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SHORT COMMUNICATION

The use of enzymatic fungal activity in the food industry - review

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ABSTRACT

Enzymes are increasingly used in the food industry, due to the fact that they allow to streamline many processes. They catalyse processes that require energy and are long-lasting. Mushrooms produce hydrolytic enzymes such as proteases, lipases and amylases. These enzymes are used in winemaking, brewing, confectionery and cheese production. Attempts have also been made to use paper industry enzymes such as laccase in the wine industry. The production of enzymes of animal origin is an expensive and complicated process, which is why they were interested in their production from microorganisms. This article attempts to review the current state of knowledge on the use of fungal enzymes in the food industry.

Keywords: amylases, fungi, laccase, protease, enyzmes

1. INTRODUCTION

Enzymes are macromolecular protein compounds that update biological responses in the environment. They reduce the energy of activation, so processes such as digestive or matter

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decomposition occur faster. Enzymes are divided into 6 classes, and from 2018 to 7 [17]. The present division includes oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases and recently the translocase. Enzymes are widely used in many industries, especially in the chemical and food industries. Brewery and viticulture and wine production are areas where there is the greatest demand for enzymes, they are also used in clarifying juices and cheese factories [26]. Especially through their catalytic abilities and specificity of action relative to the appropriate substrates. Enzymes of microbial origin replace those of animal origin. In the case of fungi, it can distinguish two types of enzymatic activity inside or outside the cell. Extracellular enzymes of fungi are important for the environment, because they are able to the decomposition of inanimate matter, whereby fungi play not only the roles of parasites in the environment, but also saprobes, for example are part of process lignin degradation by laccase and ligninase. The distribution of lignin is currently strictly dependent on the fungal enzymatic activity, currently there are no lignin-degrading enzyme-based preparations on the market. Most often, enzymes produced by fungi belong to the group of oxidoreductases and hydrolases.

2. FUNGAL EXTRACELLAR ENZYME ACTIVITY

The production of extracellular enzymes is a process that requires energy inputs. Most microorganisms are able to assimilate small molecule compounds, while fungi by enzymatic activity are able to break down biopolymers such as cellulose, lignin or starch. Each species is adapted to the degradation of specific substrates, and the expression of genes encoding enzyme production is subject to environmental regulation in the form of the availability of a given substrate [23]. Environmental conditions such as soil pH, temperature, humidity are also very important. Differences in these parameters may affect the production of enzymes. Most of the enzymes involved in litter degradation are of fungal origin. By adjusting its metabolism to the content of biogenic elements in the environment, fungi produce a mixture of oxidizing and hydrolytic enzymes [25]. The effect of this is, inter alia, effective wood distribution. In the process of degradation of dead plants, pectinase activity is very important, which breaks down pectin constituting an element of the cell wall. The degradation of lignin is, however, catalysed by laccase. These phenomena classify fungi in the food chain as saprobes. Without this activity, it would not be possible to biodegrade and transport simple chemical compounds to the environment [23].

There are various methods for determining enzymatic activity. In the case of hydrolytic enzymes, colorimetric tests based on the binding of the substrate to p-nitrophenol or fluorimetric tests are used. Oxidoreductase activity is measured as an oxidizing activity in the L-DOPA test [24].

3. OXIDOREDUCTASE

3. 1. Laccase

Oxidoreductases are an enzyme group designated as EC. 1 their function is to catalyse the oxidation and reduction reactions. The representative of this group of enzymes is laccase (EC 1.10.3.2). Laccases are polyphenol oxidases that belong to multi-oxide oxidase groups. They are compounds with a molecular weight in the range of 50-97 kDa [1]. They contain a copper atom in the catalytic centre. On the basis of the metal contained in the active centre, we distinguish three types of laccase. The copper atom is responsible for the colour of the blue colour of laccase, while the enzymes devoid of this colour are called yellow and white laccase. They are glycoproteins, currently they are obtained from fungi belonging to the *Basidiomycetes*, *Deuteromycetes* and *Ascomycetes* and fungi belonging to white rot group. In addition to the decomposition of lignin used in the paper industry and biodegradation, laccase is widely used in the food industry [3]. This enzyme is used, inter alia, in baking, viticulture and juice processing. A special application of laccase is the use of bottle corks in the production process to reduce the tart flavour that arises during long storage of wine [4], [5].

The research on the effectiveness of the laccase preparation was carried out for 3 months on 600 bottles of champagne. Bottles with corks of lower quality, which were treated with Suberase, obtained very good stability [5]. In the bakery industry, laccase improves the texture of baking, its volume and flavour through its oxidizing effect and increasing the strength of gluten structures [18]. Laccase is also used in clarifying juices. Many fruit juices contain phenols and their oxidation products. Mat has a negative effect on taste and aroma. The presence of polyphenols also causes enzyme darkening of the product, which is very undesirable for consumers, to prevent this phenomenon from getting rid of the polyphenol product [2]. The use of laccase removes phenolic compounds, while the substrate-enzyme complex can be removed by membrane filtration. The addition of laccase is also more effective than the use of ascorbic acid or sulphurisation [18].

The problem of the brewing industry is opaque, especially in some beers that are stored for a long time. This is caused by an excess of polyphenols in beer. They are usually removed with PVPP, which creates unhealthy complexes in the environment and is toxic and is hardly biodegradable. The use of laccase significantly improves the product's qualities, and the complexes created by the enzyme with polyphenols can be removed by microfiltration. The use of laccase allows removing unwanted oxygen compounds during brewing, which in turn reduces cloudiness and increases the durability of the product [1].

In the bakery industry, laccase improves the baking structure. Improves it affects the ingredients through the oxidation process, thereby improving stability of gluten in the dough. This results in an increased volume of baking and increases strength stability. Moreover, it reduces the viscosity, which improves the workability of the dough. This allows to get a good baking from poor flour, quality. The improvement of the dough's machinability is also very important [6].

Decomposition of lignin is also very important in the production of ethanol. For this purpose is used for other enzyme that degrades liginin oxidoreductase which is lignin peroxidase. Removal of lignin from the material for the production of ethanol is an important component in the process. A biological method using an enzyme is promising because of low energy requirements, mild reaction conditions and high product yield. The only drawback is the long incubation period [19].

4. HYDROLASES

Hydrolases are enzymes that catalyse the distribution of chemical bonds in the hydrolysis process that do not have a coenzyme. The hydrolases include esterases, glucosidases, proteases, nucleases including amylase, invertase, phosphatase and lipase. The biggest applications in the food industry are amylases and lipases. Their operation is important in many areas of food production.

4. 1. Lipase

Lipases (EC 3.1.1.3) can catalyse reactions such as hydrolysis, esterification and interesterification. Their characteristic feature is the ability to act on a substrate that is insoluble in water. Lipases of microbiological origin are most commonly used in the food industry [20]. Currently, they are maintained by fermentation on a solid or submerged substrate. On solid substrates, they are obtained mainly from filamentous fungi, due to the ability of these organisms to penetrate the intermolecular structure of the matrix. Lipases from the *Rhizopus*, *Aspergillus* and *Mucor* are mainly used for the production of lipases. primarily catalyse the synthesis of short-chain flavour and fragrance esters, e.g. isoamyl acetate [10]. Mainly role of this enzymes is giving stronger flavours in Italian cheeses by a modest lipolysis, increasing the amount of free butyric acid. Lipases are used in the production of cheeses, including accelerating their maturation [11]. The table below shows the properties of lipases produced by individual strains. It is worth noting that lipases are stable in various environments, which can be used in the production of very diverse products.

Table 1. Properties of lipases produced by microorganisms [20]

Species	Lipase properties
Rhizomucor variabilis	Alkaline, stable in EPS
Geotrichum candidum	thermostable
Rhizomucor endophyticus	Stable at low temperatures
Aspergillus Niger	Stable at low temperatures
Penicillium sp.	Stable at low temperatures and a wide pH range
Pleurotus ostreatus	thermostable

One of the interesting applications of lipase is the use of immobilized enzyme produced by *Rhizopus miehei* in the production of cocoa butter. The high-fat product contains palmitic and stearic acids [12]. The enzyme in the transesterification process replaces the palmitic acid contained in palm oil used for the production of cocoa butter, stearic-oleic-stearic triglyceride.

4. 2. Pectinase

Another enzyme from the group of hydrolases produced by fungi is pectinase, also known as pectinmethyl hydrolase. Its producer is *Aspergillus Niger*, which causes esterase and acidic pectinase activity. Two types of acid pectinase and alkaline pectinase can be distinguished. Its main application is to facilitate the pressing of fruit juice [21]. The specific use of pectins is described during the production of apple juice, the enzyme usually lasts from 15 minutes to 2 hours, which depends on the apple variety. During the process, the enzyme causes degradation of pectins contained in the cell walls [13]. Pectinases are also used to reduce turbidity in wines. They are used in the initial stages to increase the amount of grape juice obtained and the secretion of anthocyanins. Pectinases are also used in the fermentation of coffee and tea. The enzyme-based process removes the mucous coating from the coffee beans. Cellulases and hemicellulases contained in commercial preparations digest mucilage, which also improves the microbiological stability of coffee [14]. The addition of pectinase to teas speeds up their fermentation and stabilizes the formation of foam in instant granulated teas.

The use of pectinases brings several advantages including but not limited pumping capacity and they can shorten the free flow of juice and pulp maceration process, and carried out at low temperature which allows the use of cold processing [13]. Pectinase at a temperature of 8 ºC for 75 minutes decomposed 100% pectin, where conventional enzymes work longer, and at the same temperature are less active, pectin hydrolysis can take place even at cold maceration. The use of pectin-degrading enzymes reduces the risk of oxidation and the application of bitter substances and tannins [14].

Table 2. Stability of selected pectinases produced by microorganisms [21].

Species	Optimal pH	Optimal temperature ºC
Aspergillus niger	6.5–7.0	35
Rhizopus stolonifer	5.0	45
Amycolata sp.	10.25	30–35
Aureobasiduim pullulans	5.5 and 4.5	50

4. 3. Protease

EC protease 3.4 is a subclass of enzymes that catalyses the breakdown of peptide bonds. Due to the mechanism of action, serine, cysteine, aspartyl, metalloprotease and threonine proteases are distinguished [22]. Enzymes from this group are used in many branches of the food industry, among others for the production of milk substitute and bitter taste control in protein hydrolysates. The use of appropriately selected protease causes the correction of the unwanted trait. These enzymes are also used in fish processing [9]. The shredded remains of fish such as bones, skin and fins and are treated with a water solution with protease. By means of pasteurisation, denaturation, centrifugation, fatty substances are separated and the product is solubilized. Proteases are added to feeds to improve digestion. In bakery, the addition of proteolytic preparations to flour considerably raises the quality of the products [10].

Acid proteases are obtained in cultures *Mucor* or *Aspergillus*. In the cheese growing, instead of rennet preparations, proteases obtained from *Mucor pusillus* and *Mucor miehel* mushroom cultures are used for precipitation of casein [22]. The enzymes produced from the culture of *Aspergillus niger* and *Aspergillus oryzae* are used, among others, for the hydrolysis of soy proteins in the production of soy sauce and other hydrolysates, used as spices [15].

Table 3. Stability of selected proteases produced by microorganisms [22].

Species	Optimal pH	Optimal temperature ºC
Aspergillus awamori	5.0	55
Aspergillus clavatus	8.5	50
Aspergillus fumigates	8.0	50
Aspergillus niger	3.0	27

4. 4. Amylase

The largest group of enzymes produced by microorganisms are amylases. They are hydrolysable starches for simple sugars. Their division depends on the bond, the distribution of which catalyses. Amylases are metalloproteinase with a strong affinity for calcium ions. The amylases cleaving α-1,4-glycosidic linkages of starch (α-amylase, β-amylase, exo-α-amylase) and cleavable α-1,6 glyosidic linkages (isoamylase) are distinguished [7]. The use of amylase produced by the fungi can replace malt with 50-60%, whereas the use of amyloglucosidase can eliminate the malt in the process of distilling agricultural the saccharification of starch. Amylases are used in the brewing industry. Its action is essential in the process of mashing malt. Thanks to the use of amylolytic enzymes, the germination temperature of starch can be lowered from approx. 75-80 °C to a maximum temperature of 70 °C. Amylase is also applicable to the liquefaction and saccharification of starch, this step is to prepare the polysaccharide to fermentation by yeast. A very important effect of amylase is described in the starch industry [8]. Action enzymes used in the production of thickeners, additives for sauces, puddings, baby food ingredients. The addition of amylase to cakes results in decomposition of starch to dextrin and accelerates yeast fermentation, which has a beneficial effect on the structure and volume of baking. Amylase is also used in the production of glucose-fructose syrup. The oligosaccharide mixture is produced by digesting corn starch with an enzyme. The product obtained is usually a mixture of glucose, maltose and other oligosaccharides. The product is spray dried. It exhibits strongly hygroscopic properties, which is why it is used, among other things, as a humidity regulator. It is less sweet than sucrose and less sticky than corn syrup [7]. Due to its properties it is used as a substitute for sucrose and an anti-crystallizer in food.

Table 4. Stability of selected amylases produced by microorganisms [7].

Species	Optimal pH	Optimal temperature ºC
Thermomyces lanuginosus	6.0	50
Mucor sp.	5.0	60
Aspergillus fumigates	6.0	30
Aspergillus niger	4,95	50

5. CONCLUSIONS

Enzymes are widely used in the food industry. This review cites only the most relevant reports on the importance of fungal enzyme activity and its application in the food industry. Enzymes catalyse important processes, allow to lower the temperature of the system, because their operating optima are below 100 ºC. Enzymes are oxidoreductases and hydrolases. Currently, there are more and more works on the use of enzymes and this is the direction in which the food industry will develop.

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