{o}C with pH ranging between 3.0 to 5.0. When invertase is applied at 0.6% in a solution of sucrose 40% w / w at 40^{o}C, it inverts 80% of sucrose after 4 hours and 20 minutes.

When Cardoso et al. in 1998 added invertases to banana juice to assess its sweetness potential, they reported an increase in juice viscosity besides an increase in sweetness. Microorganisms, such as filamentous fungi, are good producers of invertase with potential application in various industrial sectors.

Microorganisms are potential producers of enzymes useful for the food industry. Biotechnological tools are available for the selection and obtaining of strains and for strains which increase enzymes' production on a large scale. Progress and achievements in this area will bring improvements in the food industry and, consequently, a better health quality for mankind.