Food nowadays

Local or global, traditional or innovative?

Conference monograph

Edited by
Monika Przeor

Wydawnictwo
Uniwersytetu Przyrodniczego w Poznaniu

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Żywność dzisiaj

lokalna czy globalna?
tradycyjna czy innowacyjna?

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traditional or innovative?

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Preface

Both, traditional and innovative food are an important element of nowadays consumers' menu. Regardless of the food processing techniques used, consumer preferences remain individual. The goal of food technology and food analysis is to monitor, on the one hand the needs of the food market, and on the other hand – the possibilities of the food industry.

Every, even the smallest aspect related to modern food should be considered scientifically in order to provide consumers with reliable nutritional information.

This monograph is a compilation of scientific work showing how far the considerations of food can be. As a result of their work, the authors of the chapters introduce the reader to the world of current analytical problems, microbiological quality, the impact of specific processing processes on a wide range of products, including mushrooms that are returning to favor.

This monograph is a compilation of interesting nutritional and technological considerations conducted during the IXth International Session of Young Scientific Staff 'Nowadays food – local vs. global? Traditional vs. Innovative?' (Poznań, 19–20th May, 2022) and shows readers in what direction young scientists working in Poland are following.

Here I would like to express my sincere thanks to all Authors for sharing their research.

Monika Przeor, PhD

Bisphenol A and its derivatives as an endocrine disrupting chemicals: occurrence, detection methods and toxicology – a critical review

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Abstract. This article is a short review about of the most frequently occurring endocrine disrupting compounds (EDCs) in our daily life. It is a group of chemicals natural or synthetic origin, that can disrupt endocrine pathways (thyroid, estrogen, and androgen) through binding with various hormone receptors, including: estrogen, androgen or even aryl hydrocarbon receptors. In the current review the main topic was bisphenol A, its analogues and derivatives such as bisphenol A diglicidyl ether, which can migrate from packaging material into the food matrix.

Keywords: endocrine disrupting chemicals, bisphenol A, bisphenol A diglycidyl ether, food packaging material

Introduction

Hazardous chemicals escape to the environment in a variety of anthropogenic ways and their release can result in adverse effects both on the human health as well as on the environment. Among the environmental pollutants, endocrine disrupting compounds/ chemicals (EDCs), able to disrupt the hormonal system, are increasingly gathering the attention of the scientific community due to their well-recognized toxicity for humans (Jatkowska et al., 2021; Russo et al., 2018). While bisphenol A (BPA) – a model EDCs – has been extensively examined much less attention has been paid to other toxic substances, including those that are used in the production of plastics.

Endocrine disrupting chemicals (EDCs)

Many studies have identified effects of various exogenous chemicals on endocrine processes and functions, exposing the important need for a shift in scientific theory. Food and Drug Administration (FDA) identified more than 1800 chemicals (natural and synthetic origin) that disrupt at least one of three endocrine pathways (thyroid, estrogen, and androgen) and can bind to and activate various hormone receptors, including: estrogen receptor, estrogen-related receptor, androgen receptor, pregnane X receptor, aryl hydrocarbon receptor, constitutive androstane receptor, glucocorticoid receptor, thyroid hormone receptor, retinoid X receptor and then mimic the natural hormone's action. A great number of EDCs seem to be able to interfere with the physiology of the hypothalamus-pituitary-gonadal axis, but every endocrine axis can be a target for each EDCs and their action is not limited to a single axis or organ. In particular, many of these dose-response relations have been non-monotonic (Kahn et al., 2020; Lauretta et al., 2019).

Many studies inform us, that environmental exposures to EDCs can provoke the so-called thrifty phenotype, in which a fetal metabolism that is conservatively programmed is maladapted to the *ex utero* environment, resulting in increased adiposity beginning in childhood (obesogens) and cardiovascular risks later in life (Kahn et al., 2020; Lobstein and Brownell, 2021). Some of EDCs are known to target the ovary and cause reproductive health problems such as infertility, premature ovarian failure, and abnormal sex steroid hormone levels (Gore et al., 2015; Patel et al., 2015) but also decrease oocyte quality, fertilization rate, implantation, embryo quality, rate of clinical pregnancy and live births (Bloom et al., 2011; Karwacka et al., 2019).

Moreover, the endocrine system is especially important for male reproductive development because androgens (like testosterone) promote the maturation of male secondary characteristics as well as the process of spermatogenesis. Male reproductive health – like sperm count and testosterone – have been declining, which is correlated with an increase in a variety of EDCs in the environment (Rehman et al., 2018).

EDCs may display different routes to contaminate the human body. Generally, food intake, inhalation, and direct contact represent the most common exposure pathways.

Food packaging material

In recent years, lifestyle changes have increased the need for ready-to-eat food and packaged/ canned foods. Among the latter, canned food products and plastics have become universal and multifunctional materials which offer several advantages, either for the producers or the consumers (ease of packaging, preservation, handling, and transportation). Still, the substances being used in polymerization processes, or at the degradation stage, are not environmentally neutral. Several of them are classified as EDCs, such as ingredients of epoxy resins, polyesters and its derivatives/analogues (Jatkowska et al., 2021).

Many factors affect the migration from packaging material into the food matrix. Sterilization was shown to have a predominant effect on the migration of bisphenol compounds in cans, other factors, including nature of food, fat concentration, presence of oxidizing agents and nitrates, porosity, tin coating thickness and surface defects, and volume of headspace may significantly affect the migration of metals and/or bisphenol compounds (Abdel-Rahman, 2015; Petropoulos et al., 2018; Wagner et al., 2018).

Bisphenol A and its analogues/derivatives

Bisphenol A (BPA). BPA, first synthesized in 1891 by Dianin, has become one of the most popular plasticizers, used to support in the polymerization of durable and relatively resistant polycarbonates and epoxy resins, which are incorporated into the inner coating of metal cans (Almeida et al., 2018; Jatkowska et al., 2021). Its estrogenic activity was discovered in 1936. It is therefore one of the oldest synthetic compounds known for its endocrine activity. Furthermore, several studies have proposed a relationship between exposure to BPA and the appearance of adverse health effects, such as cancer, infertility, diabetes, disturbance in neuronal development, chronic respiratory and kidney diseases, tooth developmental defects, reproductive disorders in both sexes and obesity, among others.

BPA can leach from food or beverage containers and is then ingested. The routes of human BPA exposure include dermal, oral and inhalation absorption (Zalko et al., 2011). Due to their endocrine disrupting effects, bisphenol compounds have been regulated with specific migration limits (SMLs) established for plastics and coatings that are intended to come in contact with foods. For example, the SML of BPA was fixed to 600 μg/ kg by the European regulations

((EU) No 10/2011), being reduced in 2018 to 50 μg/ kg according to Commission Regulation (EU) 2018/213, and expands the ban on the use of BPA in the manufacture of polycarbonate infant feeding bottles to sippy cups (Vilarinho et al., 2019).

Various analytical methods, including liquid chromatography, liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, fluorescence spectrophotometry, fluorimetry, capillary electrophoresis, immunoassay, and several novel sensors, have been used to determine BPA. The aforementioned methods can detect BPA with high sensitivity, however, may require complicated sample preparation and pre-treatment (e.g., solvent extraction, solid phase extraction, molecularly imprinted polymer solid-phase extraction, and micro-extraction techniques), high-cost instruments, and skilled operators (Sun et al., 2016; Zheng et al., 2021).

Bisphenol S (BPS) and bisphenol F (BPF). BPS (2,2-bis [4-hydroxyphenol] sulfone) and BPF (2,2-bis [4-hydroxyphenol]methane) are the most commonly used BPA substitutes (Jatkowska et al., 2021), presently not regulated and used without any restriction. However, BPA-like effects can be hypothesized because their chemical structures are similar (Eladak et al., 2015).

Compared to BPA, BPS has lower acute toxicity, less or similar endocrine disruption, similar immunotoxicity and neurotoxicity, and lower reproductive and developmental toxicity (Qiu et al., 2019). But according to Thoene and coworkers (2020) BPS works via different pathways than does BPA while causing equivalent obesogenic effects, such as activating preadipocytes, and BPS is correlated with metabolic disorders, such as gestational diabetes, that BPA is not correlated with. BPF showes the same dose-response curve as BPA with a reduction in the amount of testosterone secreted by mouse fetal testes (Eladak et al., 2015). Like BPA, BPF is absorbed by oral route and distributed to the whole organism including the reproductive tracts and the fetuses by crossing the placental barrier. BPF was found to be non-mutagenic in salmonella tester strains, however, it has been recently suggested to be potentially carcinogenic via other tests (Usman et al., 2019).

Bisphenol A diglycidyl ether (BADGE). 2,2-bis(4-(2,3-epoxypropyl) phenyl) propane, is also known as bisphenol A diglycidyl ether or BADGE. BADGE-based epoxy resin is one of the most widely used epoxy resins with an annual production amount of several million tons, due to the properties it provides (i.e. heat resistance, good mechanical and electrical properties, or low maintenance efforts)

(Jatkowska et al., 2021; Wang et al., 2021). It is a reactive pre-polymer of BPA that is synthesized through the O-alkylation of BPA with epichlorohydrin. It is comprehensively used as a coating material in beverage and food cans monomer in the production of epoxy-based polymers and an additive for the elimination of surplus hydrochloric acid in polyvinyl chloride (PVC) organosol production. The main application areas of BADGEs are primarily in sealants, packaging materials, fillers for textiles, coating, paints, or even dental filling material.

The chemical structure of BADGE is quite unique with two epoxides (electrophilic oxirane rings), resulting in its high reactivity. That is why BADGE can react with food components such as protein, amino acids and carbohydrate-rich food products which leads to its 'disappearance' in food packaging materials (Petersen et al., 2008). Cysteine was the predominant reaction center for amino acids, peptides and protein (Coulier et al., 2010).

BADGEs make numerous groups of compounds, which include H_2O adducts (BADGE·H_2O and/or BADGE·2H_2O), HCl adducts (BADGE·HCl, BADGE·HCl·H_2O and/or BADGE·2HCl), novolac glycidyl ether derivatives (NOGEs), di-, tri-, tetra- BADGEs, etc. Moreover, their easy and fast detection may be complicated by the presence of vast amounts of other compounds used in the production of epoxy resins (plasticizers, lubricating agents, crosslinking agents, curing agents, hardeners, fillers, pigments, wetting agents, antioxidants, etc.). Of course, as BADGEs are semi-volatile compounds, liquid chromatography-mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) under multiple reaction mode (MRM) have been successfully used for the analysis of BADGE conjugates in can-coating materials (Beszterda et al., 2022).

BADGE, like other plasticizers, can cross the human placenta and reach the foetus (Marqueno et al., 2019). Regarding endocrine-disrupting potentials, exhibits anti-androgenic and estrogenic effects as well as obesogenic properties. It was also reported that BADGE is mutagenic, genotoxic and cytotoxic in some cell models (Wang et al., 2015), moreover the testicular histology showed a dose-dependent effect of BADGE on spermatogenesis (den Braver-Sewradj et al., 2020).

Bisphenol F diglycidyl ether (BFDGE). Novolac glycidyl ether (BFDGE) is produced in the reaction between novolac and epichlorohydrin (Szczepańska et al., 2018). Both BADGE and BFDGE have epoxide, a cyclic ether with a three-atom ring, which makes them highly reactive. BFDGE can be easily transformed to its hydrolysis products or chlorinated products, also the same

as BADGE. The use of BFDGE was prohibited in food contact applications in 2005 in the EU (Commision Regulation no. 2011/8/EU).

BFDGE is able to induce *in vitro* morphological changes in Caco-2 cells, cell detachment from the substratum and to inhibit cell proliferation, in a time and dose-dependent manner, also exhibited anti-androgenic activity *in vitro* with comparable potencies as BPA (Vervliet et al., 2020). These chemicals and their derivatives have also been identified in plasma and adipose samples, can induce an increase in the frequency of sister chromatid exchanges and micronuclei in human peripheral blood lymphocytes and are able to induce mutagenic effects in bacterial strains (Szczepańska et al., 2018).

Conclusions

As shown in this review, more and more studies are necessary on analytical methods, occurrence, transformation and toxicity of BPA, its analogues and derivatives. A group of EDCs is now recognised as a serious and urgent threats to public health, potentially emerging as one of the leading environmental risks globally (Kahn et al., 2020). Overall many evaluations showed that for most BPA alternatives data on reproductive toxicity, carcinogenesis, mutagenic properties and endocrine disrupting potential are too limited to perform a full hazard assessment. Also, a current risk assessment does not consider mixtures of substances, which demonstrate hormonal potential. Moreover, it is yet not clear of which exposure pathway (e.g. dietary intake, dust ingestion or inhalation exposure) is more important to the entire human exposure.

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Bisfenol A i jego pochodne jako związki zaburzające gospodarkę hormonalną: występowanie, metody detekcji oraz toksyczność – przegląd literatury

Streszczenie. Artykuł poświęcono krótkiej charakterystyce substancji mających zdolność do zaburzania pracy układu hormonalnego (ang. *endocrine disrupting compounds*, EDCs), szeroko rozpowszechnionych w naszym życiu codziennym. Jest to grupa związków pochodzenia naturalnego lub syntetycznego, które mogą zakłócać szlaki hormonalne (tarczycy, estrogenu i androgenu) poprzez oddziaływanie z różnymi receptorami, np. estrogenowymi, androgenowymi czy receptorami węglowodorów aromatycznych. Główny temat rozważań stanowił bisfenol A, jego analogi i pochodne, takie jak eter diglicydylowy bisfenolu A, które ze względu na obecność w materiałach opakowaniowych przeznaczonych do kontaktu z żywnością, mogą do niej migrować.

Słowa kluczowe: związki zaburzające gospodarkę hormonalną, bisfenol A, eter diglicydynowy bisfenolu A, materiały opakowaniowe przeznaczone do kontaktu z żywnością

Derivatographic studies of the milk-containing thermostable fillings composition

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Abstract. This article is dedicated to derivatographic studies of model systems for the formation of a thermostable structure. The main purpose of the research was to clarify the data on the system water of the components for the milk-containing fillings developed in the work and the fillings themselves. The objects of these studies were the same model systems as in the research using the derivatographic method. The composition of the thermostable filling was justified. The use of gelatine, a mixture of gums and the enzyme transglutaminase was indicated. The derivatographic studies established that the main part of the water in the model systems is in a physicochemical relationship with dry substances. It was established that for model systems the phase transition of the first type of system water from an amorphous or liquid state to a gaseous state occurs in the temperature range from 115°C to 125°C. In conclusion, the obtained results confirm other results obtained by using the derivatographic method.

Keywords: milk-containing thermostable fillings, transglutaminase, gelatine, derivatographic method

Introduction

The actual task of the modern production of thermostable milk-containing fillings is the balance of the cost of raw materials and the technological process of production, low nutritional and biological value, and high content of food additives. This will increase shelf life and improve organoleptic indicators (Moroz, 2013).

Thermostable fillings are high-quality products created specifically for bakery and confectionery products, for filling products that are subject to heat treatment (Koshel et al., 2018a). They retain their shape after baking, due to their structure, they are easily machined. Fillings are part of multicomponent systems, as they consist of raw materials of various types (Lyubenko, 2013).

According to the resistance to the influence of temperature during the technological process, they are divided into thermostable and not thermostable (Yurchenko, 2013).

Thermostable fillings have a specially selected stabilization system in their composition, which ensures the resistance of the filling to the influence of high temperatures (Sarafanova, 2010).

The use of thermostable fillings is not only an additional source of improving the range of culinary products. Created on the basis of natural components (pectin, succinic acid, etc.), they improve the quality of baking, help cleanse the body of impurities and radionuclides (Bondarenko et al., 2016).

The selection of components for heat-stable milk-containing fillings with adjustable taste, aromatic and heat-stable properties allows to achieve a low cost by replacing structure-forming components with cheaper ones (Koshel et al., 2018b).

Materials and methods

The raw materials for the production of mixtures for thermostable milk-containing fillings as skimmed milk powder, white sugar, gelatine, transglutaminase, xanthan gum, tara gum, maltodextrin met the requirements of the current regulatory documentation.

These model systems are the following objects of derivatographic studies:

- model system of xanthan gum and tare gum
- model system of xanthan gum, tara gum and gelatine
- model system of xanthan gum, tara gum, gelatine and maltodextrin
- model system of xanthan gum, tara gum, gelatine, maltodextrin and skimmed milk powder
- model system of xanthan gum, tara gum, gelatine, maltadextrin, dry milk and powdered sugar
- thermostable milk-containing filling.

The determination of the moisture form was studied using the thermoanalytical method on the MOM Q-1000 (Hungary) derivatograph device. This method is based on the dependence of the rate of diffusion of various forms of moisture in the material on the rate of change in the mass of the heated sample. The device recorded thermal effects by the method of differential thermal analysis (DTA). The change in mass of the test sample was determined by

the thermogravimetric method (TG). Thermogravimetry by derivative made it possible to determine the rate of mass loss (DTG). The determination was carried out at a rate of monotonic temperature increase of 2°C for 60 s, samples were heated to 230°C.

Results

Derivatographic research is one of the most common physicochemical methods (Ivaniuk and Suprunchuk, 2018). It allows studying the behaviour of individual substances and compositions under the conditions of programmed heating. In practice, the classification and quantification of various processes that occur during heating of samples are carried out according to heat release curves and loss curves. A significant interest is the determination of kinetic parameters of these processes, as well as the evaluation of the mechanisms of their occurrence. The essence of derivatographic studies consists in the process of continuous programmed heating of the sample and recording the changes occurring in it. The mass loss (TG) due to the release of volatile components or the flow of a chemical reaction with a change in the mass of the sample was investigated, absorption or release of heat (DTA) due to phase transitions rate of mass change (DTG).

Figure 1 shows the derivatograms that containing the kinetics of temperature (T), mass loss (TG), heat absorption or release (DTA), rate of mass change (DTG). The experiment was carried out under the condition of a given monotonic temperature increasing.

As we can see at Figure 1 for all studied samples DTG and DTA are directed to the decreasing the temperature. Thus, it should be assumed that the process of heating model systems is accompanied by heat absorption. It is endothermic. Derivatograms for all samples can be divided into three main stages. They are separated on Figure 1 by a dotted line.

At the first stage, the model system is heated. This is evidenced by the monotonous change in temperature (T) over time. As a result of heating, there is an increase in the intensity of evaporation of water from the surface of the model system.

Thus, the kinetics of mass loss (TG) changes the angle of inclination relative to the axis on which time is delayed. It confirms the increase in the intensity of water evaporation and the kinetics of mass change (DTG). The angle of inclination of the curve to the abscissa axis changes at the same time.

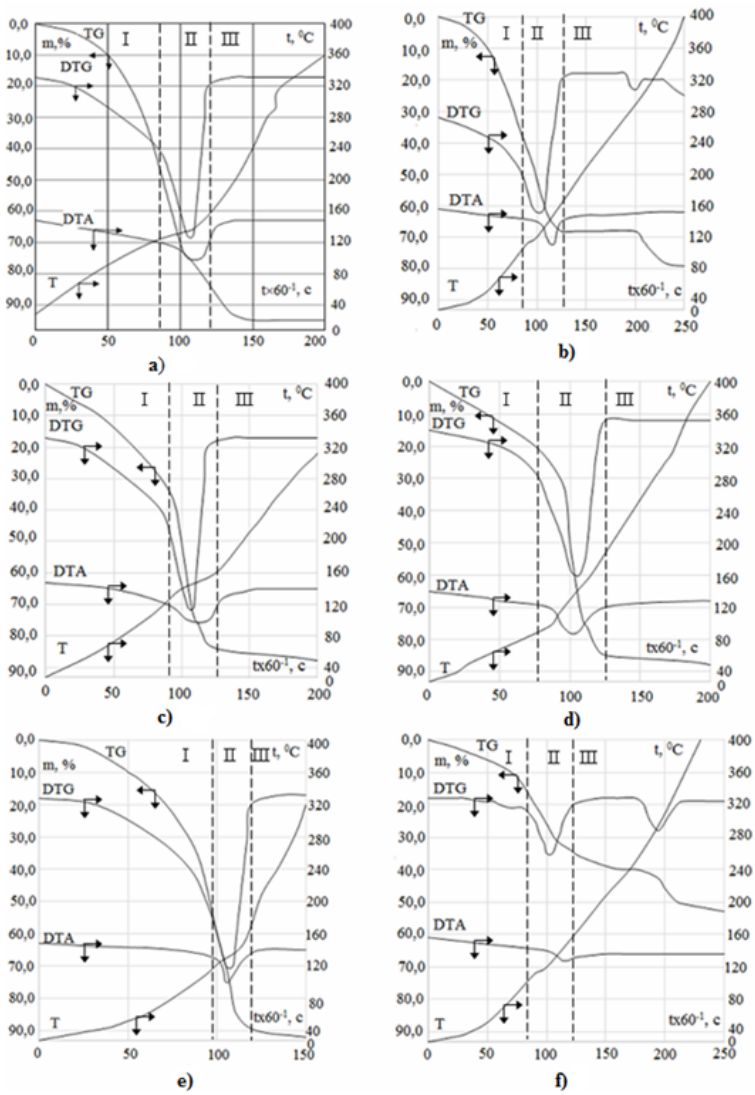

Fig. 1. Derivatograms of the model systems: a) xanthan gum and tare gum; b) xanthan gum, tara gum and gelatine; c) xanthan gum, tara gum, gelatine and maltodextrin; d) xanthan gum, tara gum, gelatine, maltodextrin and skimmed milk powder; e) xanthan gum, tara gum, gelatine, maltadextrin, dry milk and powdered sugar; f) thermostable milk-containing filling

Ryc. 1. Derywatogramy układów modelowych: a) guma ksantanowa i guma tara; b) guma ksantanowa, guma tara i żelatyna; c) guma ksantanowa, guma tara, żelatyna i maltodekstryna; d) guma ksantanowa, guma tara, żelatyna, maltodekstryna i odtłuszczone mleko w proszku; e) guma ksantanowa, guma tara, żelatyna, maltodekstryna, mleko w proszku i cukier puder; f) termostabilne nadzienie zawierające mleko

At the second stage of the process, the kinetics of mass loss (TG) and rate of mass change (DTG) have peaks characteristic of all model systems. They correspond to the intensive removal of mass from the samples due to the transition of water from liquid to gaseous state throughout the entire volume of the system. These peaks correspond to the boiling point of the liquid containing the model systems. At the time points corresponding to these peaks, the kinetics of temperature (T) and kinetics of heat absorption (DTA) change the angle of inclination to the axis on which the time is delayed. The angle of inclination of the temperature kinetics decreases. This indicates the presence of a phase transition of the first kind.

It should be noted that the width and intensity of the peaks at the second stage of the heating process are different for various model systems. This is determined by the amount of water, for which a phase transition of the first kind occurs.

The completion of the second stage is indicated by a repeated change in the angle of inclination of the temperature kinetics (T) and the leveling of the kinetics of mass loss (TG) and the rate of mass change (DTG).

At the third stage, the sample continues to be heated to the final temperature, while thermal decomposition of the substances of the studied model systems occurs with the release of gaseous substances.

Discussion

The results of studies of the degree of water binding by complex gels of sodium alginate and pectin are given. It was established that such gels have a greater amount of bound moisture and a greater activation energy compared to single-component systems of the specified hydrocolloids. This confirms the formation of additional bonds between alginate and pectin macromolecules and explains the strengthening of their gel network (Sokolovska et al., 2016).

The temperature at which the intensive transition of water from a liquid or amorphous state to a gaseous state occurs throughout the entire volume of the system for all samples lies within the range from 115°C to 125°C. It is known that the phase transition of the first kind for bulk water (so-called free water) occurs at a temperature of 100°C. At temperatures from 115°C to 125°C, physical and chemical moisture changes to a gaseous state. It is divided into adsorption and osmotically bound (Ivaniuk and Suprunchuk, 2018).

Osmotic refers to swelling moisture and moisture immobilized inside cells by a colloidal membrane. Adsorption-bound moisture includes moisture that forms a monomolecular layer with molecules of dry substances. It is more tightly bound to the substance compared to the osmotic one.

In subsequent layers, the bond energy is constantly decreasing. It is the physical and chemical connection of water with the dry substances of the model systems that is the reason why the transition of the water of these model systems to the gaseous state occurs at a temperature greater than 115°C. Since there is no clear boundary between different forms of water bonding, the temperature of the phase transition of the first kind for such moisture is determined precisely by the temperature range (Moroz, 2013).

It should be noted that there are no visible peaks on the derivative diagrams corresponding to the phase transition of free water to the gaseous state, that is, peaks at a temperature of 100°C. This fact indicates either the absence of free water. That is, all the water in the model systems is connected to dry substances by one or another mechanism. It may also indicate its small amount.

Conclusions

The derivatographic studies established that the main part of the water in the model systems is in physical and chemical connection with dry substances. At the same time, the phase transition of system water of the first kind from a liquid or amorphous state to a gaseous state occurs in the temperature range from 115°C to 125°C.

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Data availability: The dataset used during this study is the available form given author upon reasonable request.

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Derywatograficzne badania mleka zawierającego termostabilizatory

Streszczenie. Artykuł poświęcony jest badaniom derywatograficznym systemów modelowych do tworzenia struktury termostabilnej. Głównym celem pracy było wyjaśnienie danych dotyczących wody obecnej w opracowanych produktach mlecznych, z wykorzystaniem metody derywatograficznej. Wykazano, że skład wypełnienia termostabilnego był uzasadniony. Ponadto wskazano zastosowanie żelatyny, mieszaniny gum i enzymu transglutaminazy. Badania derywatograficzne wykazały, że główna część wody w systemach modelowych jest powiązana z substancjami suchymi. Ustalono, że dla systemów modelowych przejście fazowe pierwszego rodzaju wody systemowej ze stanu amorficznego lub ciekłego do stanu gazowego zachodzi w zakresie temperatur od 115°C do 125°C.

Słowa kluczowe: mleko z dodatkiem termostabilizatora, dodatki, transglutaminaza, żelatyna, metoda derywatograficzna

Genomic resistance characteristics of *Listeria monocytogenes* isolated from food

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Abstract. *Listeria monocytogenes* is one of the major microbiological hazards associated with food and processing plants where it can persist for long periods of time, which is a concern for the food industry. L. monocytogenes is characterized by high resistance to antibacterial substances, increases the possibility of survival and cross-contamination of final food products, leading to possible outbreaks of listeriosis. The aim of this study was characterization of 17 food origin L. monocytogenes isolates using whole genome sequencing (WGS). The obtained DNA od all strains was send to outer company, where WGS analysis were performed, data from the isolates were analysed to assess the genomic diversity of L. monocytogenes and to detect the absence / presence of genes encoding antimicrobial resistance using BIGSdb-Lm platform. The obtained results allowed to determine that the dominant number of strains belongs to Lineage II (72.2%), the remaining strains belong to Lineage I (27.8%). The genes coding tetracycline resistance, including the lmo0839, tetA_3 and tetC genes, were most often found in the genomes of the strains. The tetA_2 and tetA_1 genes were found in 94.1% and 72.2% of strains, respectively. In addition to the tetracycline resistance genes, all strains had lincomycin, trimethoprim and daunorubicin resistance genes. Additionally, genes encoding resistance to cadmium (cadA and cadC) and aluminium (lmo1297) were observed in each of the strains. The presence of the Tn6188 transposon was observed in four strains, containing genes encoding resistance to benzalkonium chloride, tetracyclines and macrolides. The obtained results indicate that L. monocytogenes strains isolated from food are characterized by a wide variety of genes encoding resistance to antimicrobial compounds, and additionally they may transfer resistance via mobile genetic elements.

Keywords: *L. monocytogenes*, NGS, resistance

Introduction

Listeria monocytogenes is an important food-borne pathogen responsible for human listeriosis. The disease has been widely reported to affect vulnerable consumers, including those with weakened immunity, new-borns and pregnant women, and the elderly. Listeriosis can lead to high mortality (20–30%) invasive infections such as sepsis and meningoencephalitis as well as miscarriage. In healthy adults, the infection is self-limited and limited to febrile gastroenteritis (Tîrziu et al., 2022). According to the recent European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) report, in 2020, a total of approximately 2000 confirmed listeriosis cases in humans in the European Union, with the notification rate of 0.42 per 100,000 population, were noted (EFSA…, 2021).

Recent application of whole-genome sequencing (WGS) into bacterial molecular characterization provides much different data. An evaluation of the relationship of *L. monocytogenes* from different sources is possible, it allows as well to classify strains into evolutionary lines and genetic types (Orsi et al., 2021). Based on the WGS, the multilocus sequence typing (MLST) approach allows classification of *L. monocytogenes* into clonal complexes (CCs) and sequence types (STs), which are linked with persistence of the strains in food, food production environments or with a high potential to cause listeriosis (Wagner et al., 2020).

The WGS analysis also delivers information related to the resistance to antimicrobials, which is important due to emerged of antibiotic-resistant strains of *L. monocytogenes* (Matereke and Okoh, 2020; Shamloo et al., 2019). *L. monocytogenes* most likely becomes resistant to antimicrobial agents through the acquisition of mobile genetic elements, such as plasmids, transposons, and integrons. Environmental stresses such as osmotic, acidity, oxidative, and cold also contribute to the increase of antimicrobial resistance of *L. monocytogenes* (Matereke and Okoh, 2020).

Material and methods

Bacterial strains. A total of 17 *L. monocytogenes* isolates classified species by MALDI-TOF (bioMerieux, France) were selected for the whole genome sequencing and genomic analyses. The strains came from collection of Department of Industrial and Food Microbiology at University of Warmia and Mazury

in Olsztyn. Strain were isolated from food samples and food processing plants between 2018–2019 using the standard ISO 11290-1:2017-07. Strains and source of isolation are listed in Table 1.

Table 1. List of *Listeria monocytogenes* strains used in study
Tabela 1. Lista szczepów *Listeria monocytogenes* użytych w badaniu

No.	Species	Source
Lm_1	*L. monocytogenes*	smoked salmon
Lm_2	*L. monocytogenes*	smoked salmon
Lm_3	*L. monocytogenes*	smoked salmon
Lm_4	*L. monocytogenes*	food processing plant
Lm_5	*L. monocytogenes*	smoked salmon
Lm_6	*L. monocytogenes*	food processing plant
Lm_7	*L. monocytogenes*	raw salmon
Lm_8	*L. monocytogenes*	raw salmon
Lm_9	*L. monocytogenes*	smoked salmon
Lm_10	*L. monocytogenes*	food processing plant
Lm_11	*L. monocytogenes*	smoked salmon
Lm_12	*L. monocytogenes*	food processing plant
Lm_13	*L. monocytogenes*	food processing plant
Lm_14	*L. monocytogenes*	raw salmon
Lm_15	*L. monocytogenes*	raw salmon
Lm_16	*L. monocytogenes*	smoked salmon
Lm_17	*L. monocytogenes*	smoked salmon

DNA isolation, library preparation and sequencing. Total DNA was extracted from isolated strains according to the instruction manuals of commercial DNA extraction kits (A&A Biotechnology, Gdynia Poland). Concentration and purity were measured optically using a DeNovix DS11 FX spectro-photometer / fluorometer (DeNovix Inc, Wilmington, USA) based on sample absorption at wavelengths of 260 nm and 280 nm. DNA library and sequencing were

performed by Genomed (Warszawa, Poland) on Illumina Mi-seq. Assembly quality was assessed using the number of contigs, N50 and L50 metrics.

MLST characterization. MLST (7 loci) (Ragon et al., 2008) were extracted from the assemblies using the BLASTN algorithm (Altschul at al., 1990) as previously described (Moura et al. 2016). MLST profiles were classified into sequence types (ST) and grouped into clonal complexes (CCs) as previously described (Ragon et al., 2008). Allele numbers, STs and CCs were determined according to the *Listeria* sequence typing database and its tools available on BIGSdb-Lm platform1 (Moura et al., 2016).

Antimicrobial resistance genes. Identification of antimicrobial resistance was done using BIGSdb-Lm platform. Single gene alignments were performed using BLAST3 and MEGA7 software's (Kumar et al., 2016).

Results and discussion

MLST analysis. Eleven different MLST sequence types were identified: ST6 (11.76 %, n = 2 isolates), ST8 (23.53 %, n = 4), ST9 (5.88%, n = 1), ST31 (5.88%, n = 1), ST37 (5.88%, n = 1), ST59 (5.88%, n = 1), ST77 (5.88%, n = 1), ST87 (5.88%, n = 1), ST101 (5.88%, n = 1), ST121 (17.65 %, n = 3), and ST193 (5.88%, n = 1).

L. monocytogenes isolated from food plants environment contained isolates classified to six sequence types (ST101, ST69, ST77, ST 37, ST121 and ST87) whereas isolates from food samples were classified to seven other STs (ST6, ST8, ST9, ST31, ST87, ST121 and ST193). Moreover, *L. monocytogenes* were grouped into eleven clonal complexes (CC6, CC8, CC9, CC31, CC37, CC59, CC77, CC87, CC101, CC121 and CC193). The obtained results allowed to determine that the dominant number of strains belongs to Lineage II (72.2%), the remaining strains belong to Lineage I (27.8%) (Table 2). The results of other authors suggest that dominant STs were ST155 and ST121 among *L. monocytogenes* of food origin (Wagner et al., 2020; Wang et al., 2021). It seems that such isolates may have a molecular background that allow them to survive in food and food production area (Hurley et al., 2019; Moura et al., 2016), however, our results suggest that dominant STs is ST8, explanation for that may be a small number of samples.

Presence of Antimicrobial Resistance Genes. The most prevalent genes were those coding tetracycline resistance, including the *lmo0839, tet*A_3 and *tet*C

Table 2. MLST characteristics of *L. monocytogenes* strains
Tabela 2. Charakterystyka MLST szczepów *L. monocytogenes*

Strain	MLST		
	ST	CC	Lineage
Lm_1	8	8	II
Lm_2	8	8	II
Lm_3	121	121	II
Lm_4	101	101	II
Lm_5	121	121	II
Lm_6	59	59	I
Lm_7	193	193	II
Lm_8	6	6	I
Lm_9	31	31	II
Lm_10	77	77	I
Lm_11	8	8	II
Lm_12	37	37	II
Lm_13	6	6	I
Lm_14	8	8	II
Lm_15	9	9	II
Lm_16	121	121	II
Lm_17	87	87	I

genes, which were found in all strains. The *tet*A_2 and *tet*A_1 genes were found in 94.1% and 72.2% of strains, respectively. In addition to the tetracycline resistance genes, all strains had lincomycin, trimethoprim and daunorubicin resistance genes. The presence of the Tn*6188* transposon was observed in four strains, containing genes encoding resistance to benzalkonium chloride, tetracyclines and macrolides, however, just in one gene (*ermC*) were found in all four strains. Interestingly just once strain isolated from smoked salmon had *aacA4* gene (Fig. 1)

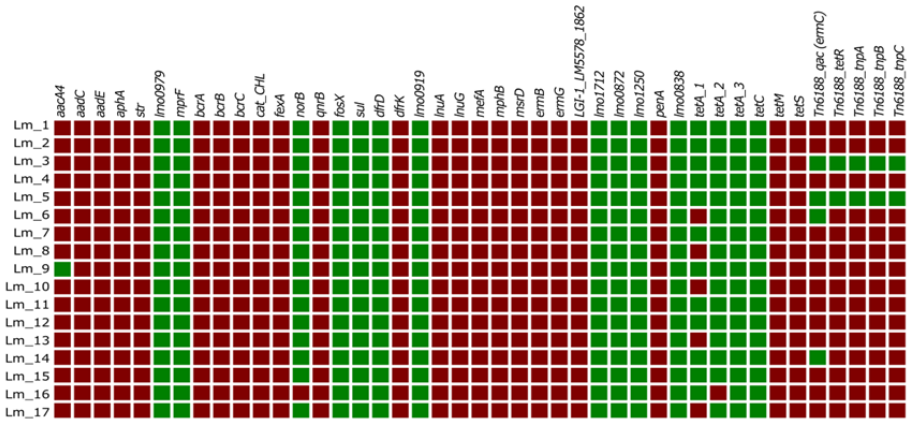
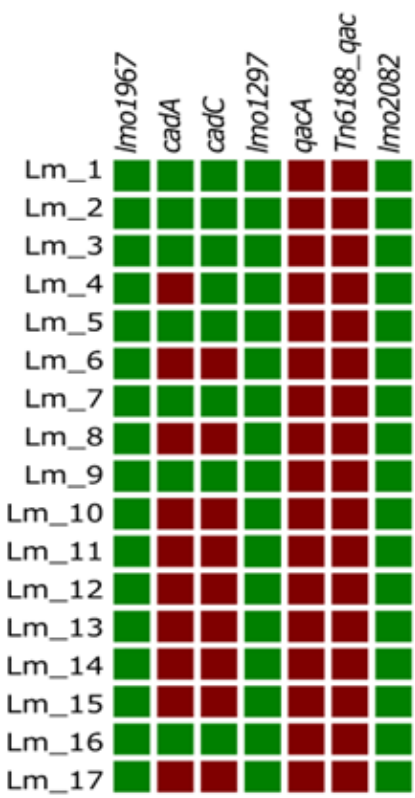


Fig. 1. Genetic characteristics of antibiotic resistance of *L, monocytogenes* strains

Ryc. 1. Genetyczna charakterystyka oporności na antybiotyki szczepów *L. monocytogenes*



Additionally, in each of the strains genes encoding resistance to toxic ions (*lmo1961*), camphor resistance protein CrcB (*lmo2082*) and aluminium (*lmo1297*) were observed. Genes encoding resistance to cadmium were observed in 47.05% (*cadC*) and 41.18% (*cadA*) (Fig. 2).

Recent studies in South America, Europe and Asia on antimicrobial resistance of *L. monocytogenes* have typically reported low levels of antimicrobial resistance in isolates from the food production environments (Li et al., 2016; Wilson et al., 2018). The latest study has reported that various antibiotic

Fig. 2. Genetic characteristics of antimicrobial resistance of *L. monocytogenes* strains

Ryc. 2. Genetyczna charakterystyka oporności substancje o właściwościach antybakteryjnych szczepów *L. monocytogenes*

resistance genes, interestingly research on WGS of *L. monocytogenes* suggest that dominat genes are *fosX*, *lin*, *mprF*, *norB*, and *mgrA* (Kurpas et al., 2020; Mafuna et al., 2021) and we found larger group of antibiotic resistance genes. In research of Mafuna et al. (2021) tetracycline resistance genes *tetM* and *tetS* were found in a few isolates, in ours research both of those genes were not found. In strains isolated from fish and food processing plants dominant genes coding tetracycline resistance genes were *tetA* and *tetC.* Although, tetracycline is believed to be the most frequent resistance trait in *L. monocytogenes* isolated from human and food processing environments.

Conclusions

The obtained results indicate that *L. monocytogenes* strains isolated from food are characterized by a wide variety of genes encoding resistance to antimicrobial compounds, and additionally they may transfer resistance via mobile genetic elements.

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Conflict of interest: The authors declare no conflict of interest.

Data availability: The dataset used during this study is the available form given author upon reasonable request.

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Genomowa charakterystyka oporności *Listeria monocytogenes* wyizolowanej z pożywienia

Streszczenie. *Listeria monocytogenes* jest jednym z głównych zagrożeń mikrobiologicznych związanych z żywnością i zakładami przetwórstwa. Może się w nich utrzymywać się ona przez długi czas, co stanowi problem dla przemysłu spożywczego. *L. monocytogenes* charakteryzuje się wysoką odpornością na substancje przeciwbakteryjne, co zwiększa możliwość przeżycia i zanieczyszczenia krzyżowego końcowych produktów spożywczych, prowadząc do możliwych ognisk listeriozy. W pracy sekwencjonowanie całego genomu (ang. *whole genome sequencing*; WGS) wykorzystano jako narzędzie do scharakteryzowania 17 izolatów *L. monocytogenes* wyizolowanych z żywności. Uzyskane dane WGS z izolatów przeanalizowano w celu oceny różnorodności genomowej *L. monocytogenes* oraz wykrycia braku / obecności genów kodujących oporność na środki przeciwdrobnoustrojowe. Uzyskane wyniki pozwoliły ustalić, że dominująca liczba szczepów należy do Lineage II (72,2%), pozostałe szczepy należą do Lineage I (27,8%). Geny kodujące oporność na tetracykliny, w tym geny *lmo0839, tet*A_3 i *tet*C, najczęściej znajdowano w genomach szczepów. Geny *tetA*_2 i *tet*A_1 znaleziono odpowiednio w 94,1% i 72,2% szczepów. Oprócz genów oporności na tetracykliny wszystkie szczepy miały geny oporności na linkomycynę, trimetoprim i daunorubicynę. Dodatkowo w każdym ze szczepów zaobserwowano geny kodujące oporność na kadm (*cad*A i *cad*C) i aluminium (*lmo1297*). Obecność transpozonu Tn*6188* zaobserwowano w czterech szczepach zawierających geny kodujące oporność na chlorek benzalkoniowy, tetracykliny i makrolidy. Uzyskane wyniki wskazują, że szczepy *L. monocytogenes* wyizolowane z pożywienia charakteryzują się szeroką gamą genów kodujących oporność na związki przeciwdrobnoustrojowe, a dodatkowo mogą przenosić oporność poprzez ruchome elementy genetyczne.

Słowa kluczowe: *L. Monocytogenes*, NGS, oporność

Effect of high pressure processing on the survival of *Lactococcus* strains from commercial starter cultures

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Abstract. Lactococci are lactic acid bacteria which are widely used in the form of starter cultures to obtain a wide range of food products, mainly in cheese and butter production. In order to extend the shelf-life of food, it must undergo preservation processes. High pressure processing (HPP) is a relatively new method of non-thermal food preservation using pressure in the range of 300–600 MPa for 30 seconds to several minutes. Currently, it is increasingly used as in food preservation, because unlike thermal methods, it does not cause changes in other food properties. It is important not to definitely eliminate microorganisms intentionally introduced into the food during the preservation processes. The tolerance of other lactic acid bacteria, mainly *Lactobacillus*, to high pressure is well known, but there is still little information about the survival rate of *Lactococcus*. Therefore, the aim of this study was to determine the survival rate of lactococci from commercial starter cultures to the high-pressure treatment depending on the pressure value and the duration of the process. The obtained results indicate that the survival of the isolates on high pressure treatment is different for individual strains. It is important information in the context of food production and its preservation using the HPP method. This suggests that the tolerance of the strains intentionally introduced into the food to the parameters of the high pressure treatment used for food preservation should be checked individually. This will ensure process optimization and allow to obtain a product of the highest possible quality.

Keywords: high-pressure processing, *Lactococcus* sp., starter cultures

Introduction

Lactococcus is one of the most popular genus forming the group of lactic acidic bacteria (LAB). Lactococci are homofermentative, their sugar fermentation product is only lactic acid. Two subspecies: *L. lactis* subsp. *lactis* and *L. lactis*

subsp. *cremoris* are predominant and the most frequently used in the food industry, mainly in dairy production. Isolates of *L. lactis* subsp. *lactis* are fast acidifiers, while isolates of *L. lactis* subsp. *cremoris* are often favored as defined starters because they tend to cause less bitterness (Fessard and Remize, 2019; Ziyaina et al., 2017).

High pressure processing (HPP) is a novel non-thermal food processing technology, and it is widely used for food preservation due to the HPP ability to eliminate pathogenic and spoilage microorganisms and retain food quality and safety. It can also inactivate the strains intentionally added to food, for example in the form of starter cultures. In consideration of the beneficial functions of starter cultures, it is expected that these microorganisms can survive high pressure treatment. It is important not to definitely eliminate microorganisms intentionally introduced into the food during the preservation processes.

Therefore, the parameters of the product pressurization process should be selected in such a way as to ensure the appropriate number of cells of strains introduced into food. The tolerance of other lactic acid bacteria, mainly *Lactobacillus*, to high pressure is well known, but there is still little information about the survival rate of *Lactococcus* in response to applied high pressures (Bucka-Kolendo and Sokołowska, 2017; Yang et al., 2021).

Therefore, the aim of this study was to determine the survival rate of lactococci from commercial starter cultures to the high-pressure treatment depending on the pressure value and the duration of the process.

Materials and methods

Strains. Strains from the collection of the Department of Food and Industrial Microbiology of the University of Warmia and Mazury in Olsztyn, described previously were selected for analysis (Zarzecka et al., 2022). A total number of 8 strains were selected for analysis. The characteristics of the strains selected for analysis are summarized in Table 1.

High-pressure processing and survival analysis. Pressure values for the analysis were chosen based on the preliminary studies. The high pressure treatment was carried out in glycol-water solution (1:1, v/v) using the high-pressure single-chamber U4040 (IWC PAN, Warsaw, Poland, Unipress Equipment Division). Temperature was maintained at 20 ± 3°C, the pressure rise rate was 300 MPa/min, and the pressure relief time was less than 5 s. The procedure was

Table 1. Strains used in the study
Tabela 1. Szczepy wykorzystane w badaniu

Strain	Identification	Origin
LAB-14	*Lactococcus lactis* ssp. *lactis*	Yoghurt starter culture/Kultura jogurtowa
LAB-16	*Lactococcus lactis* ssp. *lactis*	Kefir starter culture/Kultura kefirowa
LAB-37	*Lactococcus lactis* ssp. *lactis*	Kefir starter culture/Kultura kefirowa
LAB-39	*Lactococcus lactis* ssp. *lactis*	Kefir starter culture/Kultura kefirowa
LAB-44	*Lactococcus lactis* ssp. *lactis*	Cheese starter culture/Kultura serowarska
LAB-59	*Lactococcus lactis* ssp. *lactis*	Kefir starter culture/Kultura kefirowa
LAB-68	*Lactococcus lactis* ssp. *lactis*	Yoghurt starter culture/Kultura jogurtowa
LAB-15	*Lactococcus lactis* ssp. *lactis*	Cheese starter culture/Kultura serowarska

subjected to overnight cultures of isolates (10 mL) on de Man, Rogosa and Sharpe (MRS) broth (Merck, Germany). The analyzes were carried out in triplicate on different days. After the stress treatment was completed, the survival of the strains was checked by the plate count method. A series of ten-fold dilutions were made for pressurized and non-pressurized sample for each strain and the number of colony forming units (CFU)/mL on MRS agar was determined. The number of colonies was counted after 48 h of incubation at 30°C.

Statistical analysis. Statistical analyses were performed using STATISTICA 13.3 StatSoft® software (StatSoft, Inc., Tulsa, OK, USA). Statistical comparison of means was performed using Student's *t*-test. For all of the analyses, the significance level $p \leq 0.05$ was assumed.

Results

After the preliminary studies, the following high pressure treatment parameters were selected for the analysis: 300 MPa/3 min., 300 MPa/5 min., 400 MPa/1 min. and 400 MPa/3 min. The obtained results indicate that the survival of the isolates on high pressure treatment is different for individual strains. The differences were most noticeable the longer the pressure treatment time and the higher the pressure value. The highest recorded difference in the number of CFU/mL was 3 log cycles after applying a pressure of 400 MPa for 3 minutes

(1.20×10^{1} CFU/mL for LAB-14 strain and 1.50×10^{4} CFU/mL for LAB-15 strain) at a similar initial CFU/mL number (1.78×10^{9} CFU/mL and 1.14×10^{9} CFU/mL respectively) (Table 2).

Table 2. The number of CFU/mL of *Lactococcus* strains before and after high-pressure treatment

Tabela 2. Liczebność CFU/mL szczepów *Lactococcus* przed i po obróbce wysokociśnieniowej

Strain	[CFU/mL ± SD]				
	Control sample	300 MPa/ 3 min	300 MPa/ 5 min	400 MPa/ 1 min	400 MPa/ 3 min
LAB-14	1.36 $\pm0.08\times10^{9}$	5.56 $\pm0.2\times10^{8}$	1.73 $\pm0.07\times10^{8}$	1.00 $\pm0.13\times10^{3}$	1.20 $\pm0.13\times10^{1}$
LAB-16	8.10 $\pm0.08\times10^{8}$	2.9 $\pm0.41\times10^{8}$	1.34 $\pm0.05\times10^{8}$	6.00 $\pm0.14\times10^{3}$	2.40 $\pm0.27\times10^{1}$
LAB-37	9.82 $\pm0.1\times10^{8}$	3.03 $\pm0.05\times10^{8}$	2.18 $\pm0.16\times10^{8}$	1.70 $\pm0.10\times10^{4}$	1.80 $\pm0.07\times10^{1}$
LAB-39	8.40 $\pm0.16\times10^{8}$	4.80 $\pm0.23\times10^{8}$	1.35 $\pm0.13\times10^{8}$	1.00 $\pm0.13\times10^{3}$	2.10 $\pm0.26\times10^{1}$
LAB-15	5.54 $\pm0.15\times10^{8}$	3.60 $\pm0.29\times10^{8}$	1.90 $\pm0.17\times10^{8}$	2.30 $\pm0.08\times10^{5}$	1.50 $\pm0.17\times10^{4}$
LAB-44	1.56 $\pm0.13\times10^{9}$	1.09 $\pm0.07\times10^{9}$	6.40 $\pm0.2\times10^{8}$	3.42 $\pm0.11\times10^{5}$	1.02 $\pm0.05\times10^{4}$
LAB-59	2.10 $\pm0.09\times10^{9}$	1.42 $\pm0.10\times10^{9}$	7.50 $\pm0.23\times10^{8}$	1.76 $\pm0.05\times10^{5}$	4.63 $\pm0.09\times10^{3}$
LAB-68	7.9 $\pm0.22\times10^{8}$	5.2 $\pm0.13\times10^{8}$	2.80 $\pm0.25\times10^{8}$	2.75 $\pm0.1\times10^{5}$	3.20 $\pm0.05\times10^{3}$

Discussion

Food production processes are associated with changes in environmental conditions, which may become a stress factor for food-related microorganisms, such as lactic acid bacteria (LAB) included in starter cultures. Their ability to survive unfavorable conditions depends on the individual characteristics of bacteria. In stressful conditions microorganisms activate mechanisms allowing adaptation and might affect cells viability and bacterial technological

properties. These ability mechanisms can also originate from cross-protection systems.

High pressure processing (HPP) is recognized as one of the most promising methods of food preservation. It is non-thermal method which is possible to eliminate undesirable microorganisms while maintaining the sensory and functional product properties of the product (Li et al., 2020). It is also used to preserve food produced using starter cultures. HPP can stop or inhibit LAB growth by inactivating specific enzymes (Modugno et al., 2018). LAB respond to HPP stress with various intensity, however this response is difficult to identify. HPP response systems are very similar to the response system of other stress factors, potentially due to the presence of cross-protection systems (Liu et al., 2018).

According to the literature, HPP is able to effectively inactivate *L. plantarum* in different matrixes, achieving 5-log reductions under pressure of 400–500 MPa applied for 30 s to several minutes (Putnik et al., 2020). Castro et al. (2015) showed that high pressure treatment affects the cell viability, probably due to the cell injures.

Lactococci, as lactic acid bacteria are very valuable for the food industry, so it is important to thoroughly understand their stress survival abilities. The bacterial survival in a stressful conditions is important for the functioning of the bacteria in products obtained with starter cultures (Papadimitriou et al., 2016).

In the food industry, the plate count method is still standard method widely used for rapid microbiological analyses. However, it is noteworthy that the number of colony-forming units depends on many different parameters such as culture conditions, the physiological state of the bacteria, and sub-lethal damages occurrence (Bonomo et al., 2013).

In this study, a significant reduction in number of CFU/mL was observed in response to high pressure treatment. After exposure to 300 MPa, regardless of the treatment time, the number of CFU/mL decreased by at most 1 log cycle. Moreover application of 400 MPa caused reduction in the number of CFU/mL by as much as 8 log cycles. Obtained results are in accordance with previous studies. In study by Pega et al. (2018) required amount of starter lactic acid bacteria in yoghurt was maintained after pressure treatment of 300–400 MPa for up to 10 min. Li et al. (2010) obtained significant reduction of lactic acid bacteria number after 300 MPa for 10 min (from 7 to 4–5 log cycles). Moreover, after treatment at 600 MPa LAB were still detected at the level of 3 log cycles.

The obtained results lead to further research in this area to expand the knowledge in this field. It would allow to demonstrate which are the mechanisms of the strains survival in response to high pressure stress.

Conclusions

This study may open door to a deeper understanding of the survival of lactic acid bacteria to stresses related with food processing. Based on the obtained results, it can be suggested that there is a need for further research in this area. Currently, there is no extensive discussion in the literature on this topic, so it is important to understand in depth the mechanisms of stress survival among *Lactococcus* species.

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Conflict of interest: Authors declared no conflict of interest.

Data availability: The dataset used during this study is the available form given author upon reasonable request.

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Wpływ obróbki wysokociśnieniowej na przeżywalność komercyjnych kultur starterowych *Lactococcus*

Streszczenie. Bakterie z rodzaju *Lactococcus* to paciorkowce fermentacji mlekowej, które w postaci kultur starterowych znajdują wiele zastosowań w przemyśle spożywczym, głównie przy produkcji serów i masła. Aby przedłużyć okres przydatności do spożycia żywności, bakteria musi zostać poddana procesom utrwalania. Obróbka wysokociśnieniowa (ang. *high pressure processing* – HPP) to stosunkowo nowa metoda nietermicznego utrwalania żywności przy użyciu ciśnienia w zakresie 300–600 MPa przez 30 sekund do kilku minut. Obecnie jest wykorzystywana coraz częściej, ponieważ w przeciwieństwie do metod termicznych nie powoduje zmian innych cech żywności. Ważne jest, aby podczas procesów utrwalania nie wyeliminować definitywnie drobnoustrojów celowo wprowadzanych do żywności. Dobrze znana jest tolerancja innych bakterii fermentacji mlekowej, głównie *Lactobacillus*, na wysokie ciśnienia, ale wciąż istnieje niewiele informacji na temat przeżywalności *Lactococcus* w odpowiedzi na zastosowane wysokie ciśnienie. Z tego względu celem tej pracy było określenie przeżywalności szczepów *Lactococcus* z komercyjnych kultur starterowych do obróbki wysokociśnieniowej w zależności od wartości ciśnienia i czasu trwania procesu. Uzyskane wyniki wskazują, że przeżywalność jest różna dla poszczególnych szczepów. To ważna informacja w kontekście produkcji żywności i jej utrwalania metodą HPP. Sugeruje ona, że tolerancja szczepów celowo wprowadzonych do żywności na parametry obróbki wysokociśnieniowej stosowanej do utrwalania żywności powinna być sprawdzana indywidualnie. Zapewni to optymalizację procesu i pozwoli uzyskać produkt o najwyższej możliwej jakości.

Słowa kluczowe: obróbka wysokociśnieniowa, *Lactococcus* sp., kultury starterowe

Modern aspects of cultivated mushrooms use in the technology of sausages

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Abstract. This work is devoted to solving the problems which associated with expanding the range of food components with high biological value. Technological solutions are directed for alternative, natural and affordable sources of protein. Protein deficiency in human nutrition has led to the search for ways of increase the food biological value. It was proposed the optimal ratio of components of food, consisting of mushroom raw materials. The purpose of this combination is to achieve the composition of food to the desired approximation of the „ideal protein" with the established technological effects for this production. The main objective of this work is to evaluate the prospects of mushroom raw materials in food for expand the range of functional products processing by food enterprises. The cultivated mushroom raw material is used to create products of high biological values as an alternative, natural and affordable source of protein. In this paper it was shown that *Agaricus bisporus* is a kind of edible mushroom which is easy to browning, short storage period, low fat, high protein, delicious and widely used. It is potential to use *Agaricus bisporus* as fat replacer in meat products. The objective of this study was to investigate the effects of soybean oil and different particle size of *Agaricus bisporus* as fat replacers on the cooking yield, water holding capacity, pH, color, and textural properties of chicken sausages. The particle size of *Agaricus bisporus* was highly correlated with the cooking yield, water holding capacity, and pH value of chicken sausages.

Keywords: ideal protein, cultivated mushrooms, *Agaricus bisporus*, sausages

Introduction

Today's nutrition aims to search and involvement in the technological process of raw materials with balanced chemical composition and functional effect. It is the key to create high-quality food products that have a beneficial effect on the human body. Combining various types of raw materials to achieve a certain type of nutrient, micro- and macroelement, vitamin, and fatty acid balance is

one way of technology development. Cultivated mushrooms have a balanced composition of nutrients. They also grow quickly, without requiring expensive equipment for growing, are easily processed, and have a pleasant taste and aroma when ready (Akrashie and Stepanova, 2021).

In the research on developing healthier meat products, one of the main trends is to develop low-fat meat products (Hygreeva et al., 2014). Fat plays a crucial role in the production of emulsified meat products. Animal fat can improve the stability, flavour, cooking loss, water holding capacity and hardness of emulsified meat products. However, animal fat contains high saturated fatty acids and cholesterol, potentially harmful to human health (Varga-Visi and Toxanbayeva, 2017). The fat in emulsified meat products can be replaced partially or completely by low energy density components. e.g., soy protein, collagen, vegetable oil, pectin, cellulose, *Pleurotus eryngii*, *Agaricus bisporus* etc. (Choi et al., 2010). As this operation may adversely affect the quality of emulsified meat products, it is a large challenge for meat processors.

Materials and methods

Materials. Fresh mushrooms of *Agaricus bisporus* (Ab) species were supplied by Yufeng Edible Fungus Development Cooperative (Henan, China). Soybean oil was obtained from Shandong Luhua Group Co. Ltd. (Shandong, China), while cold fresh chicken breast and pork back fat were supplied by Henan Shuanghui Investment Development Co., Ltd (Henan, China). Salt, white pepper, sodium tripolyphosphate, and sugar were purchased from a local market.

Preparation of AB powder. The washed and drained Ab mushrooms were cut into 5 mm thick slices, dried (45°C, 8 hours) to obtain Ab mushrooms a moisture level of 7%. Then, they were ground and passed through different sieves with 80, 120 or 160 mesh in turn. The average diameter of particles (D_1, D_2 and D_3) was measured using laser particle size analyzer (BT-9300H, Sichuan Kecheng Technology Co., Ltd., Chengdu, China). The average diameter of the particles is 177.93, 99.96, and 33.73 μm, respectively. Then, the Ab powders were stored in polyethene bags at 4°C.

Preparation of chicken sausages. Connective tissue and visible fat were trimmed from cooled chicken breasts meat. The chicken breasts meat and pork back fat were cut into about 2.5 cm × 2.5 cm × 2.5 cm pieces and separately passed a grinder (6mm perforated plate). Salt and tripolyphosphate were

added to chicken breasts and mixed by a food processor (1500 rpm, 60 seconds, HR7625, Philips Corp, Hong Kong, China). A third part of totally used ice water was mixed (1500 rpm, 60 seconds). Next, pork back fat, Ab powder of D_1, D_2 and D_3 (pre-mixing with soybean oil), another 1/3 ice water portion and ingredients were added, according to the formula (Table 1) and mixed (1500 rpm, 120s). Finally, the remaining 1/3 ice water portion was added, and the whole mass was homogenized at 3000 rpm for 60 seconds. The temperature of minced meat maintained below 10°C when processing.

Table 1. The formulations of chicken batters (g/ 100 g)
Tabela 1. Skład wędlin z kurczaka (g/ 100 g)

Raw material/ ingredients	CK	D_1	D_2	D_3
Meat batters (100 g)				
Chicken meat	60	60	60	60
Pork backfat	20	8	8	8
Ice water	20	20	20	20
Ab powder (177.93 μm)		4		
Ab powder (99.96 μm)			4	
Ab powder (33.73 μm)				4
Soybean oil		8	8	8
Total	100	100	100	100
Others (% of meat batter)				
Refined salt	1.4	1.4	1.4	1.4
Sugar	0.65	0.65	0.65	0.65
Sodium tripolyphosphate	0.3	0.3	0.3	0.3
Ground white pepper	0.15	0.15	0.15	0.15

Preparation of chicken gel. A mass of 35 g emulsified chicken batters was transferred to 50 ml centrifuge tube and degassed in a centrifuge with a centrifugal force of 500 × g for 5 minutes. Next, the closed centrifuge tubes were submerged in water at 80°C for 30 minutes and ice water for 20 minutes, consecutively to obtain chicken gel. The prepared chicken gel was stored at 4°C for the determination of other indicators.

Cooking yield (CY). The cooked chicken gel was collected from the centrifuge tube, the moisture on the surface of the chicken gel was absorbed with absorbing paper, and the sample was immediately weighed. Cooking yield was calculated as a ratio (%) between the mass of the chicken batters after cooking and the mass of the chicken batters before.

Water holding capacity (WHC). The cooked chicken gel collected from the centrifuge tube, the moisture on the surface of the chicken gel was absorbed with absorbing paper, 10 g of chicken gel were wrapped with filter paper and placed in 50ml centrifuge tube to centrifuge (4℃, 8000 r/min, 10 minutes). Next, the sample was transferred from the centrifuge tube, the filter paper was carefully removed and the sample was immediately weighed. Water holding capacity represented the weight ratio (%) of chicken gel after centrifugation to that before centrifugation. The pH value was determined according to Choe et al. (2018) with some modification. After mixing 10 g of chicken batters with 100g of 0.1mol/L potassium chloride solution, the mixture was homogenized (8000 rpm, 1 min) using homogenizer (T25, IKA, Germany). The homogenate was filtered through filter paper (Whatman No. 4, Maidstone, England), and finally the pH of the filtrate was determined by pH meter (Model320, Metler-Toledo Ltd, Essex, UK).

Colour. The chicken gel was transferred to a cylinder with height of 20 mm and a diameter of 25 mm. The probe of the Minolta chromameter (CR-40, Minolta Camera Co., Japan) was placed along the surface of the sample without light leakage. The colour parameters L^*, a^*, and b^* were recorded for each sample. Whiteness was calculated as described by Wang et al. (2019).

$$Whiteness = 100 - \left[\left(100 - L^*\right) + a^{*2} + b^{*2}\right]^{\frac{1}{2}} \quad (1)$$

Textural profile analysis (TPA). Textural profile of the chicken gel was analysed in five replicates for each formulation using a texture analyser (TA-XT, Stable Micro Systems, UK) with a compression probe (P/36R) attachment. The chicken gel (25 mm diameter and 20 mm height) underwent two cycles of 50% compression with a test speed of 2 mm/s. The texture characteristics of the chicken gel were expressed as the hardness, springiness, cohesiveness, and chewiness.

Statistical analysis. SPSS 20.0 (IBM) statistical software was used to analyse the test data. The differences between factors and levels were assessed with

the help of analysis of variance (ANOVA). Duncan's multiple range tests were used to compare the means to identify which groups differed significantly from the others ($p < 0.05$). All data were presented as the means and standard deviation. Pearson's correlation coefficient were calculated to determine the correlation between the particle size of Ab and the quality of chicken batters.

Results

It was calculated CY, WHC and pH value of chicken batters. As can be seen in Figure 1, the addition of Ab mushroom powder and soybean oil significantly ($p < 0.05$) improved the cooking yield and water holding capacity of chicken batters, which could result from to abundant cellulose, hemicellulose and lignin in AB mushrooms, as showed by Kurt and Gençcelep (2018).

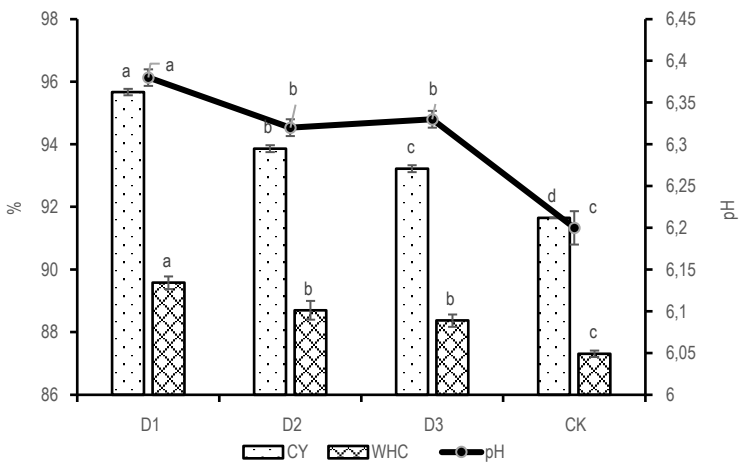

Fig. 1. Cooking yield (CY), water holding capacity (WHC) and pH values of chicken batters (D_1 – 177.93 μm, D_2 – 99.96 μm, D_3 – 33.73 μm, CK – control)

Ryc. 1. Wydajność gotowania (CY), wodochłonność (WHC) i pH badanych wędlin z kurczaka (D_1 – 177.93 μm, D_2 – 99.96 μm, D_3 – 33.73 μm, CK – kontrola)

Cellulose, hemicellulose and lignin in Ab mushrooms have oil-absorbing and water-binding properties, according to Han and Bertram (2017), and Chaplin (2007). WHC of D_1 was significantly ($p < 0.05$) higher than that of D_2, D_3 and the control sample, and there was no significant difference in WHC

between D_2 and D_3. The cooking yield increased sighnificantly ($p < 0.05$) along with the increase of the particle size of Ab. It could be attributed to that the larger size of Ab has the stronger water absorption capacity. It was also shown in previous studies (Heller et al., 1980; Jacobs et al., 2015; Sangmark and Noomhorm, 2004). The hydration properties of dietary fiber were affected by physical properties such as particle size and structure. It was one of the key characteristics of dietary fibre, as Cadden (1987) noted. Also grinding might affect hydration properties of dietary fibre, as was noted by Niu et al. (2014). The decrease of the particle size related with growth of the surface area and fibre hydration rate (Guillon and Champ, 2000). Nevertheless, grinding might also cause the matrix of the fiber that lead to collapse and reduce the water retention capacity. It was noted by Sangnark and Noomhorm (2004).

The pH values of all treatment samples were significantly ($p < 0.05$) higher than that of the control. D_1 with the largest particle size had the highest pH value, and between D_2 and D_3 no significant ($p < 0.05$) difference was found. Probably due to an opposite relation between particle size and solubility of acidic compounds of AB higher alkalisation leads to higher feasibility of meat gel formation, and the gel structure is more refined and stronger (Barbut, 1997). The changes in pH values were in line with changes in CY and WHC.

Numerous factors, incl. moisture, fat, myoglobin content, fat and nonmeat ingredients, can affect the colour of meat products (Pietrasik and Janz, 2009). In this study, the changes in colour of the treatment samples was affected by a nonmeat ingredient (Ab), because the contents of water, fat and myoglobin had no effect on all tested samples. As the content of Ab was equal in all the samples, the colour changes have to result from different L*, a* and b* values of Ab with different particle sizes.

The L* value of all samples decreased significantly ($p < 0.05$) compared with the control (Fig. 2). The L* value of chicken gel increased significantly ($p < 0.05$) with the decrease of particle size of Ab (Fig. 2).

The addition of Ab powder significantly ($p < 0.05$) reduced the whiteness value of chicken gel (Fig. 2). The whiteness value of chicken gel increased along with the decrease of Ab particle sizes. The whiteness values of each sample differed significantly ($p < 0.05$), and each sample had significantly lower whiteness than the control ($p < 0.05$), which relates with the lower whiteness of Ab itself. The sample that contained smallest particle size of Ab, had the most similar whiteness to the control. This indicates that the smallest particle size had the least impact on the whiteness of the chicken gel.

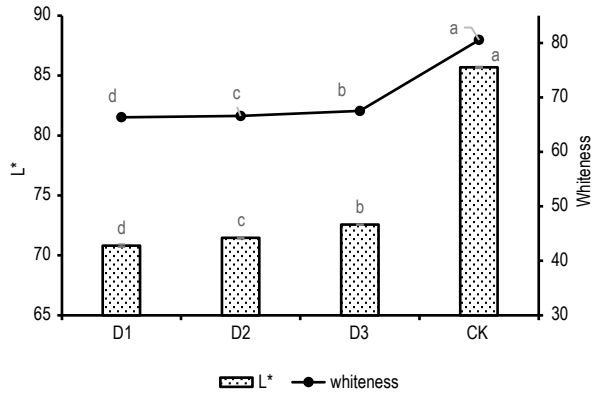

Fig. 2. L* value and whiteness of chicken sausages (D_1 – 177.93 μm, D_2 – 99.96 μm, D_3 – 33.73 μm, CK – control)

Ryc. 2. Średnie wartości parametru L* badanych parówek z kurczaka (D_1 – 177,93 μm, D_2 – 99,96 μm, D_3 – 33,73 μm, CK – kontrola)

Discussion

The particle size of Ab affected the texture characteristics of chicken gelatine (Fig. 3–Fig. 4). Soybean oil and Ab with different particle sizes significantly ($p < 0.05$) increased the hardness, springiness and cohesiveness of chicken gel. No significant difference in hardness and cohesiveness was found for samples

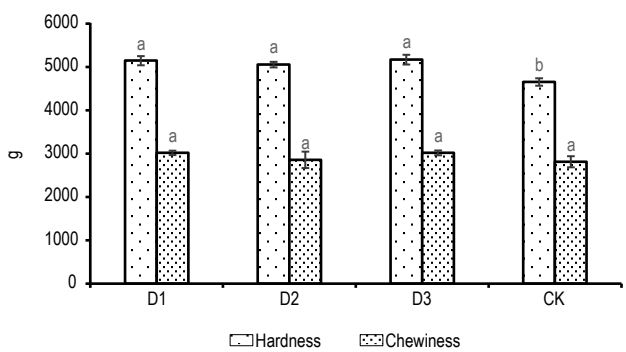

Fig. 3. Hardness and chewiness of chicken batters (D_1 – 177.93 μm, D_2 – 99.96 μm, D_3 – 33.73 μm, CK – control)

Ryc. 3. Twardość i żujność badanych wędlin z kurczaka (D_1 – 177,93 μm, D_2 – 99,96 μm, D_3 – 33,73 μm, CK – kontrola)

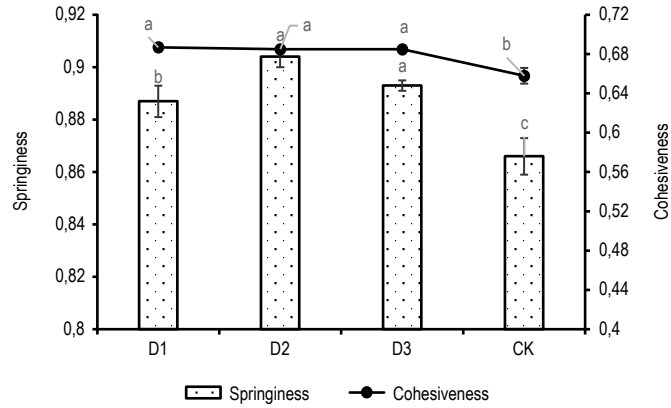

Fig. 4. Springiness and cohesiveness of chicken batters (D_1 – 177.93 μm, D_2 – 99.96 μm, D_3 – 33.73 μm, CK – control)

Ryc. 4. Sprężystość i spójność badanych wędlin z kurczaka (D_1 – 177,93 μm, D_2 – 99,96 μm, D_3 – 33,73 μm, CK – control)

with different particle sizes of Ab powder and soybean oil. Sánchez-Alonso et al. (2007) reported no significant difference in the hardness of samples with different particle sizes of dietary fibre, which was consistent with the results of this study. However, the hardness of pork meatballs grew with the decrease of rice bran particle size (Huang et al., 2005).

There was no significant difference in the chewiness between all samples and the control group, indicating that the addition of different particle sizes of Ab powder and soybean oil had no significant effect on the chewiness of chicken gels.

The springiness of D_2 and D_3 samples were significantly ($p < 0.05$) higher than that of D_1 samples, indicating that small particle size was more conducive to the springiness of chicken gel. However, soy okara with different particle sizes did not significantly ($p < 0.05$) affect the springiness value of pork gel ($p < 0.05$) (Wang et al., 2015), which was inconsistent with the results of this study and might result from the different characteristics of raw materials.

Dietary fibre and its particle size could affect the structural characteristics of meat products (Huang et al., 2005). Dietary fibre filled the protein network structure as filler, increasing heterogeneity of protein network, thereby enhancing gel strength (Sánchez-González et al., 2009). Ab powder was rich in dietary fibre (Kurt and Gençcelep, 2018). Therefore, Ab could also be used as

a filling to affect the texture characteristics of the chicken gel network. The different results might be caused by the variety of meat and dietary fibre type, content, and particle size.

Conclusions

The results of this study showed that larger particles of Ab increased the cooking yield, water holding capacity, and pH value of the chicken batters. Along with the drop of Ab particle sizes, the L* values, whiteness, and b* values of the chicken batters increased significantly ($p < 0.05$). The decrease of the particle size of Ab could improve the springiness of the batters. The particle size of Ab was highly correlated with the cooking yield, water holding capacity, L*, a*, b*value and pH value of the batters. It had a moderate correlation with the springiness. Thus, Ab may a potential additive improving the quality of chicken meat products.

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Data availability: All data are available from the corresponding author on reasonable request.

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Współczesne aspekty wykorzystania grzybów uprawnych w produkcji parówek

Streszczenie. Pracę poświęcono rozwiązaniu problemów związanych z poszerzaniem asortymentu składników żywności o wysokim potencjale bioaktywnym. Rozwiązania technologiczne ukierunkowane są na alternatywne, naturalne i przystępne cenowo źródła białka. Niedobór białka w żywieniu człowieka prowadzi do poszukiwania sposobów na zwiększenie wartości biologicznej żywności. W ramach pracy zaproponowano optymalny stosunek składników żywności opartej na grzybach. Celem było osiągnięcie składu żywności zbliżonym do tzw. „idealnego białka", ustalenie efektu technologicznego dla tej produkcji, a także określenie perspektywy stosowania grzybów w żywności w celu poszerzenia zakresu produktów funkcjonalnych. Grzyby są wykorzystywane do tworzenia produktów o wysokich wartościach biologicznych jako alternatywne, naturalne i niedrogie źródło białka. W pracy pokazano, że *Agaricus bisporus* to rodzaj grzyba jadalnego, który łatwo brązowieje, ma krótki okres przechowywania, a przy tym jest surowcem niskotłuszczowym, wysokobiałkowym, smacznym i szeroko stosowanym. *Agaricus bisporus* można zastosować jako zamiennik tłuszczu w produktach mięsnych. Zbadano wpływ oleju sojowego i różnej wielkości cząstek *Agaricus bisporus* jako zamienników tłuszczu na wydajność gotowania, zdolność zatrzymywania wody, pH, kolor i właściwości teksturalne parówek z kurczaka. Wielkość cząstek *Agaricus bisporus* była silnie skorelowana z wydajnością gotowania, zdolnością zatrzymywania wody i wartością pH uzyskanych parówek z kurczaka.

Słowa kluczowe: idealne białko, grzyby uprawne, *Agaricus bisporus*, parówki

Effect of cultivation temperature on the content of selected active components in the anatomical parts of oyster mushroom (*Pleurotus ostreatus* L.)

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Abstract. The aim of this study was to evaluate the effect of oyster mushroom (*Pleurotus ostreatus* L.) cultivation temperature on the retention of selected vitamins in the stem and cap of oyster mushrooms. The material were fruiting bodies of fungi whose fruiting bodies were obtained with and without access to light and cultivation was carried out at 10°C and 15°C. The obtained mushroom crop was fractionated into stems and caps and the extracts obtained from them were determined for vitamins B and C, phenolic acids and flavonols. It was found that cultivation has a significant effect on the content of bioactive compounds in siderophores. The mushrooms grown at 10°C had a higher content of vitamins B and C compared to mushrooms grown at a higher temperature. No flavonols were found in the mushrooms, instead it was shown that the temperature of cultivation has a decisive effect on the content of phenolic acids. It was shown that extracts from the stems had a higher content of vitamins and some phenolic acids. Therefore, it justifies that a new direction for the management of oyster mushroom stems towards the production of functional foods should be developed. Further work to confirm what technological properties the oyster mushroom stems have and how they can determine the properties of new functional products in needed.

Keywords: *Pleurotus ostreatus* L., oyster mushroom, bioactive compounds, cultivation temperature

Introduction

Mushrooms are of great importance in human nutrition, and are valued not only for their taste and aroma, but also because of the presence of biologically active compounds in them. These substances mainly exhibit anticancer, antibacterial, antifungal, antiviral, immunomodulatory, anti-inflammatory, anti-allergic, hepatoprotective, anti-atherosclerotic, cholesterol- and blood sugar-lowering properties, as well as effects on the central nervous system. They are used especially in folk medicine, however, in the last two decades there has been a growing awareness of their health-promoting effects. They are now recognized as functional foods, and their biological value has been proven in many laboratory and clinical studies (Cateni et al., 2022).

One of the most preferred and best known mushroom species by Europeans is the oyster mushroom (*Pleurotus osttreatus* L.). It is valued not only for its taste and aroma, but also for its nutritional and health value. In recent years, there has been a growing awareness and knowledge among consumers who reach for oyster mushroom fruiting bodies because of its nutritional value, described as similar to meat or milk. This mushroom is a rich source of valuable quality carbohydrates, proteins, as well as minerals and vitamins (riboflavin, thiamin or niacin) (Deepalakshmi and Mirunalini, 2014).

Oyster mushrooms, due to their rich chemical composition, are often an alternative means of prevention and therapy for many ailments. One of them is Alzheimer's disease, defined as a progressive neurodegenerative disorder with a multifactorial etiology, based mainly on the cholinergic hypothesis of its development (Sochocka et al., 2017).

Oyster mushroom is classified in the group of dietary mushrooms, and its main ingredient is water. Dry matter content ranges from 9.6% to 13.8% of the mushroom's fresh weight. The caloric value of fresh fruiting bodies is estimated at about 154 kJ. Carbohydrates are the next most abundant compound, with 6.7% of the fresh weight. The main part of these substances are polysaccharides, of which it is important to note glycogen (the spare material of mushrooms) and insoluble compounds, such as cellulose, fiber and chitin, which perform extremely important functions during the digestion process. The amount of fiber found in fresh oyster mushroom fruiting bodies is reported to be between 4.1–8.5 g, while chitin is reported to be 0.32 g. In addition, *Pleurotus ostreatus* contains significant amounts of reducing sugars (up to 30% may be present), among which are glucose, mannose, fructose and xylose,

as well as galactose, sucrose, maltose, lactose and raffinose. The presence of trehalose (about 6.5%) and even mannitol (1.8%) has also been demonstrated (Zhou et al., 2016). Edible mushrooms are also a rich source of easily digestible minerals. The fruiting bodies of *Pleurotus ostreatus* have between 6.1 and 9.9% of their dry matter. In 100 g of dry substance of oyster mushroom, the presence of 3 g of potassium, 150 mg of calcium and 125–757 mg of sodium has been demonstrated. In addition, such elements as zinc, fluorine, iodine, manganese, copper and mercury are also present in trace amounts (Deepalakshmi and Mirunalini, 2014).

Among mushrooms, *Pleurotus ostreatus* is the species containing the greatest variety of phenolic compounds. The highest amount is protocatechuic acid (3.60 mg per kg of dry weight), and in addition there are also synapinic acid, cinnamic acid, gallic acid, homogentisic acid and chlorogenic acid (Zawadzka et al., 2022). In addition, non-hallucinogenic indole compounds and their derivatives were found in the fruiting bodies of the mushroom, mainly serotonin, the amount of which in 100 g of oyster mushroom dry weight is 5.21 mg (Salata et al., 2018). In the wild, *Pleurotus ostreatus* is most often found in tropical and subtropical forests. A characteristic feature of this fungus is its ability to colonize large areas and decompose a huge number of lignocellulosic wastes in a fairly short interval of time, compared to other edible mushrooms cultivated by humans. Another significant piece of information is that oyster mushroom fruiting bodies are sporadically attacked by pests and diseases, and their cultivation is an easy and inexpensive way to obtain food (Salata et al., 2018).

Oyster mushroom grows on dying and living deciduous trees, and on dead wood (stumps, tree stumps, logs), mainly willows, poplars, beech, hornbeam, birch, acacia robinia, ash and walnut, causing white, severe rot on infested wood. When it occurs on cuts on the trunks of living trees, it is a parasite of them. However, it usually attacks old, damaged and weakened trees (Florczak et al., 2014). The oyster mushroom is also a predatory fungus, as its larvae secrete chemicals (toxin – trans-2-decenedicarboxylic acid) that penetrate the body of nematodes living in decaying wood and digest them (Landi et al., 2020).

The aim of this study was to evaluate the effect of growing conditions in the context of temperature on the retention of B and C vitamins, phenolic acids and flavonols in the stem and cap of oyster mushroom bacon (*Pleurotus ostreatus* L.).

Materials and methods

Material. The substrate for the experiment was prepared in a professional company producing oyster mushroom substrate in Łobez near the city of Jarocin (Poland).

The substrate was prepared from wheat straw cut into 4–5 cm long chaff and wheat bran. 5 kg of bran was added to 100 kg of dry straw. The mixture was moisturized with tap water to 70% moisture and pasteurized at 58–60°C for 48 hours. After cooling the substrate to 25°C, it was inoculated with the mycelium of the Spoppo strain of *Pleurotus ostreatus* (Sylvan Company).

The substrate was mixed with mycelium which constituted 3% in relation to the wet weight of the substrate and pressed into blocks in a specialized machine, which placed them in foil bags with perforation. Each block of substrate was 15 kg in weight. Incubation was conducted at the temperature of 25°C and air relative humidity 85–90%. Once the substrate was totally covered with the mycelium, it was transferred to the cultivation chambers. Different conditions for the setting and growth of fruiting bodies were applied. The conditions variants were as follows: temperature 15±1°C, light intensity of 200 lux for 8 hours a day until the appearance of fruiting buds 0.5–1 cm and then during the growth of fruiting bodies, three lighting variants 200, 50 and 10 lux for 8 hours a day. The test samples were coded according to the temperature of the crop and the intensity of the lighting used by assigning them appropriate codes as:

- **SL10** – stem from fruiting body growing up at 10 lux illumination for 8 hours, temperature 10±1°C
- **CL10** – cap from fruiting body growing up at 10 lux illumination for 8 hours, temperature 10±1°C
- **SD10** – stem from fruiting body growing up at 100 lux illumination for 0.5 hours, temperature 10±1°C
- **CD10** – cap from fruiting body growing up at 100 lux illumination for 0.5 hours, temperature 10±1°C
- **SD15** – stem from fruiting body growing at 10 lux illumination for 4 hours, temperature 15±1°C
- **CD15** – cap from fruiting body growing at 10 lux illumination for 4 hours, temperature 15±1°C
- **SL15** – stem from fruiting body growing at 200 lux illumination for 8 hours, temperature 15±1°C
- **CL15** – cap from fruiting body growing at 200 lux illumination for 8 hours, temperature 15±1°C.

Fig. 1. Photo of oyster mushroom fruiting bodies during cultivation under experimental conditions

Ryc. 1. Owocniki boczniaka w warunkach uprawy eksperymentalnej

Analysis of vitamins group B content. Vitamin content: B1, B2, B3, (thiamin, riboflavin, niacin) was analyzed with the Aquity H UPLC system equipped with a Waters Acquity PDA detector (Waters, USA) after prior enzymatic and acid extraction. The enzymatic hydrolysis was carried out for 2 hours at 50°C in the presence of tacadiastase, and acid hydrolysis with 0.6 M hydrochloric acid in contact with 90°C for one hour. An Acquity UPLC® BEH C18 column (50 mm × 2.1 mm, particle size 1.7 μm) (Waters, Ireland) was used for the determinations. The phase is formed by a precipitate of methanol and 0.05 M NaH_2PO_4 containing 0.005 M hexanesulfonic acid, pH 3.0. Elution was done in a gradient. The thiamin was converted to the thiochrome derivative with 1% potassium hexacyanoferrate (II) and immediately quantified with the Acquity column used. UPLC® BEH C18 column (150 mm × 2.1 mm, particle size 1.7 μm). The phase is formed by a precipitate of methanol and 0.05 M NaH_2PO_4 containing 0.005 M hexanesulfonic acid, pH 3.0. Elution was done in a gradient. The flow rate was 0.4 ml/min. The concentrations of the analyzed substances were determined using an internal standard at a wavelength of λ = 214 nm: for B3 nm and 267 nm for vitamins B1 and B2. Compounds

were identified by comparing the retention time of the analyzed peak with the retention time of the standard and by adding a specific amount of standard to the analyzed samples and reanalyzing. The detection level is 1 μg/ g.

Analysis of vitamin C content. In order to stabilize vitamin C, a solution of pH 3 was used, obtained by acidifying deionized water with concentrated orthophosphoric acid. 0.5 ml of the sample was transferred to a volumetric flask, vol. 100 ml and made up with the extraction solution. The obtained solutions were centrifuged in the MPW-55 ultracentrifuge, and the solution for further studies was collected from above the supernatant. Vitamin C was analyzed with the Aquity H UPLC system equipped with a Waters Acquity PDA detector (Waters, USA) at a wavelength of λ = 243 nm. Optimum chromatographic conditions were obtained using isocratic separation using an ACQUITY APC BEH Column, 200 Å, 2.5 μm, 4.6 mm × 75 mm chromatography column, with a mobile phase flow rate of 0.4 ml/ min. The mobile phase was a phosphate buffer with a conc. 40 mmol/ dm^3 at pH 3.65; acetonitrile (90:10, v/v) with the addition (1.5 mmol / dm^3) of cetyltrimethylammonium bromide as a stabilizing agent.

Phenolic compounds. Phenolic compounds in the samples were analyzed after alkaline and acid hydrolysis. The analysis was performed using a Waters Acquity Class H UPLC system equipped with a Waters Acquity Photodiode Array (PDA) detector (Waters, USA). Chromatographic separation was performed on an Acquity UPLC® BEH C18 column (100 mm × 2.1 mm, particle size 1.7 μm) (Waters, Ireland). Gradient elution was conducted using the following mobile phase composition: A: acetonitrile with 0.1% formic acid, B: 1% aqueous formic acid mixture (pH = 2). The concentrations of phenolic compounds were determined using an internal standard at wavelengths λ = 320 nm and 280 nm and expressed in mg/ 100 g dry weight. The detection level was 1 μg/ g.

Results and discussion

The extracts obtained were tested for thiamine, riboflavin, niacin and ascorbic acid, and the results are shown in Table 1. It was found that the content of the tested vitamins differed statistically significantly both according to the morphological part of the fungus and according to the cultivation temperature. Vitamins were found to be present in the order ascorbic acid > niacin > riboflavin > thiamine. The content of vitamin B1 and B2 was higher in the stems, while niacin and ascorbic acid were higher in the caps. It was shown

Table 1. Vitamin content of extracts from morphological parts of oyster mushroom (*Pleurotus ostreatus* L.) grown under different conditions

Tabela 1. Zawartość witamin w ekstraktach z części morfologicznych boczniaka (*Pleurotus ostreatus* L.) uprawianego w różnych warunkach

			Thiamine	Rybofla-vin	Niacin	Ascorbic acid
SL10	stem	Fruiting body growing up at 10 lux illumination for 8 hours, temperature 10±1°C	0.123 ±0.03[a]	4.05 ±0.095[a]	5.16 ±0.09[a]	6.35 ±0.10[a]
CL10	cap		0.048 ±0.04[b]	0.962 ±0.030[b]	5.24 ±0.18[b]	8.44 ±0.31[b]
SD10	stem	Fruiting body growing up at 100 lux illumination for 0.5 hours, temperature 10±1°C	0.111 ±0.04[c]	0.81 ±0.023[c]	3.29 ±0.36[c]	6.22 ±0.10[c]
CD10	cap		0.047 ±0.02[d]	0.36 ±0.002[d]	5.11 ±0.87[d]	7.36 ±0.01[d]
SD15	stem	Fruiting body growing at 10 lux illumination for 4 hours, temperature 15±1°C	0.156 ±0.03[e]	1.38 ±0.018[d]	2.69 ±0.13[e]	6.05 ±0.02[e]
CD15	cap		0.019 ±0.01[f]	0.03 ±0.003[d]	4.33 ±0.41[f]	7.11 ±0.11[f]
SL15	stem	Fruiting body growing at 200 lux illumination for 8 hours, temperature 15±1°C	0.127 ±0.05[g]	0.16 ±0.002[d]	4.66 ±0.31[g]	4.33 ±0.15[g]
CL15	cap		0.08 ±0.02[h]	0.74 ±0.05[e]	4.49 ±0.12[f]	8.19 ±0.10[h]

Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different (p value < 0.05)
Wartości wyrażono jako średnia (n = 3) ± odchylenie standardowe. Wartości średnie oznaczone różnymi literami (a, b, c etc.) w obrębie tej samej kolumny różnią się statystycznie (wartość $p < 0{,}05$).

that a lower temperature (10°C) of cultivation resulted in higher retention of thiamine, niacin and ascorbic acid. However, there was no significant effect of cultivation temperature on the vitamin content of the caps.

Factors affecting the course of mushroom cultivation, including oyster mushroom, include temperature, air humidity, substrate moisture and pH, and light intensity, as well as the gas composition of the atmosphere (Zawadzka et al., 2022). Not without importance are the conditions and specifics of the substrate. Ensuring the right parameters during cultivation determines the correct course of cultivation and the achievement of a high yield of good-quality fruiting bodies.

Table 2. Phenolic acids content

Tabela 2. Zawartość kwasów fenolowych

			mg/L																	
			proto-cate-chuic	Galic	2,5-di-hy-droksо-benzoic	4-hyr-doksо-benzoic	caffeic	syryn-gic	p-cou-maric	ferulic	chloro-genic	synapic	t-cin-namic	narin-genin	vitexin	rutin	quercitin	apigenin	kamp-ferol	luteolin
SL10	stem	Fruiting body growing up at 10 lux illumination for 8 hours, temperature 10 ±1°C	0.93 ±0.04^{a}	8.25 ±0.25^{a}	2.74 ±0.25^{a}	1.67 ±0.11^{a}	0.02 ±0.01^{a}	0.08 ±0.02^{a}	6.31 ±0.21^{a}	9.74 ±0.04^{a}	9.35 ±0.11^{a}	98.6 ±0.74^{a}	1.14 ±0.05^{a}	1.4 ±0.06^{a}	0	0	0	0	0	0
CL10	cap		1.69 ±0.02^{b}	9.07 ±0.05^{b}	2.39 ±0.06^{b}	1.59 ±0.11^{ab}	0.01 ±0.00^{a}	0.09 ±0.01^{a}	6.84 ±0.12^{a}	9.95 ±0.25^{abc}	9.17 ±0.06^{b}	39.97 ±0.65^{b}	1.84 ±0.02^{b}	9 ±0.02^{b}	0	0	0	0	0	0
SD10	stem	Fruiting body growing up at 100 lux illumination for 0.5 hours. temperature 10 ±1°C	0.13 ±0.03^{c}	9.12 ±0.31^{c}	2.15 ±0.04^{c}	1.07 ±0.12^{c}	0.02 ±0.01^{a}	0.08 ±0.02^{a}	6.11 ±0.11^{b}	10.11 ±0.04^{c}	9.38 ±0.07^{a}	98.6 ± 1.2^{c}	1.59 ±0.01^{c}	398.6 ±0.93^{c}	0	0	0	0	0	0
CD10	cap		0.18 ±0.02^{c}	8.62 ±0.12^{d}	2.88 ±0.1^{d}	1.54 ±0.31^{d}	0.04 ±0.01^{a}	0.09 ±0.03^{a}	5.99 ±0.02^{c}	9.38 ±0.05^{d}	8.29 ±0.12^{c}	39.97 ±0.51^{d}	1.24 ±0.02^{d}	795.2 ±1.35^{d}	0	0	0	0	0	0
SD15	stem	Fruiting body growing at 10 lux illumination for 4 hours. temperature 15 ±1°C	0.93 ±0.05^{d}	8.25 ±0.25^{ef}	2.74 ±0.11^{e}	1.67 ±0.25^{e}	0.02 ±0.01^{a}	0.08 ±0.02^{a}	6.31 ±0.05^{de}	9.74 ±0.04^{e}	9.35 ±0.06^{d}	0.16 ±0.02^{e}	1.14 ±0.06^{e}	1.4 ±0.02^{e}	0	0	0	0	0	0
CD15	cap		1.69 ±0.02^{e}	9.07 ±0.87^{f}	2.39 ±0.07^{f}	1.59 ±0.2^{f}	0.01 ±0.00^{a}	0.09 ±0.02^{a}	6.84 ±0.15^{de}	9.95 ±0.02^{f}	9.17 ± 0.21^{e}	0.27 ±0.02^{f}	1.84 ±0.02^{f}	9 ±0.04^{f}	0	0	0	0	0	0
SL15	t stem	Fruiting body growing at 200 lux illumination for 8 hours. temperature 15 ±1°C	0.69 ±0.01^{f}	8.97 ±0.51^{fg}	2.66 ±0.65^{g}	1.49 ±0.32^{d}	0.01 ±0.01^{a}	0.08 ±0.01^{a}	6.07 ±0.21^{ef}	8.62 ±0.07^{g}	9.47 ±0.04^{f}	0.07 ±0.02^{g}	99.6 ±0.71^{g}	1.7 ±0.03^{g}	0	0	0	0	0	0
CL15	cap		0.87 ±0.02^{g}	9.14 ±0.17^{h}	3.07 ±0.06^{h}	1.74 ±0.21^{g}	0.01 ±0.01^{a}	0.07 ±0.01^{a}	6.12 ±0.06^{g}	9.13 ±0.04^{h}	8.99 ±0.25^{g}	0.09 ±0.01^{g}	397.927 ±2.04^{h}	1.6 ±0.06^{h}	0	0	0	0	0	0

Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different (p value < 0.05)
Wartości wyrażono jako średnia (n = 3) ± odchylenie standardowe. Wartości średnie oznaczone różnymi literami (a, b, c etc.) w obrębie tej samej kolumny różnią się statystycznie (wartość p < 0,05)

Different species of oyster mushrooms differ in temperature requirements, the speed of entering the mycelium formation stage and the length of time for the mycelium to overgrow the substrate (Sánchez, 2010). In the wild, the fruiting bodies of oyster mushrooms appear in late autumn, and in milder climates where the winter temperature drop is small they can appear even in winter. The oyster mushroom is quite resistant to frost, but in winter periods in which the time of light is short it is difficult to get fruiting bodies because it needs a lot of light, which is scarce in winter periods, during the period of their production (Zawadzka et al., 2022). With too little of it, it does not produce fruiting bodies at all or they are small and few in number. Completed research confirms that lower temperature determines the vitamin content of mushroom stems. However, it should be borne in mind that mushrooms are also a source of other nutrients, the content of which can also depend on the growing temperature.

The study undertook an evaluation of the polyphenol content, and the results are shown in Table 2. It was found that the cultivated oyster mushrooms did not contain flavonols such as rutin, vitexin, apignin, campferol or luteolin. Instead, the presence of naringenin was measured. Importantly, it was shown that naringenin was present in mushrooms whose buds were set in the dark and the cultivation temperature was 10°C. In the other variants, the level of this compound was in trace amounts. Of the phenolic acids analyzed, gallic, ferulic and chlorogenic acids were the dominant ones. However, the highest amount of synapic acid was found among the analyzed acids in crops grown at 10°C. There was no temperature dependence in the content of the studied compounds in caps vs. stems.

There are many studies in the literature that deal with the analysis of phenolic compounds in edible mushroom species, especially the phenolic acids that predominate in them. It is with their strong antioxidant activity and ability to protect against oxidative damage of vital structures (cell membranes, structural proteins, enzymes, lipids or nucleic acids) that their wide spectrum of biological activity is associated (Jayakumar et al., 2011). The presence of phenolic acids was found in all the edible mushroom species studied, however their quantity and occurrence propriety varies and depends on many factors. The literature indicates that the species with the greatest diversity of phenolic compounds were: brown, edible peppercorn and oyster mushroom. (Jayakumar et al., 2011). Numerous scientific studies conducted around the world indicate the high therapeutic activity of extracts and compounds obtained from siderophore. Experimental studies have confirmed their therapeutic activity.

Part of the studies concerned the analysis of extracts, in which they were looking for compounds of antioxidant nature, with a quantitatively dominant group of phenolic acids. In this light, growing conditions, including irradiation or the type of substrate, also became an object of study. Based on the analysis of phenolic acids in extracts of oyster mushrooms, the presence of numerous phenolic acids was found (Zawadzka et al., 2022). Oyster mushroom has also been found to be high in other compounds. Due to its documented health-promoting properties, as well as its relatively high nutritional value and high mineral content, oyster mushroom is recommended in many countries as a supplement to the whole-food diet. Oyster mushroom protein is relatively well digested, with isoleucine being the limiting amino acid in its nutritional value.

Noteworthy is the high content of dietary fiber, far exceeding the amounts recorded in dried vegetables and fruits, as well as the high content of ash and iron, potassium, phosphorus, zinc and copper (Kortei et al., 2017). A characteristic component of the oyster mushroom is β-1,3-D-glucan, which has been shown to be effective in strengthening the body's immune system, exhibited anticancer, sugar-lowering and blood cholesterol-regulating effects (Nosál'ová et al., 2001).

Conclusion

The effect of cultivation has a significant impact on the content of bioactive components in the siderophore. Mushrooms grown at 10°C had a higher content of vitamins B and C compared to mushrooms grown at higher temperatures. No flavonols were found in the mushrooms, instead it was shown that the cultivation method has a decisive effect on the content of phenolic acids. It was shown that extracts from the stems had a higher content of vitamins and some phenolic acids. In connection with the above, it is reasonable to develop a new direction for the management of oyster mushroom stems towards the production of functional foods. Further work is needed to confirm what technological properties the oyster stems have and how they can determine the properties of new functional products.

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Wpływ temperatury uprawy na zawartość wybranych związków aktywnych w częściach anatomicznych boczniaka (*Pleurotus ostreatus* L.)

Streszczenie. Celem pracy była ocena wpływu temperatury uprawy boczniaka ostrygowatego (*Pleurotus ostreatus* L.) na retencję wybranych witamin w trzonie i kapeluszu bocznika ostrygowatego. Materiał badany stanowiły owocniki grzybów, których zawiązki uzyskano zarówno przy dostępem światła, jak i bez niego, a uprawę prowadzono w temperaturze 10°C oraz 15°C. Uzyskany plon grzyba frakcjonowano na trzony i kapelusze, a w ekstraktach z nich uzyskanych oznaczono zawartość witamin B i C, kwasów fenolowych i flawonoli. Stwierdzono, że uprawa ma znaczący wpływ na zawartość składników bioaktywnych w boczniku. Boczniaki uprawiane w temperaturze 10°C charakteryzowały się wyższą zawartością witamin z grupy B i C w porównaniu do grzybów uprawianych w wyższej temperaturze. Nie stwierdzono obecności flawonoli w grzybach, za to wykazano, że temperatura uprawy ma decydujący wpływ na zawartość kwasów fenolowych. Wykazano, że ekstrakty z trzonów zawierały więcej witamin i niektórych kwasów fenolowych. W związku z powyższym zasadne jest, aby opracować nowy kierunek zagospodarowania trzonów boczniaka jadalnego w kierunku wytwarzania żywności funkcjonalnej. Niezbędne są dalsze prace, które potwierdzą, jakie właściwości technologiczne mają trzony boczniaka i w jaki sposób mogą warunkować cechy nowych produktów funkcjonalnych.

Słowa kluczowe: *Pleurotus ostreatus* L., boczniak ostrygowaty, związki bioaktywne, temperatura uprawy

Both, traditional and innovative food are essential elements of nowadays consumers' menus. Regardless of the food processing techniques used, consumer preferences remain individual. The goal of food technology and food analysis is to monitor, on the one hand, the needs of the food market, and on the other hand – the possibilities of the food industry. This monograph is a compilation of scientific work showing how far the considerations of food can be, both – local vs. global, and traditional vs. innovative.

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