DOI : 10.2478/aucft-2024-0010

EFFECT OF BLANCHING METHODS AND PROBIOTIC BACTERIA ON THE BIOACTIVE COMPOUNDS AND PHYSICOCHEMICAL PARAMETERS OF FERMENTED BROWN *AGARICUS BISPORUS* AND *IMLERIA BADIA* MUSHROOMS

– Research paper –

Kavya VENUGOPAL, Emilia BERNAŚ*

Department of Plant Products Technology and Nutrition Hygiene, University of Agriculture in Krakow, Balicka 122 Str., 30-149 Krakow, Poland

Abstract: The brown *A. bisporus* - a cultivated species and *I. badia* – a wild species of mushroom containing health-promoting properties has been selected to create functional foods. Therefore, the study analyzed the effect of the blanching method, fermentation temperature and addition of probiotic bacteria on the course of lactic fermentation and changes in mushrooms quality. The mushroom caps were blanched in water for 30 s and 2 min, followed by microwave-blanching for 2 min. To increase the effectiveness of fermentation and bioactivity of the product a starter culture of *Lactobacillus acidophilus* strain LA-5 (Hansen) was added to the brine. The mushrooms were fermented at 21°C and 26°C. In both the species fermentation concluded within 3 days with pH < 4.5. The blanching and the addition of probiotic bacteria significantly affected bioactive compounds, compared to fermentation temperature. Concerning the mushroom's quality, blanching was necessary before fermentation. Products that were water-blanched for 2 min displayed increased dietary fiber and glucans content, while products from *A. bisporus* microwave-blanched gave organoleptic properties. The obtained products had significant amounts of B vitamins and phenols, proving that fermentation benefited the retention and enhancement of the nutritional quality of mushrooms. Fermented mushrooms can be used in nutraceuticals.

Keywords: *Lactobacillus acidophilus*, glucans, phenols, B vitamins, hardness, organoleptic quality

INTRODUCTION

Mushrooms have gained popularity in recent years not only for their unique flavor and texture but also for their nutraceutical properties. Edible mushrooms and their extracts are widely used in the pharmaceutical and cosmetic industry due to their high content of biologically active compounds that exhibit beneficial effects such as antibacterial, antifungal, antitumor, antiallergic, antioxidative, hypoglycemic, anti-inflammatory, hepatoprotective, and many more (Lindequist et al., 2005; Lan et al., 2023). To exploit these benefits, mushroom cultivation has become a major part of the world's agribusiness in the last decade, and in 2021-22, mushroom production was approx. 15.25 million tonnes globally (Kumari and Jaiswal, 2023). Nutritionally, mushrooms are rich in both macro and micro molecules. They contain a high amount of carbohydrates, protein with most of the essential amino acids, a very low amount of fat with a high proportion of polyunsaturated fatty acids, and a significant amount of vitamins (B_1, B_2, B_{12}, C, D, and E), which help maintain longevity and quality of health (Kozarski et al., 2015; Valverde et al., 2015). Mushrooms have an effective mechanism to take up minerals more efficiently than crops, making them an important tool in the supply of diet essential minerals. Additionally, they are a great source of selenium, iron, zinc, potassium, and copper, and together with the bioactive compounds, they exhibit a synergistic effect, promoting health benefits (Gebrelibanos et al., 2016; Das et al., 2023; Oyetayo, 2023).

A series of major immunomodulating compounds are found in mushrooms as polysaccharides and mainly include beta-glucans and glycoproteins; secondary metabolites such as phenolic compounds and terpenoids; vitamins, peptides, and proteins (Hobbs, 2023). Several *in vitro* studies have described mushroom extracts' strong hydrogen-donating and metal-chelating ability against superoxide and free radicals caused by

Received: 29.03.2024
Accepted in revised form: 01.07.2024

* Corresponding author.
E-Mail address: emilia.bernas@urk.edu.pl

oxidative stress acting as a strong antioxidant agent (Elmastas et al., 2007). It was found that mushroom's phenolic compounds, indole compounds, tocopherol, ascorbic acid, and carotenoids show excellent effectiveness against several chronic diseases such as cancer, rheumatoid arthritis, cirrhosis, and arteriosclerosis as well as against degenerative processes associated with aging (Cheung et al., 2003; Barros et al., 2008; Chye et al., 2008). Secondary metabolites such as terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolones, as well as some primary metabolites such as oxalic acid, peptides, and proteins in mushrooms, display antimicrobial activity. Hence, mushrooms could also be a new commercial source of these compounds (Alves et al., 2012; Bach et al., 2019).

Mushroom polysaccharides consist of a complex mixture of homopolysaccharides and heteropolysaccharides that contain chitin, hemicelluloses, glucans, mannans, xylans and galactose, and heterogalactans, which include mannogalactans, fucogalactans, and fucomannogalactans (Bhakta and Kumar, 2013; Ruthes et al., 2021). Furthermore, they have a very high amount of non-digestive dietary fibers consisting of glucans (α- and β-glucans) and chitin, which are up to 80% of the prebiotic fiber in some dried fruiting bodies that remain undigested by the digestive enzymes in the upper gut and enhance the growth and diversification of the probiotic bacteria in the large intestine while helping prevent viral infections and maintain gut health (Hobbs, 2023). Recent studies on gut microbiome have increased substantially due to the gut microbiome directly affecting brain health by enhancing the communication through the brain-gut axis and its functioning by balancing the levels of neurotransmitters such as serotonin and dopamine (Foster et al., 2016; Mu et al., 2016; Sherwin et al., 2018; Maity et al., 2021). Dietary fiber plays an important role in reducing the risks of many disorders, such as constipation, diabetes, cardiovascular diseases, diverticulosis, and obesity. A spotlight has been shown on mushroom glucans, especially 1,6-branched 1,3-β-glucans, owing to their effectiveness toward inhibiting tumor growth through stimulating the immune system via activation of macrophages. This was possible by achieving a balance of the T helper cell populations and subsequent effects on natural killer cells and also via cytokine production (Roupas et al., 2012; Nile and Park, 2014; Bozzetto et al., 2018).

Mushrooms are quite versatile in texture and flavor and are mostly consumed fresh in soups, sauces, salads, stuffings, and meat dishes or eaten in a processed form like drying, salting, marinating, canning, and freezing (Bernaś et al., 2006). In the recent surge of vegan food consumption for both health and sustainability reasons, mushrooms have proven a great alternative to meat in texture and protein content (Muliadi and Anugrahati, 2022; Paranagama et al., 2022). Both conventional and novel processing methods significantly affect the nutritional quality of mushrooms. However, novel methods possess additional economic costs, whereas conventional methods, such as drying and canning, result in the loss of soluble compounds and textural changes. Owing to the high prebiotics content, mushrooms are a great substrate for fermentation. Studies of lactic acid fermentation of cultivated and wild mushroom species have shown that it is an excellent method for extending the shelf life and preservation of the product. The fermentation process positively affects the antioxidant activity and reduces the biogenic amine content (Zheng et al., 2018; Kanellaki et al., 2022). The most commonly used bacteria in probiotic products from dairy, fruit, and vegetables are *Lactobacillus* (L.) and *Bifidobacterium* (B.). These bacteria are great starter cultures for mushroom fermentation, as the final products are characterized by good sensory properties and high antioxidant and phenolic compounds (Jabłońska-Ryś et al., 2016; Liu et al., 2016; Jabłońska-Ryś et al., 2022b). *L. acidophillus* has a homofermentative mechanism with more than 85% lactic acid production, exhibits beneficial behaviors in the fermentation of dietary fiber from vegetables and also has a high survival rate post gastro-intestinal simulation (Yari et al., 2015; Mora-Cura et al., 2017).

Our study was designed to understand the effects of probiotic bacteria – *L. acidophilus* strain LA-5, different types of pre-treatments (blanching), and the temperature of fermentation on changes in the bioactive compounds after fermentation in two mushroom species, cultivated - brown *Agaricus bisporus* and wild - *Imleria badia*. Pre-treatment is a process that halts enzymatic browning, stabilizes the color, enhances flavor retention, and maintains textural properties. The most commonly used pre-treatment methods in mushroom processing are washing in water, potassium metabisulphite (KMS), sugar, and salt either alone or in combination; blanching, blanching followed by soaking in potassium metabisulphite and citric acid and vacuum impregnation has also been examined (Walde et al., 2006; Bernaś, 2018; Bernaś and

Jaworska, 2021). Though white *A. bisporus* is more consumed than the brown variety, and even though both contain similar macronutrients, the brown variety is less researched but contains higher antioxidant activity, total phenolic compounds, and possess polysaccharides with stronger immunostimulatory and antitumor bioactivity (Zhang et al., 2014; Vunduk et al., 2018). *I. badia,* also known as *Boletus badius,* contains high contents of specific polyphenols compared to other wild species, such as protocatechuic acid and cinnamic acid. The phenolic compound Norbadione A is a dark brown dye specifically found in the caps of *I. badia* and exhibits very high antioxidant properties that show protective effects against harmful ionizing radiation, according to conducted animal studies (Le Roux et al., 2012; Muszyńska et al., 2013; Dimitrijević et al., 2017). With the presence of a high content of polysaccharides and phenolics in the selected species and the addition of probiotic bacteria during fermentation, which increases the bioavailability of these therapeutic compounds, fermented mushrooms have a very high potential to be functional foods. Vacuum-dried and powdered fermented mushrooms have been employed in nutraceutical and probiotic supplements (Reis et al., 2017; Üstün et al., 2018). Moreover, the observed increase in mushroom cultivation, including spawn production, compost production, cultivation, processing, and marketing, has generated entrepreneurial activity, which has helped the establishment of small-scale agricultural industries that have a very small carbon footprint.

MATERIAL AND METHODS

Mushrooms

The research material was fresh and fermented caps of brown *Agaricus bisporus* and *Imleria badia* mushrooms. Fresh mushrooms with a cap size of 4-8 cm in diameter were purchased early in the morning at the local market in Krakow (Poland). Damaged fruiting bodies were removed, cleaned remains from the substrate litter, and washed under running tap water of approximately 15°C temperature. Later, the caps were separated from the stalks and caps of diameter 6–8 cm were cut in half.

Sample preparation

Pre-treatment and blanching

The mushroom caps were divided into four parts. Three parts were subjected to blanching in water for 30 s or 2 min (both temp. 96-98°C), and blanching in the microwave for 2 min. The rest of the mushroom caps were fermented fresh (without any pre-treatment). Blanching in water was carried out in a stainless-steel pot with a capacity of 10 dm3, the ratio of the mass of mushrooms to water was 1:5. The blanched mushroom caps were cooled in cold tap water and then placed on sieves to drain the excess. Microwave blanching was carried out in a Panasonic NN-F621MB EPG microwave oven (1000 W, 2.45 GHz). The caps were microwave-blanched in portions of 500 g. After blanching, the material was spread out on trays in a layer of about 1-2 cm to lower the temperature of the fruiting bodies to below 25°C

Probiotic bacteria production

A defined volume (10 ml) of medium with probiotic bacteria *Lactobacillus acidophilus* strain LA-5 (Hansen) (code LbA) was introduced into 100 ml of sterile MRS medium and placed in a CO_2 incubator at 37°C and CO2 content of 15%. Samples were stirred daily to prevent the cells from settling at the bottom. After one week of cultivation, the bacterial content was determined by measuring the optical density (McFarland scale). The volume of the slurry to be added to the mushrooms was calculated so that the initial number of bacterial cells was 1000 cells/kg of fruiting bodies. The calculated volume was centrifuged and resuspended in sterile Ringer's fluid. LbA was added separately to the brine and stirred.

Fermentation conditions

The caps were fermented under relatively anaerobic conditions. Both unblanched (fresh) and blanched caps were placed in fermentation vessels, covered with a brine solution of 3% NaCl and 2.5% sucrose. The ratio of brine to the mushroom caps was 1:2. Mushroom caps were loaded with sinkers and the fermentation process was carried out in an incubator at temperatures of 21±1°C or 26±1°C until pH ≤4.5 (3 days). Then the products were transferred into a refrigerator and stored for the next 14 days at 5±1°C (the total fermentation time was 17 days). It was assumed that products with pH>4.5 after fermentation would be eliminated from organoleptic and chemical analyses. The sinkers were cleaned daily by

washing them thoroughly under cold, running tap water.

Methods

After the fermentation process was completed, the products were placed on sieves and left for 30 min at room temperature to separate mushroom caps from the brine. In fresh and fermented caps, the levels of dry matter, ash, pH, total and volatile acidity were analyzed by AOAC (1995). In addition, organoleptic analysis (5-point method) was conducted on all products. The mushroom caps were assessed for colour (CIELab) and texture (hardness) using instrumental methods. Fermented mushroom caps were freeze-dried, powdered and then used to analyze bioactive compounds like dietary fibre, beta-glucans, vitamins, and phenols. All chemical and colour analysis were performed in 4 repetitions for each product; texture measurement was performed in 6 repetitions for each product, while organoleptic analysis in 5 repetitions for each product.

Chemical analysis

The levels of dry matter, ash, pH, and total acidity (g of lactic acid/100 g fresh matter - fm) were analyzed by AOAC (1995). Volatile acidity (g of acetic acid/100 g of fm) was determined by distilling the sample in the Distillation Unit B-324, Büchi (Bączkowicz et al., 2012). All parameters were analyzed in four replications.

Vitamin B_1 and B_2 content were determined according to HPLC method (Zięba et al., 2021) in mushrooms which were freeze-dried and ground into a fine powder by a laboratory mill. Vitamins B_1 and B_2 were detected simultaneously using a High-performance liquid chromatograph with a fluorescence detector. Analysis was carried out on a Bionacom Velocity C18 PLMX column (4.6x250 mm, 5 µm), together with the precolumn (Bionacom LTD, London, UK), and was conducted at a wavelength excitation and emission: 360/503. Water and acetonitrile (t = 0 w/ac 88/12; t = 12 w/ac 0/100) were used as the mobile phase in a gradient elution with a flow rate of 0.9 cm3·min−1. The standards of thiamine (thiamine hydrochloride, purity >99.0%, Sigma-Aldrich, Germany) and riboflavin (riboflavin, purity >98.0%, Sigma-Aldrich, Germany) were used for the identification of these compounds and their quantitative analysis.

The level of total phenolics, tartaric esters, flavonols, and anthocyanins was determined in mushrooms with a spectrophotometric method (Fukumoto and Mazza, 2000). The level of all compounds was determined in 80% methanol extracts (80 mg sample/ml extract) obtained by 1-fold homogenization (90 s) at room temperature. After homogenization, the samples were centrifuged (20 min., 5000 rpm), and the supernatant was decanted. The level of total phenolics, tartaric esters, flavonols, and anthocyanins was measured at 280, 320, 360, and 520 nm. Standards used were chlorogenic acid, caffeic acid, quercetin, and cyanidin, respectively.

Determination of total, soluble, and insoluble dietary fibre was based on AOAC (1985, 1986 & 1987). Total Dietary Fibre Assay Kit from Megazyme international was used for this analysis. The measurement was performed in 4 replicates for each product.

Measurement of glucans was based on the AOAC(2012) . β-Glucan (Yeast and Mushroom) Assay Kit from Megazyme international was used for the analysis of 1,3:1,6-β-glucans and α-glucans.

Colour analysis

The colour of the homogenized caps with distilled water (1:1 w/w) was determined immediately after homogenization by reflection according to the CIE Lab system, using a MINOLTA CM-3500d with Illuminant D65 as the standard. The measurements were performed using a 30 mm Petri dish at a measurement angle of 10°. Based on the colour measurement, the total colour difference ΔE* (Mokrzycki and Tatol, 2011) was calculated by using eq. 1:

$$\Delta E^* = \sqrt{(L^* - L_f^*)^2 + (a^* - a_f^*)^2 + (b^* - b_f^*)^2} \quad [1]$$

where L*, a*, b* - are the colour parameters of unblanched fermented mushroom caps, L_f*, a_f *, b_f * are the colour parameters of blanched fermented mushroom caps.

Texture analysis

The hardness [N/mm] of the caps was determined instrumentally using a Shimadzu EZ-TEST texturometer, model EZ-LX equipped with a head with a permissible pressure of 1000 N. The measurement was carried out by the cutting method, using a Razor Blade Cutting JIG guillotine (Shimadzu) equipped with a sharp knife and a table with a groove. The mushrooms were cut at a speed of 0.5 mm/s, the deformation was 95%. The caps were placed with the gills, the cut was made across the gills through the centre of the cap.

Organoleptic analysis

The organoleptic quality of mushroom caps without brine was evaluated using a 5-point method. Mushroom quality was rated on a 5-1

scale (5 = excellent, 4 = very good, 3 = good, 2 = bad, 1= very bad) by a panel of five judges fulfilling the requirements for sensory sensitivity as described by the Polish standard. The lowest acceptable score for any characteristic assessed by the sensory panel was set at 3.0 (PN-ISO 3972, 1998).

Statistical analysis

All samples were analyzed in four (chemical analysis), five (organoleptic analysis) or six (texture) independent replications and data were expressed as means. Results were analyzed statistically using single-factor analysis of variance (ANOVA) based on Duncan's range test ($p<0.05$). Calculations were performed using Statistica 10.0 Pl (Stat-Soft).

RESULTS AND DISCUSSION

Changes in the pH, total and volatile acidity during fermentation

Lactic acid produced by *L. acidophilus* via the utilization of mushroom sugars and added sucrose increased the acidity and lowered the pH value of the fermented caps. The observed change in pH is an important factor in analyzing product fermentation (Jabłońska-Ryś et al., 2022a). pH<4.5 marked the completion of the fermentation process, rendering the food product ready for consumption after an incubation period. At the beginning of fermentation, pH of the cultivated species *A. bisporus* was slightly higher (7.4-6.3) compared to that of the wild species *I. badia* (5.9-6.2). Both species reached a pH of <4.5 after 3 days of fermentation. Though the products differed in pH values on day 1 and 2 of fermentation, there was no clear distinction in between products fermented with or without any blanching or added LbA (controls F21 and F26). The samples with added LbA in *A. bisporus* had slightly higher pH compared to those without LbA on day 2 of fermentation. Even though the decrease in pH was slower in the product added with LbA, *L. acidophilus* has been previously determined as an efficient genus of bacteria for the production of probiotic drinks. Several studies (Sharma and Mishra, 2013; Worku et al., 2021) on fruit and vegetable drinks with added *L. acidophilus* strains positively affected the pH by reducing it to <3.5 with initial pH ranges of around 5-6, which promoted their survival post storage making them excellent probiotic drinks. The acidity of the fermentation broth directly expresses the sensory quality and the consumption status of the final product (Chen et al., 2021).

The contents of total acidity and volatile acidity were 60-70% higher in *I. badia* compared to *A. bisporus* and differed significantly (Table 1). Both total and volatile acidity content were significantly affected by the type of pre-treatment on both species, wherein the effect of the pre-treatment process on volatile acidity was contradictory in both species.

Table 1. Total and volatile acidity in fermented mushrooms [g/100 g fm]

Sample	Total acidity (g of lactic acid)		Volatile acidity (g of acetic acid)	
	A. bisporus	*I. badia*	*A. bisporus*	*I. badia*
F21	0.51 ± 0.07^{ab}	1.17 ± 0.02^{de}	0.038 ± 0.002^{de}	0.046 ± 0.002^{g}
F26	0.54 ± 0.05^{ab}	1.04 ± 0.05^{cde}	0.034 ± 0.003^{cd}	0.089 ± 0.002^{m}
BW30s (21)	0.63 ± 0.05^{ab}	0.99 ± 0.02^{cd}	0.052 ± 0.005^{h}	0.062 ± 0.002^{h}
BW30s (21, LbA)	0.54 ± 0.05^{ab}	1.08 ± 0.01^{cde}	0.023 ± 0.002^{b}	0.081 ± 0.007^{l}
BW30s (26)	0.59 ± 0.05^{ab}	0.95 ± 0.05^{c}	0.033 ± 0.003^{cd}	0.054 ± 0.001^{h}
BW30s (26, LbA)	0.50 ± 0.05^{ab}	0.99 ± 0.01^{cd}	0.044 ± 0.005^{fg}	0.037 ± 0.001^{de}
BW2m (21)	0.54 ± 0.05^{ab}	0.59 ± 0.05^{ab}	0.063 ± 0.006^{i}	0.031 ± 0.005^{c}
BW2m (21, LbA)	0.41 ± 0.04^{a}	0.72 ± 0.01^{b}	0.067 ± 0.002^{ij}	0.038 ± 0.002^{def}
BW2m (26)	0.59 ± 0.05^{ab}	0.63 ± 0.01^{ab}	0.041 ± 0.005^{ef}	0.037 ± 0.001^{cde}
BW2m (26, LbA)	0.41 ± 0.04^{a}	0.68 ± 0.05^{b}	0.015 ± 0.003^{a}	0.038 ± 0.002^{def}
BM2m (21)	0.45 ± 0.05^{a}	0.94 ± 0.05^{c}	0.032 ± 0.002^{c}	0.075 ± 0.004^{k}
BM2m (21, LbA)	0.59 ± 0.05^{ab}	1.08 ± 0.01^{cde}	0.015 ± 0.003^{a}	0.089 ± 0.002^{m}
BM2m (26)	0.45 ± 0.05^{a}	1.22 ± 0.05^{e}	0.023 ± 0.002^{b}	0.096 ± 0.003^{n}
BM2m (26, LbA)	0.45 ± 0.05^{a}	0.97 ± 0.58^{cd}	0.026 ± 0.002^{b}	0.070 ± 0.002^{jk}

Legend: means with different small letters in row and columns represent significant differences at $p<0.05$, F-unblanched, BW30s-blanched in water for 30 s, BW2m-blanched in water for 2 min., BM2m-blanched in microwave for 2 min, LbA-*L. acidophilus* strain LA-5, 21 and 26 - temperature of fermentation 21°C and 26°C, respectively

In *I. badia*, total acidity and volatile acidity were significantly higher in water-blanched products for 30 s and microwave-blanched products. The products with added LbA had slightly higher total acidity content compared to those without LbA fermented under the same conditions in *I. badia*. The highest volatile acidity content was significantly higher in water-blanched products for 2 min in *A. bisporus*, and the lowest contents were observed in microwave-blanched products. No clear effects related to the fermentation temperature or the addition of probiotic bacteria were observed on total or volatile acidity.

Dry matter and ash

The moisture content in mushrooms depends on different factors such as species, substrate, storage conditions, and so on. In general, the moisture content of the studied mushrooms ranged from 85-92 g, and the ash contents ranged from 0.3-0.9 g/ 100 g of fresh weight, which was in agreement with several studies (Manzi et al., 2004; Reguła and Siwulski, 2007). Both the dry matter and ash content were higher in *I. badia* with 10.83-13.83 g/100 g and 0.67-0.94 g/100 g fresh weight, respectively, compared to *A. bisporus* and differed significantly. A clear difference in the dry matter content was observed among the different pre-treatments in *I. badia*, where the products blanched in the microwave for 2 min had the highest dry matter, and those water-blanched for 2 min had the lowest dry matter content. In *A. bisporus*, the lowest amount of dry matter and ash were found in products blanched in water for 30 s. The dry matter content was reversibly related to the moisture contents in the samples. The samples from *A. bisporus* had a higher moisture content of 89-92% than that of *I. badia*, ranging from 86 to 89%.

Dietary fibre and glucans

Dietary fiber mainly consists of complex non-starch polysaccharides that are resistant to enzymatic digestion, and include chitin and β-glucans in mushrooms. β-Glucans can be both homo- and heteroglycans with β(1→3), β(1→4), and β(1→6) glycosidic linkages branched making up to 4-13% of the total dietary fiber (TDF) with a variability of the dietary fiber fractions depending on the mushrooms species (Wasser, 2002; Dhingra et al., 2012). TDF in mushrooms is the intrinsic sum of soluble dietary fiber and insoluble dietary fiber and in general, the content of insoluble dietary fiber (IDF) is higher than soluble dietary fiber (SDF) (Kumari, 2020). Wild mushroom species contain a higher amount of TDF compared to cultivated species, amounting to up to 42.6% of dry matter (Nile and Park, 2014) which was in agreement with our results. *I. badia* showed significantly higher contents of TDF ranging from 55.35-70.52 g/100 g dm, compared to that of *A. bisporus*, which had 34.12-50.01 g/100 g dm (Table 3). The TDF content was higher in all the products with blanching compared to those without blanching in both the species. This could be due to the relative increase of dietary fibre due to the loss of some low-molecular compounds like minerals and vitamins into the blanching water (Wennberg et al., 2006). The changes in the dietary fibre can also be due to the hydrolysis of glycosidic linkages by acid environment created by LAB during the fermentation.

Table 2. Dry matter and ash content in fermented mushrooms [g/100 g fm]

Sample	Dry matter		Ash	
	A. bisporus	*I. badia*	*A. bisporus*	*I. badia*
F21	7.91±0.03^{a}	11.67±0.56^{h}	0.63±0.04^{efgh}	0.86±0.06^{k}
F26	8.02±0.03^{a}	11.98±0.06^{ij}	0.74±0.07^{ij}	0.94±0.03^{l}
BW30s (21)	8.72±0.02^{b}	11.67±0.03^{h}	0.45±0.02^{b}	0.70±0.02^{hij}
BW30s (21, LbA)	8.66±0.04^{b}	12.14±0.10^{j}	0.33±0.06^{a}	0.71±0.04^{hij}
BW30s (26)	8.65±0.04^{b}	13.83±0.08^{m}	0.48±0.01^{bc}	0.68±0.05^{ghij}
BW30s (26, LbA)	8.51±0.05^{b}	11.91±0.14^{hij}	0.50±0.05^{bc}	0.69±0.06^{ghij}
BW2m (21)	10.81±0.06^{f}	10.83±0.03^{f}	0.55±0.01^{cde}	0.69±0.02^{hij}
BW2m (21, LbA)	10.26±0.08^{de}	10.90±0.23^{f}	0.60±0.04^{efg}	0.70±0.00^{hij}
BW2m (26)	9.95±0.06^{c}	11.16±0.19^{g}	0.61±0.03^{efg}	0.67±0.01^{ghi}
BW2m (26, LbA)	10.24±0.07^{d}	11.78±0.17^{hi}	0.59±0.01^{def}	0.69±0.03^{ghij}
BM2m (21)	10.47±0.07^{de}	12.89±0.25^{k}	0.52±0.08^{bcd}	0.75±0.05^{ij}
BM2m (21, LbA)	10.35±0.04^{de}	13.53±0.12^{l}	0.59±0.08^{def}	0.73±0.08^{ij}
BM2m (26)	10.52±0.09^{e}	14.15±0.10^{n}	0.64±0.04^{fgh}	0.76±0.05^{j}
BM2m (26, LbA)	10.21±0.06^{d}	13.75±0.19^{lm}	0.64±0.02^{fgh}	0.76±0.04^{ij}

Legend: see Table 1

Table 3. Dietary fibre in fermented mushrooms [g/100 g dm]

Sample	TDF		IDF		SDF	
	A. bisporus	*I. badia*	*A. bisporus*	*I. badia*	*A. bisporus*	*I. badia*
F21	36.27±0.45^{ab}	55.68±0.21^{fg}	31.58±0.07^{a}	47.55±1.12^{efg}	4.69±0.39^{b}	8.13±1.33^{bc}
F26	34.12±0.04^{a}	59.85±1.82^{ghi}	30.38±0.19^{a}	51.68±2.56^{ghi}	3.74±0.15^{a}	8.17±0.74^{bc}
BW30s (21)	40.81±0.31^{bc}	58.95±2.50^{gh}	36.93±0.45^{b}	46.82±3.46^{defg}	3.89±0.14^{a}	12.13±0.96^{cde}
BW30s (21, LbA)	41.29±0.21^{bc}	63.78±5.95^{hijk}	38.08±0.17^{b}	47.25±1.65^{efg}	3.21±0.05^{a}	16.53±4.30^{f}
BW30s (26)	42.51±3.03^{bcd}	55.35±1.43^{fg}	38.80±3.50^{bc}	42.32±0.72^{bcde}	3.71±0.47^{a}	13.03±2.15^{def}
BW30s (26, LbA)	44.86±1.33^{cde}	66.73±5.67^{ijk}	40.90±0.98^{bcd}	51.36±4.14^{ghi}	3.96±0.34^{a}	15.37±1.53^{ef}
BW2m (21)	49.06±3.25^{def}	66.21±4.26^{ijk}	44.12±3.26^{cdef}	54.09±0.13^{ghi}	4.95±0.01^{ab}	12.12±4.38^{cde}
BW2m (21, LbA)	50.01±0.73^{ef}	70.52±0.34^{k}	46.88±1.23^{defg}	56.49±4.33^{ij}	3.13±0.50^{a}	14.03±3.99^{def}
BW2m (26)	49.91±2.5^{ef}	63.93±0.62^{hijk}	46.73±2.53^{defg}	51.99±1.48^{ghi}	3.18±0.03^{a}	11.94±0.86^{cde}
BW2m (26, LbA)	46.00±1.02^{cde}	68.76±3.32^{jk}	42.78±0.57bc^{de}	58.9±1.50^{j}	3.22±0.45^{a}	9.85±1.82^{cd}
BM2m (21)	42.30±1.83^{bcd}	63.74±4.50^{hijk}	38.79±2.32^{bc}	51.52±1.78^{ghi}	3.51±0.48^{a}	12.22±2.72^{cde}
BM2m (21, LbA)	44.39±6.73^{cde}	67.33±4.37^{jk}	42.93±5.10b^{cde}	54.24±4.12^{hij}	1.46±1.63^{a}	13.09±0.25^{def}
BM2m (26)	45.87±0.69^{cde}	62.61±2.26^{hij}	42.68±0.39b^{cde}	49.63±4.50^{efg}	3.20±0.30^{a}	12.99±2.23^{def}
BM2m (26, LbA)	45.40±2.38^{cde}	62.45±5.29^{hij}	42.52±2.29b^{bcde}	51.29±2.43^{ghi}	2.88±0.09^{a}	11.15±2.86^{cde}

Legend: see Table 1

In both species, the products blanched in water for 2 min had 5-9% higher TDF compared to the other pre-treatments; and samples with added *L. acidophilus* had slightly higher contents of TDF compared to those without the addition of bacteria. 79-86% of TDF consisted of IDF in *I. badia* and 87-94% in *A. bispous*, with the remaining being SDF. We observed a significant positive influence of processing (added probiotic bacteria and pre-treatment) on TDF content, compared to either mushrooms fermented without any pre-treatment or added probiotic bacteria. Processing had contrary effects on SDF contents of the studied species. Compared to those fermented without any blanching or added probiotic bacteria (F21 and F26), there was an increase of 18-50% of SDF in *I. badia* and a decrease of 30-70% in *A. bisporus*, but this was considered insignificant. *I. badia* proportionally had a higher content of SDF compared to *A. bisporus*. The highest content of SDF was observed in water blanched product for 30 s fermented at 21 °C with LbA was 16.53 g/100 g dry matter. microwave-blanched product fermented at 21 °C with added LbA in *A. bisporus* and water-blanched mushrooms at 26 °C with added LbA in *I. badia* had the lowest SDF content. The total glucan content in *I. badia* was above 2 times higher compared to that of *A. bisporus* (Table 4). An increase in the total glucans was observed in certain products that were blanched or added LbA compared to the products without blanching or added LbA (F21 and F26) in both species. The total glucans in *I. badia* ranged from 23.66-28.02 g/100 g dm and in *A. bisporus* from 9.33-13.59 g/100 g dm. These results were in agreement with those reported by Jaworska et al. (2015) and Sari et al. (2017).

Table 4. Glucans in fermented mushrooms [g/100 g dm]

Sample	α-glucans		β-glucans		Total glucans	
	A. bisporus	*I. badia*	*A. bisporus*	*I. badia*	*A. bisporus*	*I. badia*
F21	1.04±0.01^{abc}	2.84±0.02^{fgh}	9.55±0.06^{a}	23.90±0.38^{bc}	10.59±0.06^{a}	26.74±0.54^{bc}
F26	0.86±0.01^{abc}	3.20±0.03^{gh}	10.42±0.06^{a}	20.46±0.41^{b}	11.29±0.08^{a}	23.66±0.31^{b}
BW30s (21)	1.09±0.04^{abc}	2.45±0.02^{efgh}	12.10±0.12^{a}	22.53±0.09^{bc}	13.19±0.22^{a}	24.98±0.78^{bc}
BW30s (21, LbA)	0.81±0.02^{ab}	2.77±0.02^{efgh}	11.41±0.03^{a}	22.03±0.19^{bc}	12.22±0.16^{a}	24.80±0.63^{bc}
BW30s (26)	0.89±0.02^{abc}	2.44±0.06^{efgh}	11.10±0.01^{a}	24.74±0.23^{bc}	11.99±0.41^{a}	27.18±0.88^{bc}
BW30s (26, LbA)	1.10±0.02^{abcd}	3.20±0.11^{gh}	12.50±0.04^{a}	25.63±0.46^{c}	13.60±0.35^{a}	28.83±1.02^{c}
BW2m (21)	1.75±0.02^{bcde}	2.80±0.03^{efgh}	9.07±0.04^{a}	22.22±0.67^{bc}	10.82±0.07^{a}	25.02±0.75^{bc}
BW2m (21, LbA)	0.92±0.01^{abc}	3.39±0.18^{h}	9.99±0.06^{a}	23.16±0.28^{bc}	10.91±0.07^{a}	26.55±0.93^{bc}
BW2m (26)	1.19±0.03^{abcd}	3.18±0.05^{gh}	10.49±0.06^{a}	25.98±0.84^{c}	11.67±0.24^{a}	29.16±0.29^{c}
BW2m (26, LbA)	1.92±0.04^{cdef}	2.48±0.03^{efgh}	8.70±0.08^{a}	24.93±1.09^{bc}	10.61±0.33^{a}	27.42±0.58^{bc}
BM2m (21)	0.33±0.02^{a}	2.99±0.04^{gh}	9.00±0.08^{a}	25.03±0.37^{bc}	9.33±0.09^{a}	28.02±0.60^{bc}
BM2m (21, LbA)	1.20±0.02^{abcd}	2.67±0.02^{efgh}	10.94±0.04^{a}	23.96±0.84^{bc}	12.15±0.34^{a}	26.63±0.82^{bc}
BM2m (26)	1.19±0.04^{abcd}	2.52±0.02^{efgh}	9.60±0.01^{a}	24.60±0.44^{bc}	10.80±0.15^{a}	27.12±0.48^{bc}
BM2m (26, LbA)	1.02±0.03^{abc}	2.13±0.03^{defg}	11.67±0.18^{a}	23.75±0.36^{bc}	12.69±0.10^{a}	25.88±0.57^{bc}

Legend: see Table 1

Though 2/3rds of the products with added probiotic bacteria and fermented at 26 °C showed slightly higher total glucans content compared to the other products, no significant difference or clear trends were observed.

It was found that 85-92% of the total glucans in both species consisted of β-glucans. There are several studies (Mongkontanawat and Phuangborisut, 2019) that show that blanching increases β-glucan content but decreases α-glucans content. However, the reason for this phenomenon is unknown. Though the products did not differ significantly, in *I. badia,* all products fermented at 26 °C displayed higher β-glucans content compared to those fermented at 21 °C, where microwave blanching was the most favorable to increase β-glucan content; and in *A. bisporus* products blanched in water for 30 s contained slightly higher β-glucans than other products.

Vitamin B_1 and B_2

Studies examining vitamin content in different mushroom species confirmed that the mushrooms contain higher amounts of vitamin B_1 and B_2 (Figures 1 and 2). Though it is known that blanching in water results in loss of water-soluble vitamins, vitamin B_1 and B_2 content of *A. bisporus* and vitamin B_1 content of *I. badia* were higher in water-blanched products compared to those fermented without blanching. This increase in vitamin B content can be the result of production of these vitamins by LAB (LeBlanc et al., 2011). Both vitamin B_1 and B_2 content were generally higher in *A. bisporus* than in *I. badia*. Vitamin B_1 content was ~70% higher in *A. bisporus* (0.65-0.86 mg/100 g dm) than *I. badia* (0.11-0.32 mg/100 g dm).

Additionally, a similar trend was found in vitamin B_2 content, where, in general, the content was higher in *A. bisporus* (0.47-4.27 mg/100 g dm) compared to *I. badia* (1.05-2.64 mg/100 g). The obtained results were in agreement with the studies on vitamin content in mushrooms by Furlani and Godoy (2008) and Mattila et al. (2001). In *A. bisporus*, the highest vitamin B_1 content was observed in mushrooms blanched in water for 2 min and fermented at 26 °C with added LbA. In general, vitamin B_1 content was higher in mushrooms blanched in water for 30 s and 2 min than in microwave blanching in *A. bisporus*. In *I. badia,* water-blanched mushrooms for 2 min and microwave blanching had higher vitamin B_1 content, whereas the highest content was observed in mushrooms blanched in water for 2 min and fermented at 21 °C. In *A. bisporus*, two products blanched in water for 2 min displayed increased vitamin B_2 content of ~88%, compared to their controls (F21 and F26). An opposite trend was found in *I. badia* where mushrooms blanched in water for 2 min had the lowest vitamin B_2 content. However, no clear effects of either temperature of fermentation or addition of LbA were observed on both the contents of vitamin B_1 and vitamin B_2 but the type of blanching had different effects. Furthermore, the type of pre-treatment can be specifically chosen for a particular species to enhance the vitamins B contents in mushrooms.

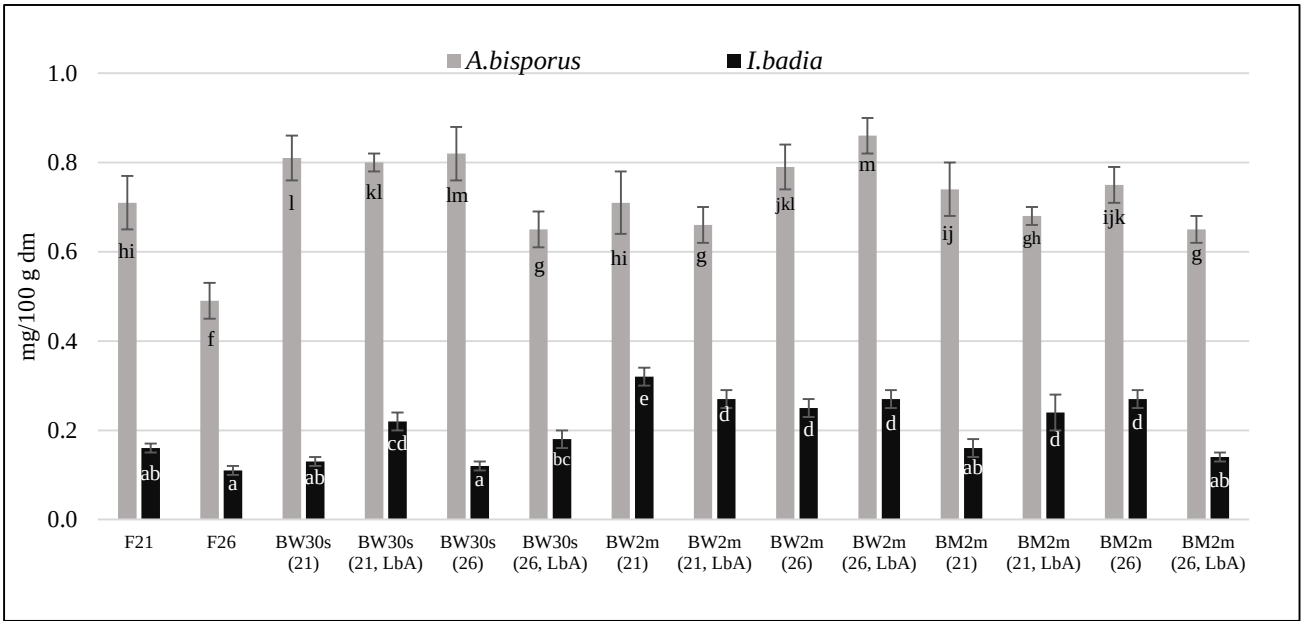

Figure 1. Vitamin B_1 content in fermented mushrooms. Small letters in the graph indicate significantly different samples; F-unblanched, BW30s-blanched in water for 30 s, BW2m-blanched in water for 2 min., BM2m-blanched in microwave for 2 min, LbA-*L. acidophilus* strain LA-5

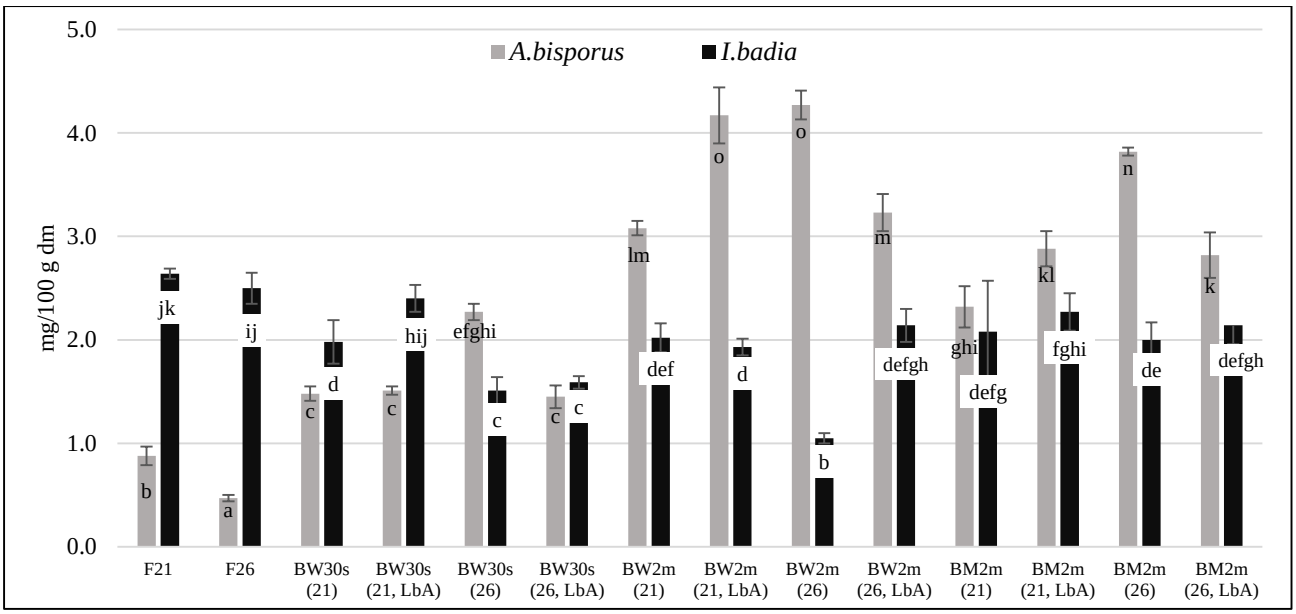

Figure 2. Vitamin B_2 content in fermented mushrooms]. Small letters in the graph indicate significantly different samples; F-unblanched, BW30s-blanched in water for 30 s, BW2m-blanched in water for 2 min., BM2m-blanched in microwave for 2 min, LbA-*L. acidophilus* strain LA-5

Phenol content

Mushrooms have proven to contain high contents of phenolic compounds like cinnamic acid, p-hydroxy-benzoic acid, protocatechuic acid and caffeic acid (Çayan et al., 2020; Mattila et al., 2001). Total phenols in both the species were comparable, but *I. badia* contained slightly higher phenols in most of the products than *A. bisporus* (Table 5). Product from both species fermented without any blanching or added LbA (F21 & F26) contained the highest phenols, which shows that processing has a negative effect on the phenolic content. In *A. bisporus*, microwave-blanched samples contained the lowest amount of all phenol compounds while *I. badia* the highest. A study by Sun et al. (2014) on the effects of different cooking methods on the phenolic contents of 4 species of boletus mushrooms showed that microwaved mushrooms retained the highest content of total phenolics, compared to other methods which agrees with our results.

Table 5. Phenol content in fermented mushrooms [in 100 g fm]

Sample	Total phenols [mg chlorogenic acid]		Total phenylpropanoids [mg caffeic acid]		Total flavonoids [mg quercetin]		Total anthocyanins [mg cyanidin]	
	A. bisporus	*I. badia*	*A. bisporus*	*I. badia*	*A. bisporus*	*I. badia*	*A. bisporus*	*I. badia*
F21	334.3 ± 11.3^{ghi}	463.3 ± 32.9^{l}	42.4 ± 1.0^{fg}	62.0 ± 5.3^{j}	58.7 ± 1.6^{jk}	80.7 ± 5.8^{l}	15.5 ± 0.6^{jk}	25.1 ± 1.7^{n}
F26	375.5 ± 16.4^{j}	494.7 ± 16.2^{m}	47.2 ± 2.6^{hi}	69.8 ± 1.1^{k}	61.1 ± 0.9^{k}	88.0 ± 4.1^{m}	15.9 ± 1.6^{kl}	29.9 ± 1.5^{o}
BW30s (21)	262.3 ± 14.6^{d}	362.4 ± 31.6^{ij}	27.3 ± 2.1^{d}	41.2 ± 3.3^{efg}	40.5 ± 3.2^{e}	52.0 ± 4.8^{fghi}	$6.0\pm1.0b^{cde}$	16.9 ± 1.1^{kl}
BW30s (21, LbA)	312.5 ± 22.0^{efg}	333.1 ± 22.3^{fghi}	36.9 ± 2.8^{e}	40.1 ± 2.6^{efg}	47.0 ± 2.0^{f}	49.7 ± 3.9^{fgh}	7.4 ± 1.0^{de}	14.7 ± 1.2^{ijk}
BW30s (26)	344.2 ± 28.0^{ghij}	354.9 ± 11.0^{hijj}	39.3 ± 3.0^{ef}	42.3 ± 2.2^{fg}	57.0 ± 5.6^{ijk}	54.8 ± 2.7^{hij}	13.6 ± 1.0^{hij}	16.4 ± 1.4^{kl}
BW30s (26, LbA)	352.8 ± 22.8^{hij}	409.6 ± 34.3^{k}	42.7 ± 3.8^{fg}	49.3 ± 4.5^{i}	56.8 ± 5.7^{ijk}	54.9 ± 3.2^{hij}	11.2 ± 2.1^{fg}	19.4 ± 1.9^{m}
BW2m (21)	327.7 ± 19.2^{fgh}	206.9 ± 19.8^{a}	39.5 ± 2.6^{ef}	14.9 ± 1.6^{ab}	54.1 ± 3.0^{hij}	18.5 ± 1.5^{b}	14.8 ± 0.6^{ijk}	5.5 ± 0.6^{abcd}
BW2m (21, LbA)	313.0 ± 22.3^{efg}	291.4 ± 25.2^{e}	36.9 ± 2.9^{e}	25.4 ± 1.8^{cd}	48.3 ± 4.7^{fg}	34.3 ± 2.1^{cd}	11.6 ± 1.9^{fgh}	10.1 ± 1.0^{f}
BW2m (26)	241.0 ± 15.3^{bcd}	228.7 ± 11.0^{abc}	24.2 ± 2.3^{cd}	16.8 ± 1.4^{b}	36.1 ± 3.8^{de}	18.2 ± 0.7^{b}	8.0 ± 1.0^{e}	4.0 ± 0.5^{ab}
BW2m (26, LbA)	301.1 ± 15.6^{ef}	198.9 ± 7.2^{a}	39.5 ± 3.4^{ef}	12.5 ± 0.4^{a}	52.9 ± 5.5^{ghij}	16.1 ± 1.5^{a}	13.0 ± 1.6^{ghi}	3.8 ± 0.5^{a}
BM2m (21)	197.9 ± 17.7^{a}	351.4 ± 19.2^{hij}	17.2 ± 1.6^{b}	40.9 ± 2.7^{efg}	27.1 ± 2.2^{b}	49.5 ± 3.3^{fgh}	6.4 ± 1.5^{cde}	17.8 ± 1.4^{lm}
BM2m (21, LbA)	255.7 ± 19.6^{cd}	363.9 ± 17.8^{ij}	22.8 ± 1.8^{c}	42.5 ± 3.4^{fg}	33.4 ± 3.8^{cd}	53.2 ± 2.7^{ghij}	6.8 ± 1.3^{de}	16.2 ± 1.2^{kl}
BM2m (26)	220.6 ± 17.3^{ab}	486.8 ± 7.9^{lm}	22.1 ± 2.3^{c}	64.9 ± 2.2^{j}	30.2 ± 3.5^{bc}	83.4 ± 3.3^{lm}	4.5 ± 1.3^{abc}	26.0 ± 1.9^{n}
BM2m (26, LbA)	256.2 ± 7.9^{cd}	370.7 ± 31.4^{j}	27.2 ± 1.9^{d}	44.5 ± 2.7^{gh}	36.8 ± 1.7^{de}	56.0 ± 4.5^{ijk}	7.4 ± 1.3^{de}	16.1 ± 1.4^{kl}

Legend: see Table 1

In *A. bisporus* mushrooms blanched in water for 30 s and in *I. badia* microwave-blanched mushroom had the highest content of phenols. There was no significant effect observed due to different temperature of fermentation or the addition of LbA on the contents on total phenols in both the species. The content of total phenyl propanoids, flavonoids and anthocyanins in *A. bisporus* ranged from 17.19-47.21 mg/100 g dm, 27.05-61.16 mg/100g dm and 4.46-15.89 mg/100g dm respectively and in *I. badia* 12.5-69.8 mg/100g dm, 16.1-88.0 mg/100 g dm and 3.8-29.9 mg/100g dm respectively. For comparison Yildiz et al. (2015) showed that different species of mushrooms contain a high level of vanillic acid, protocatechuic acid and p-hydroxybenzoic acid and among 3 other species *Morchella esculenta* had high amounts of chlorogenic acid and quercetin. In turn, Kim et al. (2008) showed that among 10 edible mushroom species *A. bisporus* and *Flammulina velutipes* were regarded as medicinal due to the presence of high contents of phenolic compounds.

Organoleptic analysis

A trained organoleptic panel analyzed all the samples for color, consistency, smell, and taste, and a total score was calculated (Table 6). *A. bisporus* products, which were blanched in water for 2 min, had better color, compared to other pre-treatments. However, the products that were microwave-blanched had the best consistency. There were no trends observed for smell and taste. Among the total scores, the products from *A. bisporus* microwave-blanched scored 5.0/5.0, and the rest of the products scored between 4.4 and 4.8 (Table 6). The type of pre-treatment mostly influenced the overall score of the products from *A. bisporus*. In *I. badia*, the products blanched in water for 2 min scored 5.0/5.0 for the color and also scored the highest 4.5/5.0 for consistency and taste. There were no trends observed for smell. The products with added LbA scored slightly higher than products without LbA, and the products blanched in water for 2 min was the best pre-treatment with a score of 4.4-4.7/5.0. The products that were not blanched or had LbA added scored the least, as well as the other parameters except smell. Hence, for wild mushrooms, *I. badia* blanching was very important to obtain a fermented product with good sensory properties. The addition of LbA and different pre-treatments had different effects on the sensory quality of the products and requires further research on water blanching for 2 min and microwave blanching for 2 min mushrooms. In general, *A. bisporus* had higher scores in all parameters compared to *I. badia*.

Colour and texture

The color difference (ΔE) of *A. bisporus* and *I. badia* products was 1.99 – 11.16 and *I. badia* 5.79-14.20, respectively (Table 6). Products from *I. badia* showed a greater difference in color from their control (F21 and F26) than from *A. bisporus*. In both species, this parameter was the highest in the products blanched in water for 2 min, which showed that blanching had a substantial change on the mushroom's color. In *A. bisporus*, the lowest changes in color were observed for water-blanched products for 30 s and *I. badia* in microwave-blanched products. As the authors' previous research has shown influence on the mushroom colour had PPOs activity (Bernaś, 2018).

Table 6. Selected quality determinant of fermented mushrooms

Sample	General organoleptic assessment (points)		Colour difference (ΔE)		Hardness (N)	
	A. bisporus	*I. badia*	*A. bisporus*	*I. badia*	*A. bisporus*	*I. badia*
F21	5.0	2.9	*	*	11.72 ± 0.66^{d}	1.42 ± 0.25^{a}
F26	5.0	2.9	*	*	13.43 ± 1.23^{e}	1.38 ± 0.26^{a}
BW30s (21)	4.4	3.3	4.60	8.17	16.49 ± 1.28^{g}	2.57 ± 0.48^{a}
BW30s (21, LbA)	4.8	3.4	4.85	9.97	15.29 ± 1.58^{fg}	2.45 ± 0.43^{a}
BW30s (26)	4.4	3.5	8.73	8.43	14.68 ± 1.44^{ef}	2.67 ± 0.37^{a}
BW30s (26, LbA)	4.6	3.5	1.99	7.13	15.52 ± 1.47^{fg}	2.50 ± 0.37^{a}
BW2m (21)	4.4	4.4	8.49	14.20	18.06 ± 1.79^{h}	8.84 ± 1.16^{c}
BW2m (21, LbA)	4.4	4.5	8.66	11.79	15.62 ± 1.25^{fg}	5.27 ± 0.76^{b}
BW2m (26)	4.8	4.4	11.16	13.22	20.76 ± 1.28^{j}	8.00 ± 1.19^{c}
BW2m (26, LbA)	4.4	4.7	4.78	13.47	26.24 ± 2.21^{k}	5.58 ± 0.75^{b}
BM2m (21)	5.0	3.0	6.21	5.71	14.42 ± 1.30^{ef}	4.64 ± 0.59^{b}
BM2m (21, LbA)	5.0	3.1	7.76	10.00	18.22 ± 1.53^{ef}	5.18 ± 1.02^{b}
BM2m (26)	5.0	2.9	6.08	5.79	18.83 ± 1.22^{hi}	4.16 ± 0.56^{b}
BM2m (26, LbA)	5.0	3.5	7.06	8.61	17.41 ± 1.85^{gh}	4.60 ± 0.61^{b}

Legend: see Table 1, * not applicable

Extending the blanching treatment time from 30 s to 2 min probably limited the activity of PPOs, which is why the fermentation process and consequently the colour of the fruit bodies in the case of 30 s blanching was more similar to the colour of unblanched fermented mushrooms.

The addition of LbA or fermentation temperature change did not affect the color. The instrumental analysis of texture showed that overall, *A. bisporus* was significantly harder than *I. badia*. Water-blanched products for 2 min in both species were the hardest, and unblanched products were the softest.

CONCLUSION

The studied mushroom species were determined as suitable for fermentation by reaching a pH< 4.5 in 3 days and were a source of bioactive compounds. Additionally, they were usually characterized by good organoleptic quality, color, and texture. The type of pre-treatment and addition of probiotic bacteria had a significant influence on the change in bioactive compounds and the organoleptic parameters. Both species behaved differently under different fermentation conditions, which proved that mushrooms are a complex food product and that each species differs at the cellular level. Hence, the technological procedure of fermentation must be tailored specifically to each species to maximize the nutritional content, maintaining the best taste and textural properties. Though processing negatively affected the phenol content in both species, blanched products had a higher content of dietary fiber and β-glucan, compared to unblanched mushrooms, and also retained significant contents of vitamin B_1 and B_2. According to our results, for fermented mushrooms to become an everyday-consumable functional food, water blanching for 2 min or microwave-blanching is recommended because they gave the best sensory results with *A. bisporus*.

ACKNOWLEDGMENTS

The study was financed by the Polish Ministry of Science and Higher Education statutory grant.

REFERENCES

1. Alves, M., Ferreira, I. F. R., Dias, J., Teixeira, V., Martins, A., & Pintado, M. (2012). A review on antimicrobial activity of mushroom (basidiomycetes) extracts and isolated compounds. *Planta Medica*, *78*(16), 1707-1718. DOI: 10.1055/s-0032-1315370.
2. AOAC (1985). Association of Official Analytical Chemists. 14th ed., 1st suppl. Secs. 43, A14-43, A20, p.399.
3. AOAC (1986). Association of Official Analytical Chemists. Changes in methods. *Journal of AOAC*, 69, 370.
4. AOAC (1987). Association of Official Analytical Chemists. Changes in methods. *Journal of AOAC*, 70, 393.
5. AOAC (1995). Association of Official Analytical Chemistry. *Official Methods of Analysis*, 16th ed. Arlington, Virginia, USA.
6. AOAC (2012). Association of Official Analytical Chemistry. *Official Methods of Analysis*, 19th ed. Rockville, Maryland, USA.
7. Bach, F., Zielinski, A. A. F., Helm, C. V., Maciel, G. M., Pedro, A. C., Stafussa, A. P., Ávila, S., & Haminiuk, C. W. I. (2019). Bio compounds of edible mushrooms: In vitro antioxidant and antimicrobial activities. *LWT-Food Science and Technology*, *107*, 214-220. DOI: 10.1016/j.lwt.2019.03.017.
8. Bączkowicz, M., Fortuna, T., Juszczak, L., & Sobolewska, J. (2012). Basics of food quality analysis and assessment - edited by T. Fortuna. Publishing House of the University of Agriculture in Krakow. ISBN 978-83-60633-59-5.
9. Barros, L., Falcão, S., Baptista, P., Freire, C., Vilas-Boas, M., & Ferreira, I. C. F. R. (2008). Antioxidant activity of *Agaricus sp*. mushrooms by chemical, biochemical and electrochemical assays. *Food Chemistry*, *111*(1), 61-66. DOI: org/10.1016/j.foodchem.2008.03.033.
10. Bernaś, E. (2018). Comparison of the mechanism of enzymatic browning in frozen white and brown *A. bisporus*. *European Food Research and Technology*, 244(7), 1239-1248. DOI: 10.1007/s00217-018-3039-y.
11. Bernaś, E., & Jaworska, G. (2021). Onion juice and extracts for the inhibition of enzymatic browning mechanisms in frozen *Agaricus bisporus* mushrooms. *Journal of the Science of Food and Agriculture*, *101*(10), 4099–4107. DOI: 10.1002/jsfa.11045.

12. Bernaś, E., Jaworska, G., & Kmiecik, W. (2006). Storage and processing of edible mushrooms. *Acta Scientiarum Polonorum Technologia Alimentaria*, *5*(2), 5-23.
13. Bhakta, M., & Kumar, P. (2013). Mushroom polysaccharides as a potential prebiotics. *International Journal of Health Sciences and Research*, 3(8), 77-84.
14. Bozzetto, L., Costabile, G., Della Pepa, G., Ciciola, P., Vetrani, C., Vitale, M., Rivellese, A. A., & Annuzzi, G. (2018) Dietary fibre as a unifying remedy for the whole spectrum of obesity-associated cardiovascular risk. *Nutrients*, 10(7), 943. DOI: 10.3390/nu10070943.
15. Chen, Z., Gao, H., Wu, W., Chen, H., Fang, X., Han, Y., & Mu, H. (2021). Effects of fermentation with different microbial species on the umami taste of shiitake mushroom (*Lentinus edodes*). *LWT-Food Science and Technology*, *141*, 110889. DOI.org/10.1016/j.lwt.2021.110889.
16. Cheung, L. M., Cheung, P. C. K., & Ooi, V. E. C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*, *81*(2), 249-255. DOI: 10.1016/S0308-8146(02)00419-3.
17. Chye, F. Y., Wong, J. Y., & Lee, J. S. (2008). Nutritional quality and antioxidant activity of selected edible wild mushrooms. *Food Science and Technology International*, *14*(4), 375–384. DOI: 10.1177/1082013208097445.
18. Das, T., Roy, B., & Bhattacharyya, S. (2023). Edible mushrooms and their holistic approach on health. *Archive of Food and Nutrition Science*, *7*, 078-087. DOI: 10.29328/journal.afns.1001053.
19. Dhingra, D., Michael, M., Rajput, H., & Patil, R. T. (2012). Dietary fibre in foods: A review. *Journal of Food Science and Technology*, *49*(3), 255. DOI: 10.1007/s13197-011-0365-5.
20. Dimitrijević, M., Stankov Jovanović, V., Cvetković, J., Mitić, M., Petrović, G., Đorđević, A., & Mitić, V. (2017). Phenolics, antioxidant potentials, and antimicrobial activities of six wild *Boletaceae* mushrooms. *Analytical Letters*, *50*(10), 1691-1709.DOI.org/10.1080/00032719.2016.1242133
21. Elmastas, M., Isildak, O., Turkekul, I., & Temur, N. (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis*, 20(3-4), 337-345. DOI: 10.1016/j.jfca.2006.07.003.
22. Foster, J. A., Lyte, M., Meyer, E., & Cryan, J. F. (2016). Gut microbiota and brain function: An evolving field in neuroscience. *International Journal of Neuropsychopharmacology*, *19*(5), 1–7. DOI: 10.1093/ijnp/pyv114.
23. Fukumoto, L. R., & Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of Agriculture and Food Chemistry*, *48*(8), 3597-3604. DOI: 10.1021/jf000220w.
24. Furlani, R. P. Z., & Godoy, H. T. (2008). Vitamins B_1 and B_2 contents in cultivated mushrooms. *Food Chemistry*, *106*(2), 816-819. DOI: 10.1016/j.foodchem.2007.06.007.
25. Galoburda, R., Kuka, M., Cakste, I., & Klava, D. (2015). The effect of blanching temperature on the quality of microwave-vacuum dried mushroom *Cantharellus cibarius*. *Agronomy Research*, 13(4), 929-938. DOI: 10.1007/s13197-012-0827-4
26. Gebrelibanos, M., Megersa, N., & Taddesse, A. M. (2016). Levels of essential and non-essential metals in edible mushrooms cultivated in Haramaya, Ethiopia. *International Journal of Food Contamination*, *3*(1). DOI: 10.1186/s40550-016-0025-7.
27. Hobbs, C. (2023). The health and clinical benefits of medicinal fungi. *Advances in Biochemical Engineering/Biotechnology*, *184*, 285-356. DOI: 10.1007/10_2023_230.
28. Jabłońska-Rys, E., Sławińska, A., Radzki, W., & Gustaw, W. (2016). Evaluation of the potential use of probiotic strain *Lactobacillus plantarum* 299v in lactic fermentation of button mushroom fruiting bodies. *Acta Sci. Pol. Technol. Aliment.*, *15*(4), 399–407. DOI: 10.17306/J.AFS.2016.4.38
29. Jabłońska-Ryś, E., Sławińska, A., Skrzypczak, K., & Góral, K. (2022a). Dynamics of changes in pH and the contents of free sugars, organic acids and LAB in button mushrooms during controlled lactic fermentation. *Foods*, *11*(11), 1553. DOI: 10.3390/foods11111553.
30. Jabłońska-Ryś, E., Sławińska, A., Skrzypczak, K., Kowalczyk, D., & Stadnik, J. (2022b). Content of biogenic amines and physical properties of lacto-fermented button mushrooms. *Applied Sciences*, *12*(18), 8957. DOI: 10.3390/app12188957.
31. Jaworska, G., Pogoń, K., Skrzypczak, A., & Bernaś, E. (2015). Composition and antioxidant properties of wild mushrooms *Boletus edulis* and *Xerocomus badius* prepared for consumption. *Journal of Food Science and Technology*, *52*(12), 7944-7953. DOI: 10.1007/s13197-015-1933-x.
32. Kozarski, M., Klaus, A., Jakovljević, D., Todorovic, N., Vunduk, J., Petrović, P., Niksić, M., Vrvić, M. M., & Van Griensven, L. (2015). Antioxidants of edible mushrooms. *Molecules*, *20*, 19489-19525. DOI: 10.3390/molecules201019489.
33. Kumari, K. (2020). Mushrooms as source of dietary fiber and its medicinal value: A review article. *Journal of Pharmacognosy and Phytochemistry*, *9*(2), 2075-2078.
34. Kumari, J., & Jaiswal, R. (2023). Scientific research and its approach towards the mushroom production and industrial developments. *International Journal of Home Science*, *9*(1), 157-162.

35. Lan, J. H., Wei, J., Yang, Y. Q., Liu, X. M., Cao, J. F., Quan, C. X., Liu, S. W., Ding, Z. G., & Ding, J. H. (2023). Chemical constituents and antitumor activities of the edible mushroom *Cortinarius rufo-olivaceus*. *Chemistry and Natural Compounds*, *59*(5), 903-905. DOI: 10.1007/s10600-023-04146-w.
36. LeBlanc, J. G., Laiño, J. E., Del Valle, M. J., Vannini, V. V., van Sinderen, D., Taranto, M. P., de Valdez, G.F., de Giori, G.S. & Sesma, F. (2011). B-Group vitamin production by lactic acid bacteria–current knowledge and potential applications. *Journal of Applied Microbiology*, *111*(6), 1297-1309. DOI: 10.1111/j.1365-2672.2011.05157.x.
37. Le Roux, A., Josset, E., Benzina, S., Nadal, B., Desage-El Murr, M., Heurtaux, B., Taran, F., Denis, J.M., Le Gall, T., Meunier, S. and Bischoff, P. (2012). Evaluation of the radioprotective potential of the polyphenol norbadione A. *Letters in Drug Design and Discovery*, *9*(1), 48-53. DOI: 10.2174/157018012798192900.
38. Li, J., Li, S., Wang, A., Chen, J., & Tian, G. (2014). Appropriate water blanching treatment to keep quality and color of *Agaricus bisporus* slices. Transactions of the Chinese Society of Agricultural Engineering, 30(7), 268-273. DOI: 10.3969/j.issn.1002-6819.2014.06.032
39. Lindequist, U., Niedermeyer, T. H. J., & Jülich, W. D. (2005). The pharmacological potential of mushrooms. *Evidence-Based Complementary and Alternative Medicine*, *2*(3), 285–299. DOI: 10.1093/ecam/neh107.
40. Liu, Y., Xie, X. X., Ibrahim, S. A., Khaskheli, S. G., Yang, H., Wang, Y. F., & Huang, W. (2016). Characterization of *Lactobacillus pentosus* as a starter culture for the fermentation of edible oyster mushrooms (*Pleurotus spp*.). *LWT-Food Science and Technology*, *68*, 21-26. DOI: 10.1016/j.lwt.2015.12.008.
41. Maity, P., Sen, I. K., Chakraborty, I., Mondal, S., Bar, H., Bhanja, S. K., Mandal, S. & Maity, G. N. (2021). Biologically active polysaccharide from edible mushrooms: A review. *International Journal of Biological Macromolecules*, *172*, 408-417. DOI: 10.1016/j.ijbiomac.2021.01.081.
42. Manzi, P., Marconi, S., Aguzzi, A., & Pizzoferrato, L. (2004). Commercial mushrooms: nutritional quality and effect of cooking. *Food Chemistry*, *84*(2), 201-206. DOI: 10.1016/S0308-8146(03)00202-4.
43. Mattila, P., Könkö, K., Eurola, M., Pihlava, J.M., Astola, J., Vahteristo, L., Hietaniemi, V., Kumpulainen, J., Valtonen, M. & Piironen, V. (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *Journal of Agriculture and Food Chemistry*, *49*(5), 2343-2348. DOI: 10.1021/jf001525d.
44. Mokrzycki, W. S., & Tatol M. (2011). Colour differenceΔ EA survey. *Machine Graphics and Vision*, *20*(4), 383-411.
45. Mongkontanawat, N., & Phuangborisut, S. (2019). Product development of mushroom beverage with high β-glucan content from local mushrooms. *International Journal of Agricultural Technology*, 15(6), 959-974. ISSN 2630-0192.
46. Mora-Cura, Y. N., Meléndez-Rentería, N. P., Delgado-García, M., Contreras-Esquivel, J. C., Morlett-Chávez, J. A., Aguilar, C. N., & Rodríguez-Herrera, R. (2017). Fermentation of dietetic fiber from green bean and prickly pear shell by pure and mixture culture of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* 450B. *Current Microbiology*, *74*, 691-701. DOI: 10.1007/s00284-017-1228-8
47. Mu, C., Yang, Y., & Zhu, W. (2016). Gut microbiota: The brain peacekeeper. *Frontiers in Microbiology*, *7*, 345. DOI: 10.1155/2024/9341184
48. Muliadi, J., & Anugrahati, N. A. (2022). Utilization of mushroom as the main ingredient of vegan petis with different types of flour. *GCISTEM Proceeding*, 1, 132-142. DOI: 10.56573/gcistem.v1i.2
49. Muszyńska, B., Sulkowska-Ziaja, K., & Ekiert, H. (2013). Phenolic acids in selected edible basidiomycota species: *Armillaria mellea, Boletus badius, Boletus edulis, Cantharellus cibarius, Lactarius deliciosus* and *Pleurotus ostreatus*. *Acta Scientiarum Polonorum. Hortorum Cultus*, *12*(4), 107-116.
50. Nile, S. H., & Park, S. W. (2014). Total, soluble, and insoluble dietary fibre contents of wild growing edible mushrooms. *Czech Journal of Food Science*, *32*(3), 302-307. DOI: 10.17221/226/2013-CJFS
51. Oyetayo, V. O. (2023). Mineral element enrichment of mushrooms for the production of more effective functional foods. *Asian Journla of Biological Sciences*, *16*(1), 18-29. DOI: 10.17311/ajbs.2023.18.29
52. Paranagama, I., Wickramasinghe, I., Somendrika, D., & Benaragama, K. (2022). Development of a vegan sausage with young green jackfruit, oyster mushroom, and coconut flour as an environmentally friendly product with cleaner production approach. *Journal of Microbiology, Biotechnology and Food Sciences, 11*(4), e4029-e4029. DOI: 10.55251/jmbfs.4029
53. PN-ISO 6658. (1998). Polish Committee for Standardization, Sensory Analysis. Methodology. General Guidelines [in Polish].

54. Reguła, J., & Siwulski, M. (2007). Dried shiitake (*Lentinulla edodes*) and oyster (*Pleurotus ostreatus*) mushrooms as a good source of nutrient. *Acta Scientiarum Polonorum. Technologia Alimentaria*, *6*(4), 135-142.
55. Reis, F. S., Martins, A., Vasconcelos, M. H., Morales, P., & Ferreira, I. C. F. R. (2017). Functional foods based on extracts or compounds derived from mushrooms. *Trends Food Science and Technology*, *66*, 48-62. DOI: 10.1016/j.tifs.2017.05.010
56. Roupas, P., Keogh, J., Noakes, M., Margetts, C., & Taylor, P. (2012). The role of edible mushrooms in health: Evaluation of the evidence. *Journal of Functional Foods*, *4*(4), 687-709. DOI: 10.1016/j.jff.2012.05.003
57. Ruthes, A. C., Cantu-Jungles, T. M., Cordeiro, L. M. C., & Iacomini, M. (2021). Prebiotic potential of mushroom d-glucans: Implications of physicochemical properties and structural features. *Carbohydrate Polymers*, *262*, 117940. DOI: 10.1016/j.carbpol.2021.117940
58. Sari, M., Prange, A., Lelley, J. I., & Hambitzer, R. (2017). Screening of beta-glucan contents in commercially cultivated and wild growing mushrooms. *Food Chemistry*, *216*, 45-51. DOI: 10.1016/j.foodchem.2016.08.010
59. Sharma, V., & Mishra, H. N. (2013). Fermentation of vegetable juice mixture by probiotic lactic acid bacteria. *Nutrafoods*, *12*(1), 17-22. DOI: 10.1007/s13749-012-0050-y
60. Sherwin, E., Dinan, T. G., & Cryan, J. F. (2018). Recent developments in understanding the role of the gut microbiota in brain health and disease. *Annals of the New York Academy of Sciences*, *1420*(1), 5–25. DOI: https://doi.org/10.1111/nyas.13416
61. Sun, L., Bai, X., & Zhuang, Y. (2014). Effect of different cooking methods on total phenolic contents and antioxidant activities of four *Boletus* mushrooms. *Journal of Food Science and Technology, 51*(11), 3362–3368.
62. Üstün, N. Ş., Bulam, S., & Pekşen, A. (2018). The use of mushrooms and their extracts and compounds in functional foods and nutraceuticals. *Türkmen, A.(ed.)*, 1, 1205-1222. ISBN: 978-975-2481-10-7
63. Valverde, M. E., Hernández-Pérez, T., & Paredes-López, O. (2015). Edible mushrooms: Improving human health and promoting quality life. *International Journal of Microbiology*, Article Id:376387. DOI: 10.1155/2015/376387
64. Vunduk, J., Djekic, I., Petrović, P., Tomašević, I., Kozarski, M., Despotović, S., Nikšić, M., & Klaus, A. (2018). Challenging the difference between white and brown *Agaricus bisporus* mushrooms: Science behind consumers choice. *British Food Journal*, *120*(6), 1381-1394.
65. Walde, S. G., Velu, V., Jyothirmayi, T., & Math, R. G. (2006). Effects of pretreatments and drying methods on dehydration of mushroom. *Journal of Food Engineering*, *74*(1), 108-115. DOI: 10.1016/j.jfoodeng.2005.02.008
66. Wasser, S. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, *60*(3), 258-274. DOI: 10.1007/s00253-002-1076-7
67. Wennberg, M., Ekvall, J., Olsson, K., & Nyman, M. (2006). Changes in carbohydrate and glucosinolate composition in white cabbage (*Brassica oleracea* var. *capitata*) during blanching and treatment with acetic acid. *Food Chemistry*, *95*(2), 226-236. DOI: 10.1016/j.foodchem.2004.11.057
68. Worku, K. F., Kurabachew, H., & Hassen, Y. (2019). Probiotication of fruit juices by supplemented culture of *Lactobacillus acidophilus*. *International Journal of Food Science and Nutrition Engineering*, *9*, 45-48. DOI: 10.5923/j.food.20190902.03
69. Yari, M., Fooladi, J., & Motlagh, M. A. K. (2015). Microencapsulation and fermentation of *Lactobacillus acidophilus* LA-5 and Bifidobacterium BB-12. *Applied Food Biotechnology*, *2*(4), 27-32. DOI: 10.1002/fsn3.2470
70. Zhang, Y., Ma, G., Fang, L., Wang, L., & Xie, J. (2014). The Immunostimulatory and anti-tumor activities of polysaccharide from *Agaricus bisporus* (brown). *Journal of Food and Nutrition Research*, *2*(3), 122-126. DOI: 10.12691/jfnr-2-3-5
71. Zheng, H. G., Chen, J. C., & Ahmad, I. (2018). Preservation of king oyster mushroom by the use of different fermentation processes. *Journal of Food Processing and Preservation*, *42*(1), e13396. DOI: 10.1111/jfpp.13396
72. Zięba, P., Sękara, A., Bernaś, E., Krakowska, A., Sułkowska-Ziaja, K., Kunicki, E., Suchanek, M., & Muszyńska, B. (2021). Supplementation with magnesium salts - a strategy to increase nutraceutical value of *Pleurotus djamor* fruiting bodies. *Molecules*, 26(11), 327. DOI: 10.3390/molecules26113273