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SOME ASPECTS OF MANAGEMENT OF BIOLOGICAL AGENTS USED IN FOOD BIOTECHNOLOGY

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Abstract

Food sector is one of the major beneficiaries of biotechnology development, being able to ensure in the near future most of the elements needed of the human feeding. Most technological processes for obtaining food are biotechnologies that are based on the use of microorganisms or their metabolites. Food production must be carried out in accordance with domestic and international hygiene standards and in accordance with consumer requirements. The role of biotechnology is overwhelming in the food industry, becoming, at the same time, one of the most dynamic areas of research. Thus, global problems related to food, health, population aging and sustainable development can find many answers in the field of biotechnology. In order to make products or improve technological processes, food biotechnology involves the industrial processing of various raw materials with the help of added biological agents, the most important of which are enzymes and bacteria. Biotechnologies in the food industry have developed impressively through the use of exogenous enzymes and starter crops. This paper aims to provide a brief description of the most common enzymes and bacteria used in food biotechnology and some aspects about their management in the context of food safety and consumer protection. The most used bacteria in the food industry belong to the genera Streptococcus, Lactobacillus, Leuconostoc, Pediococcus, Streptomyces and Propionibacterium. From the point of view of the management of enzymatic preparations used in the food industry, they must be produced under conditions similar to a good food manufacturing practice, and by their use there should be no increase of the total number of germs.

Key words: *food, biotechnology, enzymes, bacteria, safety*

INTRODUCTION

The loss of genetic diversity in the last century can threaten food security and EU policies on sustainable agriculture, biodiversity protection and climate change mitigation strategies. Biotechnology is the one that supports the solution of this desideratum. It is a modern field, in full development, based on the application of biology in agriculture, in the food industry, but also other fields, which use biological systems, living organisms or their derivatives to obtain or modify products or processes for specific use.

The conventional biotechnology refers to the biotechnological classic concepts, applied in food industry, agriculture [8, 19], medicine or for environmental protection [3, 4, 20, 21], while the modern biotechnology presents biotechnological concepts based on advanced studies of molecular biology, ecology [11], genetics and genetic engineering, phytopathology [17, 18], Biochemistry [5, 22], etc.

According to the European Federation of Biotechnology-EFB, *"biotechnology is an integrated application of natural and engineering sciences with the aim of using living organisms, cells and their component parts for products and services"* [9].

On the one hand, research in food biotechnology aims to ensure food quality and availability (food safety and security) and on the other hand, ensuring the nutritional value and amplifying the biological effects of food.

Food biotechnologies have a vast field of exploitation throughout the world. World production of food preservatives, dyes, sweeteners, etc. has increased which gives the products a higher quality, and due to which the products have a longer storage period.

In the conditions of climate change, advanced soil degradation and lack of water, increasing food production will be almost impossible to sustain through conventional processes.

With the beginnings dating back to the time of domestication of animals and plants, biotechnology has become one of the most dynamic fields of research. Starting from genetics, microbiology or embryology, the science of modifying living organisms allows the production of superior drugs, the discovery of treatments for rare diseases or the efficient use of agricultural resources. For the first time, global issues related to health, population aging, nutrition and sustainable development can find answers in the field of biotechnology.

MATERIALS AND METHODS

This is a short review about the most common enzymes and bacteria used in food biotechnology and some aspects about their management in the context of food safety and consumer protection. To this end, we have briefly selected some of the most representative scientific communications in the field, given the importance of modern biotechnology in the food industry and ensuring the globally food security.

RESULTS AND DISCUSSIONS

The enzymes specific to plant and animal tissues are essential in the transformations offered by agri-food products: ripening of fruits and vegetables, cheeses and meat. However, enzymes can also have a deteriorating role with implications in changing the sensory characteristics and nutritional value of agri-food raw materials until their thermal processing. Microorganisms are also involved in fermentation of vegetable products (cabbage, olives, cucumber, etc.).

Biotechnologies in the food industry are based on the use of exogenous enzymes and starter crops in the dairy, beer, starch, meat, bakery, etc. industries. With the enzymes help, biochemical processes can be accelerated, production processes and food quality can be improved and the degree of diversification of food production can be increased.

The classification and nomenclature of enzymes are based on the principles and rules established by the International Union of Pure and Applied Chemistry (I.U.P.A.C.), namely: oxidoreductases, which catalyzes redox reactions by transferring hydrogen or electrons, or by combining a substrate with oxygen; transferases, which catalyzes the transfer of different chemical groups from one donor substrate to another acceptor substrate; hydrolases, which catalyzes the hydrolytic cleavage of different substrates, by adding water to different chemical groups; lyase, which catalyzes the addition or removal of chemical groups from substrates, by different mechanisms than hydrolysis; isomerases, which catalyzes intramolecular rearrangement reactions; ligases (synthetases), which catalyzes the synthesis of new bonds by combining two compounds into one, using nucleoside triphosphates as energy source [10].

Enzymatic preparations used in various biotechnological processes in the food industry are considered as processing aids. FAO and WHO Committee of Experts on Food Additives established some general rules for enzymatic preparations used in food industry. Thus, *"…the enzymatic preparations, used as additives in the food industry, are obtained from raw materials of animal origin (liver, pancreas, stomach or intestinal mucosa, heart, kidneys, brain), vegetables (seeds, germinated and ungerminated cereals, roots, leaves and in some cases even bark) or microbial (bacteria, yeasts, moulds), being made up of whole cells, parts of cells or extracts completely devoid of cells"* [10].

Enzymatic preparations are obtained from several categories of raw materials, which must meet certain requirements:

- Tissues of animal origin must comply with the veterinary rules applied to meat and their handling must meet the requirements of good hygiene practice;
- Materials of plant origin, used as sources of enzymes or as ingredients in the preparation of culture media for enzyme-producing micro-

organisms, must not release any residue harmful to health under normal conditions of use;

- Enzymatic preparations of microbial origin must be produced by the controlled use without penetration of microorganisms likely to lead to the appearance of toxic substances or other undesirable products.

From the point of view of the management of enzymatic preparations used in the food industry, they must be produced under conditions similar to a good food manufacturing practice, and by their use there should be no increase in the total number of germs and an increase in salt content above the permitted limits for a given foodstuff. Sources rich in the desired enzymes are usually used to obtain the enzymatic preparations, which are cheap, accessible and easily processed.

Enzymes obtained as crude or partially purified preparations, in liquid, semi-liquid or dried form, are used in the food industry as such, being added and acting in the media to be transformed as free enzymes, respectively solubilized in aqueous media and their activity, after having carried out the desired transformations, is usually stopped by various treatments, especially by thermal or chemical means (by acidification or alkalization) and in the finished product.

For the production of enzymatic preparations of microbial origin, two basic techniques can be applied:

- Surface fermentation, which uses a solid medium on which the microorganism is grown. This technique has the advantage that the culture medium will have a very high enzymatic activity;
- Deep fermentation, which takes place in reactors with liquid media, case in which all the parameters of fermentation (temperature, pH, aeration, concentration of C, N sources, etc.) are perfectly controlled.

Microorganisms are used in the food industry to obtain starter or pure cultures. They are used in the form of production crops (leavens) for the fermentation of acidic dairy products or cheeses, and in the form of concentrated starter cultures they are used in the processing of food products from meat, bread, etc.

Starter cultures of microorganisms are used to trigger biochemical processes that ensure the product a certain degree of safety and conservation; to improve sensory/nutritional properties. Concentrated starter cultures are cultures developed under controlled conditions, concentrated in a small volume and preserved by freezing or drying for storage and transport.

The management of starter crops in the food industry involves attention to the following aspects:

- It must contain as few undesirable microorganisms as possible [5, 6];
- The metabolic products must not present a danger to the health of consumers [14];
- The new species (strains) that are introduced into production must be registered and stored in collections with nomenclature; before use in production, they must be tested for safety in accordance with the legislation in force. Moreover, even if the strains have been declared safe, they must be inspected at regular intervals by specialized institutes for their purity;
- The species recognized as having toxicogenic potential must be rigorously monitored for each strain, with long-term toxicity, carcinogenicity and mutagenicity studies.

Another type of enzyme preparation (biomass) can be used as an ingredient for fermentation (baking yeast) or for enriching some protein foods, respectively as protein feed for birds, fish and pigs.

Molecular formula of baking yeast is C19H14O2 (Fig. 1); Molecular Weight: 274.3 g/mol. Synonyms: 11H-benzo[a]fluoren-11-ylacetic acid; 2-(11H-benzo[a]fluoren-11-yl)acetic acid or 2-{11H-benzo[a]fluoren-11-yl}acetic acid [1].

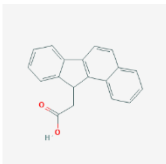

Fig. 1. The baker's yeast structure
Source: [1].

Yeasts are mainly used for alcoholic fermentation, obtaining biomass, in the meat industry and in the dairy industry. Among the yeasts, for the food industry are those belonging to the family *Saccharomycetaceae*, the genus *Saccharomyces* which includes alcoholic yeasts used in the beer, wine, bread, alcohol industry, the genus *Kluyveromyces* which ferments lactose, the genus *Debaryomices* used in the meat industry. Also useful are the yeasts from the *Cryptococcaceae* family (genus *Torulopsis* for example) that are used as fermentation agents and biomass producers.

Metabolism consists of closely coordinated series of chemical reactions mediated by enzymes that take place in the plant organism, resulting in the synthesis and use of a wide variety of molecules in the category of carbohydrates, amino acids, fatty acids, nucleotides and polymers derived from them (polysaccharides, proteins, lipids, DNA, RNA, etc.). All of these processes are defined as primary metabolism and the respective compounds, which are essential for plant survival, are described as primary metabolites [16].

Among the primary metabolites can be listed: ethyl alcohol, preservatives (acetic acid, lactic acid, propionic acid, ethyl alcohol); antioxidants and synergists (ascorbic acid, citric acid, gallic acid, tartaric acid, etc.); thickeners (xanthan, dextran, mannitol, glycerol); flavour enhancers (glutamate); acidulants (acetic acid, citric acid, malic acid, gluconic acid, lactic acid, tartaric acid, fumaric acid); amino acids (lysine, tryptophan, phenylalanine, etc.); vitamins (riboflavin, cyanocobalamin, vitamin C, vitamin D); gas (CO2).

Secondary metabolites can be stimulant additives (gibberellins); preservatives (bacteriocins and antibiotics); peptide compounds.

The most used bacteria in the food industry belong to several genera. Within the *Streptococcus* genus, useful bacteria in the food industry can be mesophilic (*S. lactis, S. cremoris, S. diacetilactis*) and thermophilic (*S. thermophilus*). They are homofermentative, produce lactic acid and have fermentative activity (ferment lactose and glucose) and proteolytic activity.

The *Leuconostoc* genus includes Gram-negative bacteria, optionally anaerobic, which are necessary for the development of vitamins (nicotinic acid, thiamine, biotin) and fermentable sugars. This genus includes species: *L. cremoris; L. lactis; L. dextranicum; L. mezenteroides*. In dairy products, leukonostocs have two basic functions: they produce flavour compounds (diacetyl, acetoin); CO2 formation in some types of cheese (eg Goude cheese).

The *Pediococcus* genus comprises, in terms of importance for the food industry, the bacteria *P. acidilacti* and *P. pentosaceus*. The metabolism of these bacteria is predominantly fermentative, homolactic. Racemic lactic acid is produced from glucose, fructose and mannose. These bacteria exert an inhibitory action against pathogenic and altering microorganisms: staphylococci, botulins, bacilli, gram-negative enterobacteria, etc. Also, the lactic acid produced contributes to the denaturation of meat proteins, which contributes to the achievement of a firm texture of the finished product [2, 7, 13].

Bacteria of the *Lactobacillus* genus are asporogenic, immobile, Gram positive, anaerobic or facultative anaerobic. They have low proteolytic and lipolytic activity. The best fermented carbohydrates are lactose, maltose, sucrose (especially in the development phase), then hexoses (glucose, fructose, galactose).

Depending on the optimal temperature development, lactobacilli can be thermophilic (*L. lactis*, *L. helveticus*, *L. bulgaricus, L. acidophilus*, the optimum temperature being 37-45^{0}C) or mesophilic (*L. casei, L. plantarum, L. brevis* etc., the optimal development temperature being 26-30^{0}C).

Among the species of bacteria of the *Micrococcus* genus are *M. aurantiacus* and *M. varians*, useful in the meat industry, for their ability to reduce nitrates to nitrites, their catalase activity and acidification activity, proteolytic and lipolytic.

For the dairy industry, micrococci form the main part of the non-dairy population in raw milk and cheeses made from raw milk, respectively. *Micrococcus freundenreichii* was isolated from Cheddar cheese made from raw milk, which was later used as a pure culture in order to accelerate the formation of the aroma of cheese made from pasteurized milk due to proteolytic and lipolytic activity [15].

Of the *Staphylococcus* genus, the most interest present the non-pathogenic bacteria *S. carnosus, S. xilosus* and *S. simulans*. Efficient management of combinations of micrococci and staphylococci are effective for nitrate-reductase and catalase activity, because in these combinations, *S. carnosus* acts better than micrococci in color formation, reducing nitrates to nitrites and nitrites to nitric oxide respectively, even under conditions of high acidity of the substrate. The aroma of the products in which the starter culture of *Staphylococcus carnosus* is used is superior [12].

Bacteria of the *Streptomyces* genus can alter food, producing unpleasant odors and tastes [23, 24]. The *Propionibacterium* genus is represented by bacteria that ferment carbohydrates, the most useful for food industry being *P. freundreichii, P. theonii, P. acidipropionici* and *P. jensenii*.

CONCLUSIONS

The food industry is a priority area of the national economy, food being of strategic importance. By biotechnological means, using selected bacterial cultures or their mutants are obtained products with a higher economic value: proteins, amino acids, lactic acid, acetic acid, etc.

From the point of view of the management of enzymatic preparations used in the food industry, they must be produced under conditions similar to a good food manufacturing practice, and by their use there should be no increase in the total number of germs and an increase in salt content above the permitted limits. Some sources rich in the desired enzymes, which are inexpensive, easily accessible and easy to process, are usually used to obtain the enzymatic preparations.

The management of starter crops in the food industry assumes that they contain a certain number of viable microorganisms and as few undesirable germs as possible and the primary and secondary metabolic products do not pose a danger to the health of consumers.

In the current century, a century of biotechnologies, modern strategies to approach this concept will certainly lead to obtaining of the new food products with improved qualities, but also new food pigments, food flavours, food preservatives, etc.

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