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## YEAST AS AN ALTERNATIVE METHOD FOR FUNGAL CONTROL IN FOOD INDUSTRIES

### Summary

**Background.** The quality of the final product in industries such as bakery, winemaking, brewing, and sausage and cheese making often deteriorates due to the development of food spoilage microbes. Filamentous fungi and their toxic metabolites, known as mycotoxins, are among the factors that significantly reduce food quality and seriously threaten food safety. Mycotoxins cause biochemical, physiological and pathological changes in living organisms and have a toxic effect even at low concentrations. The threat to food safety posed by pathogenic fungi and their metabolites has prompted the search for new ways to reduce their entry into the food chain.

**Results and conclusions.** In recent years, much attention has been paid to biopreservation methods. Biopreservation refers to extending shelf life and enhancing food safety using indigenous or added microorganisms and their antimicrobial metabolites. Starter cultures of yeast antagonistic against toxigenic fungi can contribute to the product's safety, primarily by inhibiting the growth of pathogens during the fermentation process and improving the stability of this process. This review describes the main problems related to the occurrence of fungi in food industries and the strategies for reducing the presence of fungi and mycotoxins in food. Focus is placed in particular on the use of yeast as antifungal microorganisms tested for food bioprotection, and their mechanisms of action.

**Keywords:** biopreservation, biocontrol, food safety, control of fungi, mycotoxins

### Introduction

Food losses are a major global problem today, especially in view of the ever-growing world population and the fact that about one-third of all food produced for human consumption is either lost or wasted [23]. The reasons for this huge global food

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loss are multifarious, but the main cause/factor is microbial spoilage, which affects the organoleptic quality of products (appearance, taste and aroma) and has a significant impact on food safety. Among food spoilage microorganisms, fungi pose a serious problem at every stage of the food chain due to their ability to grow under various, even harsh, conditions. In addition to the negative impact on food quality, some fungal genera, most notably *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*, have the ability to produce secondary metabolites (mycotoxins) that can be toxic to humans and animals. Mycotoxins cause biochemical, physiological and pathological changes in living organisms and produce a toxic effect even at low concentrations [31]. They rarely lead to acute food poisoning, but by accumulating in the body, they cause chronic poisoning. Literature reports indicate that mycotoxins are stable and remain mostly intact during food production [3].

Quality is an important distinguishing feature of a product, and there is now an increasing interest in food quality among consumers and producers alike. The term food quality is understood as the health quality of food products, which depends on the nutrients and bioactive components they contain and on the presence of foreign substances that may be harmful to human health and cause many diseases. Commercial quality means the characteristics of an agri-food article regarding its organoleptic, physicochemical and microbiological properties in terms of production technology, size or weight, as well as requirements arising from the manner of production, packaging, presentation and labeling. Food producers should make it their top priority to provide consumers with high-quality food, ensuring a high degree of safety, so that their products are both tasty and healthy [46]. Food can be preserved to avoid spoilage. Benzoic, sorbic, lactic and acetic acids are some of the major organic acids that have been used in food preservation. These compounds act against a variety of spoilage microorganisms, including some fruit spoiling fungal pathogens [39]. Propionic acid and some of its salts, mainly sodium and calcium, as well as sorbic acid and its derivatives, are approved for the preservation of bread. The limits of 0.2 % (w/w) and 0.3 % (w/w) have been established for sorbate and propionate addition, respectively [58]. It was observed, however, that the addition of high concentrations of these preservatives, though desired for antifungal activity, delays the fermentation of the dough, and in the case of products made of light flours, it changes their aftertaste [38]. There are also reports in the literature of urticaria and contact dermatitis after consuming products with the addition of sorbic acid [60].

Demand for natural, less processed products, without the addition of chemical preservatives, prompts the search for and development of new food preservation methods allowing to eliminate of harmful microorganisms and their metabolites. Biological control is considered a safe method and is gaining more and more popularity, not only in agriculture but also in the food industry [55]. Microorganisms such as yeast, which

produce growth-inhibition metabolites, present a biological alternative for food and beverage preservation.

### **Fungal spoilage in food industries**

Fungi are some of the most resistant spoilage microorganisms, and they are able to evade the control strategies used by the food industry. Many of them are highly tolerant to abiotic environmental factors and develop in a wide temperature range from 0 to 30 °C. Fungi are well-adapted to particular ecological niches and are able to contaminate and spoil commercially processed foods [68]. Food products are also susceptible to fungal spoilage due to their nutrient-rich composition. However, this susceptibility depends on various factors: (a) the nature of the food matrix (living material or not, liquid or solid), its biological, physical, and chemical parameters (natural microbiota, water activity, pH); and its composition (nutrient content, solute type); (b) management during harvesting of vegetables and fruits (maturity, handling) and storage of raw material (hygrometry, temperature and duration); (c) technological processes used during production (heating, drying, fermentation, preservative addition); and (d) post-production storage conditions (temperature, relative humidity). The intrinsic properties of the food matrix, as well as the external factors associated with them, control the development of certain fungal genera or species. This usually results in the selection or dominance of one or more fungal species on a given matrix [31].

Fungal contamination can lead not only to visual defects but also to various organoleptic defects, including gas production, off-flavours and texture changes. Another huge problem that carries a serious health risk is the possible production of mycotoxins by some species of fungi [51]. The synthesis of mycotoxins by fungi is genetically determined and related to the basic metabolic pathways. Phenotypically, it is determined by environmental factors, which include the chemical composition of the substrate, the presence of micronutrients, temperature, moisture and the presence of competitive microflora [44]. Mycotoxins exhibit various toxic effects: genotoxic, cytotoxic, neurotoxic, hepatotoxic, or immunosuppressive, depending on their structural parameters [6]. The most important mycotoxins are aflatoxins, ochratoxin A, patulin, zearalenone, fumonisins, T-2 and HT-2 toxins, and deoxynivalenol. The presence of mycotoxins in processed foods is a serious problem as these metabolites are usually resistant to technological processes [31]. Table 1 lists the important mycotoxins, main producers and some commonly contaminated food commodities.

Table 1. The important mycotoxins in food commodities  
 Tabela 1. Najważniejsze mykotoksyny w produktach spożywczych

Mycotoxin / Mykotoksyna	Fungal species / Gatunki grzybów	Food commodity / Środek spożywczy
Aflatoxins B1, B2, G1, G2 / Aflatoksyny B1, B2, G1, G2	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>	Maize, wheat, rice, sorghum, almond, peanut, figs, pistachio, cottonseed / Kukurydza, pszenica, ryż, sorgo, migdały, orzeszki ziemne, figi, pistacje, nasiona bawełny
Aflatoxin M1 / Aflatoksyna M1	Derivative of aflatoxin B1 / Pochodne aflatoksyny B1	Milk, milk products / Mleko, produkty mleczne
Citrinin (citroviridin) / Cytrynina (cytrewirydyna)	<i>Penicillium citrinum</i> , other spp. from the genera <i>Aspergillus</i> and <i>Monascus</i>	Cereals / Zboża
Fumonisin B1, B2, B3 / Fumonizyny B1, B2, B3	<i>Fusarium verticillioides</i> , <i>F. proliferatum</i>	Maize, maize products, sorghum, asparagus / Kukurydza, produkty kukurydziane, sorgo, szparagi
Ochratoxin A / Ochratoksyna A	<i>Aspergillus ochraceus</i> ; <i>A. niger</i> ; <i>A. carbonarius</i> , <i>Penicillium verrucosum</i>	Legumes, grapes, cereals, coffee beans, dried vine fruit, wine, grapes / Rośliny strączkowe, winogrona, zboża, ziarna kawy, suszone owoce winorośli, wino, winogrona
Patulin / Patulina	<i>Penicillium expansum</i> , <i>P. patulum</i> and other spp. from the genera <i>Aspergillus</i> and <i>Byssoschlamys</i>	Apples, grapes, pears, other fruits / Jabłka, winogrona, gruszki i inne owoce
Trichothecenes / Trichoteceny	<i>Fusarium graminearum</i> , <i>F. sporotrichioides</i> , <i>F. culmorum</i> , <i>F. poae</i> and spp. from the genera <i>Myrothecium</i> , <i>Phomopsis</i> , <i>Tricoderma</i> , <i>Trichothecium</i>	Wheat, maize, barley, oats / Pszenica, kukurydza, jęczmień, owies
Zearalenone / Zearalenon	<i>F. graminearum</i> , <i>F. culmorum</i> , <i>F. cerealis</i> , <i>F. equiseti</i> , <i>F. crookwellense</i> , <i>F. semitectum</i>	Cereals, cereal products, maize, wheat, barley, sorghum / Cereals, cereal products, maize, wheat, barley, sorghum

Explanatory notes/ objaśnienia:

authors' elaboration based on literature [3, 6, 27, 40, 44] / opracowanie autorskie na podstawie literatury [3, 6, 27, 40, 44]

Heat treatment, especially acidic foods, usually destroy fungi. However, the ascospores of some species, such as *Byssoschlamys nivea*, are resistant and can spoil fruit purees or juices [73]. Fungal spoilage is a serious and costly problem for bakeries. Fungal spores are generally killed by the baking process in fresh bread and other baked product. This means that all spoilage problems caused by fungi must occur after baking. Therefore, for bread to become mouldy, it must be contaminated from bakery surfaces, equipment, air or raw ingredients after baking during the cooling, slicing or

packing operations [19]. Another significant problem in baking is the mycotoxin contamination of flour. As mycotoxins are temperature stable, they do not degrade significantly after baking [3]. Fungi also commonly contaminate meat and meat products. They can cause spoilage and produce mycotoxins, making meat dangerous for consumption. Nasser [42] found fungi of the genera *Aspergillus* and *Penicillium* in canned beef and poultry samples. Xerophilic *Aspergillus*, *Eurotium* and *Penicillium* species tolerate low pH and high salt concentrations, which is why they can grow on the surface of dry-cured meat products [40]. Plavsic et al. [53] detected *Penicillium aurantiogriseum* and *Penicillium commune* in dry-smoked meat products. Alía et al. [2] listed *Cladosporium oxysporum*, *Cladosporium cladosporioides* and *Cladosporium herbarum* as causes of black spots on dry-cured ham, and Lozano-Ojalvo et al. [36] reported that *Cladosporium oxysporum* formed black spots on dry-cured fermented sausages. Fungi can also spoil dairy products. In some cases, moulds are identified on the surface of the product, while in others, they form metabolites causing off-odours, discolouration, and an altered consistency [20]. Cheeses are most often contaminated by *Penicillium* spp. They convert sorbic acid and potassium sorbate to trans-1,3-pentadiene, thus, leading to a „kerosene” off-odor [70].

### **Fungus prevention and removal of mycotoxins from raw materials**

There are two main strategies for reducing the presence of fungi and mycotoxins in food: (a) preventing contamination before and after harvest; (b) detoxification of mycotoxins present in raw materials [51]. Most of the mycotoxins found in food are already synthesized during plant growth in the field. Preventing the production of fungal toxins requires appropriate preventive measures, which consist of appropriate treatment of the raw material already during vegetation, and then during harvesting, transport, storage and processing [27]. Such preventive actions include: selection of appropriate varieties resistant to fungal infections; minimizing the exposure of plants to stress, e.g. lack of water; careful use of plant protection products; the use of insecticides and herbicides; timely harvest; avoiding mechanical damage and soil contamination; appropriate soil management (plowing) to remove, destroy or bury infected crop residues; crop rotation; and proper storage of raw materials in appropriate temperature and humidity conditions [37].

Unfortunately, it is not always possible to minimize the production of toxins by mould fungi. If the raw materials are contaminated with mycotoxins, they should be decontaminated. There are several methods with varying degrees of practical use: mechanical separation of contaminated grains (sorting); use of physical factors, e.g. high temperature, UV or gamma radiation; application of chemical agents, e.g. ammonia, H<sub>2</sub>O<sub>2</sub>; and biological methods [54]. Effective reduction of food contamination with mycotoxins is one of the most difficult tasks of modern food processing and technolo-

gy. Chemical and physical decontamination methods may have product safety limitations or negatively affect product quality. Due to these limitations, the interest in microorganisms as detoxifying agents has been recently increasing. Currently, scientific research is focused on searching for organisms that can be used in biotechnological processes during the production of e.g. fermented food, where the raw material may be contaminated with mycotoxins. Such microorganisms can be found among lactic acid bacteria and yeast, which are widely used in food production. In addition to appropriate technological features, they have the ability to reduce the content of toxins, making the final product safer for the consumer [51].

Decontamination of mycotoxin-containing food by microbial binding is a very diverse process that depends on many factors, including the strain used, the physiological state of the cells, environmental conditions, and the initial concentration of the toxin. Yeasts are able to detoxify mycotoxins in different ways: bioadsorption, biodegradation, or the inhibition of mycotoxin production [43]. It has been suggested that adsorption to the cell surface is the main mechanism responsible for removing toxins produced by fungi. Many authors have described this ability in live yeasts and yeasts inactivated by exposure to a high temperature or an acid. It has even been reported that mycotoxins can be bound by prepared cell walls [48]. One of the most promising features of yeasts applied against mycotoxins is biodegradation. Literature reports demonstrate that some yeasts can break down toxins into less toxic or even non-toxic substances. Vekiru et al. [75] demonstrated that *Trichosporon mycotoxinovorans* could convert zearalenone into a non-estrogenic ZOM-1 product. This metabolite did not show estrogenic activity, even at a concentration 1000-fold higher than that of zearalenone, and did not interact with the human estrogen receptor *in vitro*. Schatzmayr et al. [62] reported that yeasts from the genus *Trichosporon*, *Rhodotorula*, and *Cryptococcus* spp. were able to split the amide bond of the ochratoxin molecule and release non-toxic ochratoxin  $\alpha$  (OT $\alpha$ ). Yeasts can also produce metabolites that have a significant suppressing effect on the expression of genes related to mycotoxin biosynthesis [50].

### **Role of antagonistic microorganisms in ensuring food safety**

Biological control of pathogenic fungi and the mycotoxins produced by them is considered a safe method and, as such, is gaining popularity in the food industry [44]. This method uses microorganisms or their antimicrobial metabolites in order to extend the shelf-life of food products and increase food safety [41]. Most of the research on antagonistic microorganisms and their applicability in food technology is focused on lactic acid bacteria (LAB). Many species of these bacteria have GRAS (Generally Recognized As Safe) status, established by the Food and Drug Administration (FDA), as well as QPS (Qualified Presumption of Safety) status, established by the European

Food Safety Authority (EFSA). Lactic acid bacteria are widely used not only in the food industry but also in animal production, including as feed additives or silage preparations [22], and in agriculture as safe and effective biological agents for combating bacterial and fungal phytopathogens. The mechanism of their action is the production of lactic and acetic acids, bacteriocins, reuterin and other antimicrobial compounds, as well as the removal of reactive oxygen species [33]. In recent years, scientists' interest has also concentrated on the search for microorganisms other than LAB, capable of inhibiting the growth of pathogenic microorganisms. More and more scientific reports concern the use of yeast occurring naturally in the environment. Finding yeast strains that synthesize antifungal compounds and have the ability to lower the mycotoxin content would make it possible to extend the shelf life of food products and improve their quality [4, 55].

Microorganisms antagonistic to pathogens should exhibit a number of characteristics, such as rapid growth, a greater ability to survive and develop than the pathogen, effectiveness against a wide range of pathogens, ability to survive in adverse environmental conditions, effectiveness at low concentrations, and genetic stability; they should also be nutritionally undemanding, safe for consumers and should not produce metabolites that are toxic to humans [65]. It is often difficult to select a strain with a broad spectrum of activity against many pathogens. Therefore, compatible strains are sought to ensure the necessary differentiation of action [55]. The use of a mixture of antagonists has certain advantages: it increases their effectiveness, broadens the spectrum of their activity, and allows to combine of various biological features without passing foreign genes through genetic transformation. The absence of antagonism between components of an antagonist mixture must also be ensured in choosing those microorganisms. The selection of components which show mutually beneficial interactions (mutualism) allows for making more effective use of their antagonistic potential [65].

### **Mechanisms of antagonistic yeast action**

The main mechanisms of yeast action that play a key role in the biological control of pathogenic fungi are (a) competition for nutrients and space; (b) secretion and release of antimicrobial compounds, such as killer toxins and volatile organic compounds (VOCs), and (c) secretion of enzymes that degrade the fungal cell wall. The mechanisms of yeast action on fungi are usually used simultaneously, thus enhancing the antagonistic effect [11, 41].

#### *Competition for nutrients and space*

Competition for nutrients and space is considered one of the main mechanisms of antagonistic yeast action, as it involves the nutritional requirements of both the antago-

nist and the pathogen. These two types of competition are commonly considered together without assigning an appropriate level of significance to each [55]. Antagonistic yeast can effectively fight pathogenic fungi by outgrowing their mycelium, especially if applied before the appearance of the pathogen. Yeast quickly depletes glucose, fructose or sucrose, preventing the growth of undesirable microorganisms, a method that has been used in the fermentation of foods and beverages involving *S. cerevisiae* [41, 71]. Competition for sugars has also been demonstrated between the yeast *Candida valida*, *Rhodotorula glutinis* and *Trichosporon asahii*, all of which have the ability to colonize sugar beet roots, and the pathogen *Rhizoctonia solani* [16]. Competition for nitrogen compounds, especially in the carbon-rich environment of fruit wounds, has been reported for the species *Candida guilliermondii* against *P. expansum* [63]. The results obtained by Zhang et al. [76] also indicate that competition for nutrients played a significant role in the biocontrol activity of *Aureobasidium pullulans* PL5 against *M. laxa*, *B. cinerea*, and *P. expansum*. The ability of *A. pullulans* PL5 to inhibit the growth of pathogen mycelium was significantly better when co-cultured with pathogens on a medium with a lower nutrient concentration. A specific example of inhibition of fungal growth by yeast is competition for iron, which is necessary for the proper functioning of cells. This mechanism is based on the synthesis of siderophores, compounds chelating iron ions from an environment poor in this element, which, when chelated, are unavailable to pathogenic microbes [71].

#### *Production of antifungal metabolites and volatile compounds*

Many microorganisms effectively affect other microbes by producing antibiotic agents. This, however, is not commonly observed in yeast. Some strains of *Pseudozyma fusiformata* have been found to secrete ustilaginic acid – a glycolipid active against various species of yeast and filamentous fungi [77]. The inhibitory effect of aureobasidin A produced by *A. pullulans* against the pathogens *Candida albicans*, *Aspergillus nidulans*, *M. fructicola*, *P. expansum*, *P. digitatum*, *P. italicum* and *B. cinerea* has also been observed [8, 25, 35]. This antibiotic is an inhibitor of one of the key enzymes in the sphingolipid biosynthesis pathway, and its effects include morphological changes in mycelium and disturbances in the conidia maturation process [25].

Some yeast secretes killer toxins which can also be used to reduce pathogenic fungi. They are produced by the yeast *S. cerevisiae* and others of the genera *Kluyveromyces*, *Candida*, *Debaryomyces*, *Pichia*, *Hansenula*, *Kloeckera*, *Torulopsis*, *Williopsis*, *Zygosaccharomyces*, and *Cryptococcus* [41, 64]. The mechanisms of action of killer toxins on fungi include inhibition of  $\beta$ -glucan synthesis or hydrolysis of  $\beta$ -glucan in the cell wall of susceptible strains, disruption of cell division by blocking DNA synthesis, tRNA cleavage, blocking calcium uptake, and ion leakage caused by the formation of channels in the cytoplasmic membrane [41]. Da Silva Portes et al. [66] showed that



killer toxins isolated from the yeast of the genus *Kluyveromyces* could be used in the biocontrol of the pathogenic fungi *P. expansum* and *A. ochraceus*, and that they exerted their action by inhibiting spore germination and limiting mycelium growth. The activity of the yeast *S. cerevisiae*, possibly related to its ability to secrete killer toxins, was also used to control the toxigenic fungi *A. flavus* and *A. parasiticus* [49]. The yeast *D. hansenii* and *Wickerhamomyces anomalus* showed killer activity against fungi from the genus *Monilinia*, which cause moniliosis, also known as brown rot. Grzegorzczuk et al. [21] demonstrated that the infestation of peaches and plums with *M. fructicola* was reduced after applying the yeast *W. anomalus* BS91 to the infection site.

Among a wide range of antimicrobial molecules, VOCs are currently attracting the attention of scientists because of the advantages of their potential application. VOCs typically consist of a mixture of volatile metabolites that can exert strong inhibitory activity against other organisms. Their potent bioactivity, along with the lack of reports of significant effects on consumer health and the environment, has prompted the development of research on the subject [47]. Fernandez San Millan et al. [18] identified several new antifungal compounds produced by the yeast *M. pulcherrima*, with 3-amino-5-methylhexanoic acid, biphenyl-2,3-diol and sinapaldehyde showing the greatest activity (with reductions of up to 90 ÷ 100 % in tomato and apple infections by *B. cinerea*). Núñez et al. [45] demonstrated that the yeast isolate **Debaryomyces hansenii** produced 2-methyl-1-butanol, other volatiles, and unidentified diffusing molecules, which were active against *P. verrucosum*, a spoilage fungus from dry-cured ham.

#### *Production of lytic enzymes*

The cell wall of filamentous fungi is a complex made of numerous polymers, including  $\beta$ -1,3- $\beta$ -1,6-glucan, chitin, proteins and lipids. It is a very dynamic structure which undergoes constant changes, e.g. during morphogenesis, cell division or growth, and is slightly different for different species of fungi [10]. Yeasts produce hydrolytic enzymes, especially chitinases,  $\beta$ -1,3-glucanases and proteases, which can degrade cell wall polymers, thus providing an effective means for biocontrol phytopathogenic fungi [11]. Chitinases not only allow efficient degradation of the cell wall of plant pathogens but can also stimulate natural plant immune processes by degrading chitin and producing chitooligosaccharides [28]. The ability to produce lytic enzymes has been demonstrated in many yeast species including *M. guilliermondii*, *Cyberlindnera saturnus*, *R. glutinis*, *Candida shehatae*, *C. tropicalis*, *C. fluvialis*, *P. anomala*, *P. membrani-faciens*, *Cryptococcus laurentii*, *C. carnescens*, *A. pullulans*, *T. albescens*, *T. pallescens* [17, 55].

## Yeast in bioconservation of food and beverages

The discovery of the mechanisms of the antagonistic action of yeasts against fungal pathogens and their ability to remove mycotoxins is of great importance to many branches of the food industry. The quality of products such as bread, cold cuts, cheese, wine and beer is directly related to the growth of spoilage microorganisms. The use of antagonistic yeasts or their antimicrobial metabolites may contribute to increasing the safety and shelf life of food by inhibiting the growth of pathogens [59].

The most commonly used yeast in the food industry is *S. cerevisiae*. This species has gained its "popularity" in the food industry mainly due to its ability to tolerate a wide range of stress conditions in the conversion processes, especially ethanol, osmotic and oxidative stress while demonstrating very efficient fermentation performance [9]. As an ingredient of leaven, *S. cerevisiae* has been used for centuries for baking bread and cakes. Carbon dioxide, released in large volumes during the fermentation, the yeast carries out, loosens and raises the dough. In brewing, this yeast is used in the production of beer, with the two most common strains being the top-fermenting yeast *S. cerevisiae* var. *cerevisiae* and the bottom-fermenting *S. cerevisiae* spp. *uvarum* var. *carlsbergensis*. Molasses distillation uses many strains of *S. cerevisiae* obtained from scientific institutes or isolated in various distilleries and adapted for industrial purposes. Their main features include the ability to quickly and dynamically produce alcohol and the ability to resist a 10 ÷ 12 % alcohol concentration, and the high osmotic pressure caused by the presence of sugar and inorganic substances in molasses. They are also resistant to the action of organic acids such as tartaric, malic, citric and other non-volatile acids [32, 57].

*S. cerevisiae*, in addition to the appropriate technological characteristics, has antagonistic properties against pathogenic bacteria and fungi and the ability to reduce the content of toxins. Soboleva et al. [69] found that *S. cerevisiae* RCAM 01730, when used in bread baking, inhibited the growth of undesirable bacteria. This isolate also accelerated the production of gas, intensifying the maturation of the dough and improving the organoleptic properties of the finished bread. *S. cerevisiae* has been proven to have detoxification properties towards mycotoxins such as aflatoxin B1, deoxynivalenol, fumonisins, T-2 toxin and zearalenone [13]. The results obtained by Piotrowska and Masek [52] indicate that polysaccharide components of the yeast *S. cerevisiae* cell wall are responsible for the adsorption of ochratoxin A. This phenomenon may find applications in industry, such as in oenology or as dietary supplements for humans and animals. Literature reports indicate that yeasts other than *S. cerevisiae* are also effective in reducing the occurrence of mycotoxins. Var et al. [74] demonstrated that a *C. famata* strain removed about 30 % of ochratoxin A from wine. Reduction of the deoxynivalenol, nivalenol and zearalenone by selected non-conventional yeast strains (*C.*

*shehatae*, *C. fluviatilis*, *C. tropicalis*, *R. glutinis*, *C. carnescens*, *C. saturnus*) in wheat grains and bread was demonstrated in a study by Podgórska-Kryszczuk et al. [56].

Currently, more and more research is focusing on applying non-conventional yeast strains in food technology. The great potential of various non-*Saccharomyces* yeasts species in the modern baking industry to increase the aromatic complexity of bread [5] and as potential leavening agents has been observed [78]. Such yeasts, by producing many aromatic secondary metabolites such as esters, aldehydes and ketones, can significantly affect the flavour profile of the finished product [72]. Timmermans et al. [72] studied the fermentation properties of non-conventional yeast strains from various food industries in the sweet dough with 14 % sucrose. The scientists showed that 22 non-conventional yeast strains were able to ferment in the sweet dough, and several strains produced more positive flavour compounds compared to the reference baker's yeast.

The literature indicates that using yeasts that produce killer toxins prevents the growth of wine spoiling bacteria and yeast [14]. Killer toxins secreted by *Tetrapisispora phaffii* (former *Kluyveromyces phaffii*) are potent inhibitors of *Hanseniaspora uvarum*, which is why they may have biopreserving properties potentially useful for the wine industry [61]. Comitini et al. [14] noted that the glycosylated 33 KDa protein produced by *K. phaffii* bound  $\beta$ -1,3-glucan, leading to the formation of pores in the cell walls of the wine-spoiling yeast. Scientists also showed that the killer toxins secreted by *W. anomalus* and *Kluyveromyces wickerhamii* inhibited the growth of *Dekkera* and *Brettanomyces* – odor-causing yeasts found in wine. Yeasts that produce killer toxins can also be used in the production of beer and sake [41], in the dairy sector to prevent deterioration of cheese and yoghurt [34], in olive fermentation [24], and in the production of salted fermented foods such as salted vegetables and miso [12]. The main factors to consider when using killer toxins in food processes are the pH and the temperature range at which they exhibit a high activity, as inappropriate conditions can limit their effectiveness, especially in the food fermentation processes. Although there are large differences in the pH and temperature conditions under which the toxins are active, they all exhibit optimal activity between pH values of 4.0 and 5.4 and at temperatures below 30 °C [7]. Da Silva et al. [67], for example, isolated the killer toxin CnKT (*Candida nodaensis* Killer Toxin) from the extremely halotolerant yeast *Candida nodaensis*, which was active in the range of pH 2.6 ÷ 6 and temperatures from 18 to 30 °C.

Today, consumers prefer minimally processed products obtained through natural methods such as fermentation, so the consumption of fermented vegetables has increased by almost 60 %, mainly in developed countries. Most fermented products have a long stability and low production costs compared to other products with similar characteristics [29]. While yeasts are commonly present in traditionally fermented or acidi-

fied foods, there is little data on their isolates, with most research being focused on the presence of lactic acid bacteria. Lazar et al. [30] isolated yeasts naturally occurring in sauerkraut, cucumber pickles and fermented vegetable mixtures. Strains of the species *S. cerevisiae* and *Y. lipolytica* turned out to be the dominant ones, constituting 38.7 % and 25.6 % of the total number of isolates, respectively. Strains belonging to the species *Pichia etchellsii*, *P. ohmerii*, *Candida holmii*, *C. pelliculosa*, and *Shizosaccharomyces japonicus* were also isolated from the pickled products. Large amounts of traditionally fermented foods and drinks are produced in African, Asian and South American countries from raw materials such as corn, wheat, cassava, rice, soybeans and various fruits. Fermentation is essential to the quality and safety of these products. The participation of bacteria and yeast also has a significant impact on food parameters such as taste, texture, smell and nutritional value [1, 26]. Selected yeasts can also be used as antifungal agents in dry-fermented foods to inhibit the growth of pathogenic fungi. Their ability to compete for nutrients and space with fungi as well as to produce antifungal metabolites such as volatile compounds and proteins, explains their antifungal properties [15].

## Conclusions

1. The growing demand for "clean label", less processed food products with high safety and quality levels and a long shelf-life, prompts scientists to develop new food preservation methods. The increasing consumer requirements and competitiveness force manufacturers to use high quality agricultural raw materials and produce high quality products.
2. Yeast can contribute to the safety of food products by inhibiting the growth of undesirable pathogens, through various mechanisms of action, during the fermentation process. Unfortunately, most studies on the use of antagonistic yeast against toxicogenic fungi are laboratory scale investigations due to the short list of GRAS/QPS yeasts that are unequivocally recognized as safe for use in food production.
3. Current research on the activity of microorganisms involved in the removal of mycotoxins is mainly of scientific importance. It provides a better understanding of strains, their properties and the mechanisms of the processes taking place. Further research is needed to identify strains that could be used in the industry without compromising consumer safety.

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## DROŹDŹE JAKO ALTERNATYWNA METODA ZWALCZANIA GRZYBÓW W PRZEMYŚLE SPOŻYWCZYM

### Streszczenie

**Wprowadzenie.** Jakość produktu końcowego w branżach, takich jak piekarnictwo, winiarstwo, browarnictwo oraz w przemyśle mięsny i serowarski często pogarsza się z powodu rozwoju drobnoustrojów powodujących psucie się żywności. Grzyby strzępkowe i ich toksyczne metabolity, znane jako mikotoksyny, należą do czynników, które w znacznym stopniu obniżają jakość żywności i poważnie zagrażają jej bezpieczeństwu. Mikotoksyny powodują biochemiczne, fizjologiczne i patologiczne zmiany w organizmach żywych, a działanie toksyczne wykazują już nawet w niskich stężeniach. Zagrożenie dla bezpieczeństwa żywności stwarzane przez patogenne grzyby i ich toksyczne metabolity skłania do poszukiwania nowych sposobów na ograniczenie ich przedostawania się do łańcucha pokarmowego.

**Wyniki i wnioski.** W ostatnich latach wiele uwagi poświęcono metodom biokonserwacji. Biokonserwacja odnosi się do wydłużenia okresu przydatności do spożycia i zwiększenia bezpieczeństwa żywności przy użyciu rodzimych lub dodanych mikroorganizmów oraz ich metabolitów o działaniu przeciwdrobnoustrojowym. Kultury starterowe drożdży antagonistycznych w stosunku do grzybów toksynotwórczych mogą przyczynić się do zwiększenia bezpieczeństwa produktu, głównie poprzez hamowanie wzrostu patogenów podczas fermentacji i poprawę stabilności tego procesu. Niniejszy przegląd opisuje główne problemy związane z występowaniem grzybów strzępkowych w przemyśle spożywcym oraz strategie ograniczania ich obecności i produkowanych przez nie mikotoksyn w żywności. W szczególności skupiono się na wykorzystaniu drożdży jako mikroorganizmów przeciwgrzybiczych testowanych pod kątem biochrony żywności oraz na mechanizmach ich działania.

**Słowa kluczowe:** biokonserwacja, biokontrola, bezpieczeństwo żywności, zwalczanie grzybów, mykotoksyny ✂