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OBTAINING BIOTECHNOLOGICAL PRODUCTS ON THE BASIS OF ENZYMATIC CONVERSION OF PLANT WASTE AND PROSPECTS FOR THEIR USE

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Abstract

Enzyme preparations play a significant role in biotechnological processes for obtaining food products. The classification of enzymes, the specificity of their action on various substrates, and the processes for obtaining enzyme preparations based on microorganisms producing enzymes are presented. The monitoring of the world and domestic market of enzyme preparations used in the food industry was carried out. The issues of effective bioconversion of various types of plant raw materials to improve the quality of obtained juices, fruit drinks, reduce viscosity and increase the yield of biologically valuable components for the production of functional food products are considered; microbial raw materials - for obtaining protein-amino acid, vitamin food fortifiers, as well as food ingredients; animal raw materials - for the intensification of technological processes, processing of waste from the meat and dairy industries, as well as in cheese making to improve the quality of products. The proposed review is of scientific and practical interest for specialists in the field of biotechnology for the production of food products obtained on the basis of the enzymatic conversion of various types of agricultural raw materials.

Keywords: enzyme preparations, biotechnology, substrate specificity, conversion, resource-saving technology, agricultural raw materials, food products

A promising direction for improving technological processes in the processing industries of the food industry is the use of highly active biological catalysts that contribute to a significant increase in yield, improve quality and extend the shelf life of finished products [1, 2]. In addition, enzymatic catalysis makes it possible to radically change the functional and technological properties of raw materials at various stages of its processing, thereby opening up wide opportunities for creating fundamentally new easily digestible products, including specialized food products.

Most food technologies are based on biocatalytic methods for the conversion of agricultural raw materials [3–5]. Enzyme preparations of microbial origin are most

widely used in alcohol and brewing (about 60% of the total volume of enzyme preparations), baking, confectionery, starch-treacle, cheese-making (up to 20%) industries. The use of domestic biocatalysts allows not only to intensify the existing biotechnological process in the food industry, but also to create a new generation of competitive products with desired properties, to produce import substitution.

Enzymes: classification and specificity

Enzymatic catalysis of substrates of plant, animal and microbial origin provides a radical change in functional properties and fractional composition of raw materials at various stages its processing, opening up wide opportunities creation of fundamentally new types of food products.

According to the modern classification adopted by the Committee on Nomenclature of the International Union of Biochemists and Molecular Biologists (NC-IUBMB), all enzymes are divided into 6 main classes according to the type of catalyzed reactions [6]: oxidoreductases (redox reactions); transferases (group transfer reactions); hydrolases (reactions of addition or elimination of a water molecule); lyases (reactions of cleavage or addition of groups by a non-hydrolytic way along a double bond); isomerases (isomerization reactions); ligases, or synthetases (reactions of attachment of two molecules to each other, coupled with the cleavage of the pyrophosphate bond).

Most of the enzymes used in the food industry belong to the 3rd class - hydrolases, which includes 11 subclasses [7]. Hydrolases catalyze hydrolytic reactions in the bioconversion of plant, animal, and microbial substrates. The name of hydrolases is formed according to the form: “substrate–hydrolase”.

Hydrolytic enzymes of the 3rd class are divided into subclasses depending on the specificity of their action in the catalytic cleavage of certain bonds: 3.1 - ester bonds; 3.2 - glycosidic bonds; 3.3 - ether bonds; 3.4 - peptide bonds; 3.5, C–N bonds other than peptide bonds; 3.6 - acid-anhydride bonds; 3.7, C–C bonds; 3.8 - haloalkyd bonds; 3.9, P–N bonds; 3.10 - S–N bonds; 3.11 - C-P bonds.

Of greatest interest to specialists in the field of food biotechnology are 3 subclasses of enzymes of the class of hydrolases (3.1, 3.2 and 3.4). These include esterases (pectinesterase acts on pectin in plant substrates); glycosidases (amylases, hemicellulases catalyzing the hydrolysis of glycosidic bonds in poly- and oligosaccharides); proteases that catalyze the hydrolysis of proteins.

Substrates for hydrolytic enzymes are polymers that serve as the object of action on them of enzymes with the appropriate substrate specificity. During enzymatic hydrolysis, an enzyme-substrate complex is formed, which undergoes intramolecular rearrangement under the influence of the active center of the enzyme [8]. The catalyzed cleavage of the anhydride bond of the substrate leads to the release of one

of the reaction products from the enzyme-substrate complex. The second product is isolated after groupings associated with the addition of water.

The "recognizability" of a polymeric substrate by an enzyme can be achieved by a large number of contacts. Each polymer substrate molecule actually represents a whole range of reaction centers with different reactivity. In this case, the reactivity of polymers, as a rule, decreases in the course of its enzymatic degradation.

The specificity of the action. Enzymes have a high selective ability to interact with the substrate and high specificity with respect to the catalyzed reactions. At the same time, stereospecificity, absolute and relative specificity are distinguished.

Enzymes exhibiting relative or group specificity act on a group of similar the structure of the substrates. In addition, each individual enzyme exhibits its own characteristic features. Supposed, that the active center of the enzyme is conditionally present two sections: sorption and catalytic. Wherein enzymes are variable in the structures of sorption sites of centers and are strictly conservative in structures catalytic sites [9].

Subclass 3.1 hydrolases (esterases) are characterized by relative substrate specificity, i.e. the ability to hydrolyze ester bonds between kinds of radicals. Esterases cleave mono-, di-, triacylglycerols and other compounds containing an ester bond. Splitting rate depends on the structure of the substrate.

Lipases exhibit positional specificity: preferably hydrolyze the ester bond at C1 and C3 of glycerol, and also show selectivity in relation to the chain length of the cleaved fatty acid residues.

Proteases (subclass 3.4) are group specific for proteins and peptides. In this case, pepsin preferentially catalyzes the cleavage of the peptide bond between tyrosine and phenylalanine, especially in the presence of a free carboxyl groups. Chymotrypsin acts on peptide bonds formed by carboxyl groups of aromatic amino acids.

Enzymes have the ability to act not only on various substrates, but also to catalyze various biochemical reactions. For example, trypsin catalyzes the hydrolysis of peptide bonds, formed by the carboxyl group of arginine or lysine, as well as amide bonds and ester bonds between amino acid and alcohol [10].

Glycosidases (subclass 3.2) are stereospecific. For example, amylases and β-glucanases catalyze the hydrolysis of glycosidic bonds of a certain spatial configuration (α- or β), but not both at the same time. Less stringent selectivity is manifested for various types of α- or β-glycosidic bonds. So, glucoamylase cleaves α-1,4 and α-1,6 bonds; yeast glucanase - β-1,3 and β-1,4 bonds, etc.

Glycosidases are specific in terms of chain length: high molecular weight substrates (dextrins) are preferred for mold glucoamylase, while lower molecular weight oligosaccharides are preferred for yeast. The rate of hydrolysis of carbohydrates may

depend on the presence of certain substituent groups in carbohydrate residues. Some glycosidases only hydrolyze linear polymers, and so on.

Hydrolytic enzymes are subdivided into 2 types according to the nature of the process of substrate cleavage: endo- and exo-actions [7–10]. Endoenzymes catalyze the disordered cleavage of intramolecular bonds of a polymer molecule with the formation of large fragments of various sizes in the initial stage of hydrolysis. That is, substrate bonds located at a sufficient distance from the ends of the polymer molecule are attacked. These include many hydrolase enzymes (α-amylase, pullulanase, endo-β-glucanase, proteinase, etc.). Exoenzymes catalyze the sequential cleavage of fragments of the substrate molecule, more often monomers or dimers, from a certain end of the polymer chain. It is possible that the active center of such enzymes is arranged in the form of a pocket directed deep into the protein molecule; it can accommodate no more than a certain number of monomeric substratum links. These include glucoamylase, exoβ-glucanase, peptidases, β-xylosidases, etc.

For the deep degradation of polymers of agricultural raw materials, the action of enzymes is mutually enhanced, achieved by the fact that one endodeaction enzyme supplies a substrate for the exodeaction enzyme.

Promising directions for the development of enzyme technologies in the food industry

An analysis of the global biotechnological market shows that enzyme preparations are the main commercial product. Their production is constantly increasing. The volume of production of domestic enzyme preparations is currently about 1,000 tons per year, and the need is about 18,000 tons per year. As a result, the country annually imports enzyme preparations worth about 500 million US dollars. The most widely used enzyme preparations of microbial origin, especially hydrolytic action.

The key factor in the biotechnology of enzyme preparations is the strain that produces the target enzymes necessary for the efficient conversion of polymers of agricultural raw materials. Recently, highly active strains of microorganisms producing industrial enzymes have been obtained by genetic engineering and induced mutagenesis, the activity of which has been significantly increased [11–16]. A promising direction is the development of advanced biotechnologies based on new highly active recombinant and mutant strains of competitive enzyme preparations for targeted purposes, which are necessary for the practical implementation of enzyme technologies in the food industry. When creating biocatalytic technologies, not only the polymer composition of agricultural raw materials is taken into account, but also the substrate specificity of synthesized enzymes and the mechanism of their action.

As a result of the identified regularities in the processes of biocatalysis of plant and animal polymer and microbial substrates are being developed scientifically

fundamentals of biotechnology of enzyme preparations for improving the efficiency of biotechnological processes in processing industries, to create new types of food products, food ingredients, dietary supplements. As a result target enzyme preparations have been created for their application in biotechnological processes food production [17–26]. For example, for efficient biocatalysis of polymers of grain raw materials in alcohol, brewing, starch-treacle, baking and other industries, enzymatic systems are needed that carry out conversion of starch (α-amylase, glucoamylase, pullulanase), non-starch polysaccharides (xylanase, β-glucanases and cellulases) and proteins (proteases) [15, 27], which makes it possible to intensify biotechnological processes, improve the yield and quality products.

For the confectionery industry complex enzyme preparations of amylolytic and proteolytic action instead of chemical reagents to increase dough elasticity in the production of crackers, intensification of technological processes, improving the quality of confectionery products [17]. For juice and alcoholic beverages industry - enzymatic systems (polygalacturonases, pectinesterases, pectin lyases, hemicellulases and proteases) that carry out the destruction polymers of fruit and berry raw materials [18, 20]. The use of complex enzyme preparations of the target purpose allows you to increase the yield of juice and its organoleptic characteristics, increase the durability drinks during storage. In the dairy industry a wide range of enzyme preparations are used proteolytic action, regulating the functional properties of dairy products, and correcting their structural indicators at certain stages technological processes [19].

For the hydrolysis of microbial biomass, optimal enzymatic systems have been selected that allow carry out a regulated process of biocatalysis of cell wall polysaccharides (β-glucanases, mannanases, proteinases and chitinases), protoplasmic proteins and nucleic acids (peptidases, proteinases and nucleases) to obtain functional biologically active additives, protein-amino acid and vitamin food fortifiers, food ingredients.

For the hydrolysis of animal raw materials in the cheese-making, dairy, and meat industries, enzyme preparations are widely used as sources of a complex of acidic and neutral proteases in order to intensify technological processes, improve product quality, and efficiently process waste [27].

The use of lipolytic enzymes is promising in those branches of the food industry that require partial or complete hydrolysis of fats [38–40]. The catalytic feature of lipases is the ability to redistribute fatty acids in the reaction mixture and replace them with others that are part of glycerides, thus carrying out esterification reactions.

Biocatalytic processes in food technologies

Enzyme preparations are an important factor contributing to the deep processing of agricultural raw materials, increasing the yield, quality and safety of finished products. Enzymatic catalysis of substrates provides a radical change in the functional properties and fractional composition of raw materials at various stages of its processing, expands the possibilities for improving traditional food technologies, as well as creating new types of food products.

Using genetically modified strains of microorganisms synthesizing enzymes with different substrate specificity and mechanism of action, complex target enzyme preparations have been developed. The theoretical justification for the selection of an enzyme system is based on knowledge of the composition of raw materials and the presence of substrates in it for biocatalytic conversion by enzymes, as well as predicted results on a given degree of degradation and the expected composition of hydrolysis products.

Biocatalytic conversion of starch-containing raw materials

For the hydrolysis of starch, amylolytic enzymes are used. These include enzymes of thinning, dextrinating and saccharifying effects on starch. These enzymes can be conditionally divided into 3 groups: α-amylases, glucoamylases, and pullulanases (table). Of these, α-amylase and pullulanase are endothermic enzymes, and glucoamylase is exoacter.

The role of amylolytic enzymes in hydrolysis starch is exceptionally large. They attack not only gelatinized, but also native starch, destroying starch grains [25]. Acting on the whole starch grain, α-amylase attacks it, loosening the surface and forming channels and grooves, i.e. as if splitting grain in pieces. Hydrolysis of starch occurs with the formation of products that are not stained with iodine, consisting of mainly from low molecular weight dextrins. α-Amylases act on α-1,4-glucosidic bonds, cleaving amylose within its chain, i.e. are endoamylases. As a result of the multistage hydrolysis of starch, α-dextrins are formed, then tetra- and trimaltose, the hydrolysis of which further gives maltose and glucose.

Glucoamylase is designed to saccharify partially cleaved starch polymers to form glucose. Glucoamylase is an enzyme with an exogenous mechanism of action on the substrate, catalyzes sequential cleavage of terminal residues glucose from the non-reducing end of the substrate. Glucoamylase is characterized by the ability to more rapidly hydrolysis of high molecular weight dextrins than oligosaccharides. Many glucoamylases have the ability to catalyze as quickly as the α-1,4 bond hydrolysis of α-1,6-glucosidic bonds. But it's happening only when an α-1,6 bond is followed by an α-1,4- bond, therefore, for example, dextran is not hydrolyzed.

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Pullulanase catalyzes internal α-1,6 bonds in amylopectin and limiting dextrins with the formation of maltooligosaccharides. Like α-amylase, pullulanase is an endogenous enzyme, but unlike from it is able to randomly hydrolyze α-1,6 bonds in pullulan, amylopectin, glycogen and limiting dextrins obtained by the combined action of α- and β-amylases on starch and glycogen. Characteristic the substrate for pullulanase is a polysaccharide pullulan, which is a glucan in which maltotriose molecules are interconnected α-1,6- connections. Pullulan contains α-1,4 and α-1,6-glucan bonds, which to some extent brings it closer to starch, making them a common substrate for such an enzyme as pullulanase.

Amylopectin and β-limit dextrins pretreated with pullulanase are more deeply hydrolyzed by amylolytic enzymes than these. same substrates in the native state. Yes, joint action of pullulanase and β-amylase on amylopectin and glycogen leads to their complete hydrolysis. The attack of amylopectin also increases when using a complex of enzymes containing pullulanase, glucoamylase, and α-amylase [15]. The synergy of these enzymes allows you to increase the degree and speed hydrolysis of starch.

Biocatalytic conversion protein-containing raw materials

For the hydrolysis of proteins, proteolytic enzymes are used, which, according to .the mechanism of action, origin and effectiveness of the impact on protein polymers are divided into 2 main groups: peptidases EC 3.4 – 11–15 and proteinase EC 3.4 – 21–24.

In the 1st group of proteolytic enzymes, subdivision is carried out on the basis of the mechanism of cleavage of peptide bonds in peptides. Proteases belonging to the group of peptidases, are mainly exoactive enzymes that catalyze hydrolysis peptide bond with the N- and (or) C-terminus of the peptide chain and subdivided into subclasses:

– α-aminoacylpeptide hydrolases (EC 3.4.11) – aminopeptidases;

– hydrolases of peptidylamino acids or acylamino acids (EC 3.4.12) – carboxypeptidases;

– dipeptide hydrolases (EC 3.4.13) – dipeptidases;

– dipeptidyl peptidhydrolase (EC 3.4.14) and peptidyl dipeptide hydrolase (EC 3.4.15). Their products hydrolysis are amino acids and low molecular weight peptides.

The 2nd group of proteolytic enzymes - proteinases - has 4 subclasses, in which all enzymes are subdivided depending on the features of the mechanism of catalysis, established by the functioning the active site of the enzyme, as well as the effect of pH on its activity. Proteinases catalyze the hydrolysis of peptide bonds to form peptides with different molecular weight: serine (EC 3.4.21), thiol (EC 3.4.22), carboxyl (EC 3.4.23) and metal-containing (EC 3.4.24). The joint catalytic effect of proteolytic enzymes on the protein substrate provides the highest degree of its conversion to free

amino acids and low molecular weight peptides [16]. Many researchers note that bioactive peptides are formed in hydrolysates that exhibit immunomodulatory and antioxidant properties.

Recently, the food industry has been using an enzyme preparation as a source of transglutaminase (EC 2.3.2.13). The enzyme was first described in 1959 [50]. Transglutaminase catalyzes the formation of covalent bonds between free amino groups (for example, associated with a protein or lysine peptide) and γ-carboxamide groups of glutamine.

In this case, the formation of connections can occur as between proteins of the same origin (for example, vegetable), and between proteins that differ by type (lactic and vegetable), which makes it possible use transglutaminase in the production of mixed products. Effective range enzyme action: from 30 to 60 ° C, pH - from 3.0 to 9.0. At temperatures above 70 ° C, inactivation begins enzyme.

Biocatalytic conversion of polysaccharides vegetable raw materials

Enzymes that catalyze the hydrolysis of non-starch polysaccharides of plant materials include cellulolytic, hemicellulase enzymes and pectolytic action (see table). These drugs reduce the viscosity of grain must, increase availability of starch for the action of amylolytic enzymes, which leads to an increase in the concentration soluble carbohydrates and promotes more intensive thinning and improvement of rheological properties [2]. Enzyme preparations of hemicellulase and cellulolytic action are necessary for the processing of rye and barley raw materials in production alcohol, beer and feed. These raw materials are characterized high content of cellulose, hemicellulose and gum substances, leading to gel and gel formation, an increase in the viscosity of the wort and its deterioration rheological indicators.

Almost all hemicellulase enzymes can divided into three groups: β-D-glucanases, β-xylanases and β-glucosidase: β-D-glucanases include a group of enzymes that catalyze the breakdown of β-glucans from β-1,2-, β-1,3-, β-1,4 and β-1,6 bonds. This group includes 6 enzymes: cellulase, or endo-1,4-β-glucanase, endo-1,3-β-glucanase, endo-1,6-β-glucanase, laminarinase, lichenase and endo-1,2-β-glucanase. β-xylanases include a system of enzymes that catalyze the cleavage of β-glucosidic bonds in β-xylans. β-Glucosidases (cellobiases) are enzymes of exogenous actions, catalyze the cleavage from the irreducible end of the β-1,4 bond in β-D-glucosides, releasing β-D-glucose.

When processing plant materials, enzymes hemicellulase action (β-glucanase and xylanase), catalyzing the hydrolysis of polysaccharides with the formation of glucose and pentoses, perform their specific function associated with their specificity and mechanism of action [1, 7].

As a result of the analysis of a large array of experimental data, the dependence of the rheological and biochemical characteristics of grain wort was revealed and indicators of mash from the concentration of hemicellulases. At the same time, it was found that the use of enzyme preparations - sources of β-glucanases - as a result of enzymatic depolymerization of grain glucans, it allows to increase the glucose content in the reaction medium and thereby promote increase the yield of the target product. Application xylanolytic enzyme preparations provides a reduction in the viscosity of the wort and improves its rheological indicators, which contributes to the intensification of the process of bioconversion of grain polymers raw materials.

When creating resource-saving technologies for deep processing of grain raw materials, it is necessary to take into account not only the starch content, but also the composition proteins and non-starch compounds. For increasing the efficiency of polymer bioconversion grains use specially selected target multi-enzyme compositions, which, along with with traditionally used amylases included complexes of proteinases and peptidases, β-glucanases, xylanases, cellulolytic enzymes. Synergy the action of enzymes with different substrate specificity contributes to the improvement of rheological indicators of grain wort, increasing the fermentation yeast activity, acceleration of generation processes yeast and alcoholic fermentation, increasing the yield target product [11, 16, 23].

In the processing of fruit and berry raw materials in the juice-and-mortar and wine-making industries, pectolytic enzymes are most widely used, including polygalacturonase, pectinesterase, etc. [4, 7, 12, 18]. In the works of a number of researchers, data on the effectiveness of the complex impact pectolytic enzymes with enzymes that catalyze the hydrolysis of proteins and polysaccharides [20, 24, 25].

As a result of the enzymatic degradation of polymers fruit and berry raw materials, the yield of juices increases, increases their quality and stability during storage.

Therefore, biotechnology is one of the the most promising areas of science, ensuring the development of processing industries agro-industrial complex focused on food production and the environment. The problem of fully providing the nutritional needs of the population can be solved with the involvement of valuable ingredients obtained on the basis of enzymatic conversion of plant, animal and microbial raw materials.

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