

EVALUATION OF THE POTENTIAL USE OF PROBIOTIC STRAIN *LACTOBACILLUS PLANTARUM* 299V IN LACTIC FERMENTATION OF BUTTON MUSHROOM FRUITING BODIES

Ewa Jabłońska-Ryś✉, Aneta Sławińska, Wojciech Radzki, Waldemar Gustaw

Department of Fruits, Vegetables and Mushrooms Technology, University of Life Sciences in Lublin
Skromna 8, 20-704 Lublin, Poland

ABSTRACT

Background. The available literature does not provide data on the application of probiotic strains in mushroom processing. The aim of the study was to evaluate the potential to use the *L. plantarum* 299v strain with documented probiotic properties in the process of lactic fermentation of button mushroom fruiting bodies (*Agaricus bisporus*).

Materials and methods. Fresh button mushroom fruiting bodies and cultures of lactic acid bacteria *L. plantarum* Ib and a probiotic strain *L. plantarum* 299v were the material analysed. Sensory evaluation was performed with a 5-point scale, an instrumental method of colour measurement based on the CIA $L^*a^*b^*$ scale, total phenolic compounds were determined with the Folin method, antioxidant properties were assayed with the DPPH radical test, and reducing power was determined using the FRAP method.

Results. After a week-long lactic fermentation, the pH value in the samples declined to a level of 3.6 (*L. plantarum* Ib) and 3.75 (*L. plantarum* 299v); these values persisted or decreased slightly during the period of maturation of the fermented samples under refrigeration. Fermented mushrooms were assigned high grades in the organoleptic evaluation. The colour analysis revealed significant changes in the values of the $L^*a^*b^*$ parameters in the fermented product, in comparison with fresh mushrooms. Blanching contributed to a significant decrease in the content of total phenolic compounds in the mushroom fruiting bodies and to a decline in antioxidant activity. Mushrooms fermented with the probiotic strain were characterised by higher phenolic compound content and higher antioxidant activity.

Conclusion. *L. plantarum* 299v strain with documented probiotic properties can be applied in fermentation of button mushroom fruiting bodies. Products obtained with the use of both strains were characterised by good sensory properties. The type of strain used in the lactic fermentation of mushroom fruiting bodies had an effect on the phenolic compound content and antioxidant properties of the final product.

Key words: *Agaricus bisporus*, fermented mushroom, probiotic bacteria, phenolic compound, antioxidant activity, colour

INTRODUCTION

Mushrooms are consumed primarily for their taste. However, there is a growing interest in their health-enhancing or medicinal properties associated with their chemical composition (Rajewska and Bałasińska,

2004). Mushrooms should be eaten or processed immediately after harvesting, as then they have the highest nutritional value. The oldest method of mushroom processing is drying. Other popular methods include

✉ ewa.jablonska-rys@up.lublin.pl, phone/fax +48 81 462 3305

freezing and producing marinades and sterilized mushrooms canned in brine (Bernaś et al., 2006). Formerly, using table salt at a concentration of 15–25% was a popular method for preserving mushrooms; in some Asian and European countries, including Poland, fermentation was a traditional method. The popularity of this method is evidenced in the *Kuchnia Polska* cookbook (compiled in 1977). Red pine mushrooms (*Lactarius deliciosus*), boletus (*Boletus edulis*), slippery jack mushrooms (*Suillus luteus*), and some mushrooms belonging to the genus *Tricholoma* were recommended for fermentation. The method was used not only in households but also on an industrial scale. In a comprehensive monograph on mushroom processing, Mering (1955) describes the technology of lactic fermentation thereof. As shown by the author, red pine mushrooms were usually fermented on an industrial scale in Poland, and the method was most popular in the countries of the former Soviet Union and Czechoslovakia. Mushrooms were fermented in open casks or, on a smaller scale, in 200 L oak barrels. Red pine mushrooms were fermented raw, while other species were blanched. The fermentation mixture contained 1.5% salt and 1.5% sugar, as well as an inoculum of 0.5 L of sour milk per 100 kg of mushrooms. Similar formulations can be found in the very few scientific publications available.

Mushrooms contain low amounts of total soluble sugars. Tsai et al. (2007) found that mannitol was the major soluble sugar in fresh fruiting body of *Agaricus bisporus*. Glucose represented the second highest proportion with its contents in the range of 17.6–28.1 mg/g dry matter. The fruiting body of button mushrooms also contains fructose (0.62–6.02 mg/g dry matter), lactose (0–3.8 mg/g dry matter), sucrose (0.41–1.48 mg/g dry matter), and trehalose (0.88–5.31 mg/g dry matter). Additionally, lactic acid bacteria, including probiotic strains, exhibit varied degrees of the utilisation of these carbon sources (Hedberg et al., 2008). Available sources recommend the addition of sugar in the fermentation process (Jabłońska-Ryś and Sławińska, 2012; Jabłońska-Ryś et al., 2005; Jabłońska-Ryś et al., 2016; Joshi et al., 1996; Mering, 1955; Milanović et al., 2010; Niksic et al., 1997; Skapska et al. 2008; Stojanovic et al., 1994); only Kreß and Lelley (1991) carried out lactic fermentation of mushrooms without the addition of sugar. The majority of authors indicate

the need for the brief cooking of mushrooms prior to the fermentation process and the use of starter cultures in the natural form of supplementation with sour milk (Mering, 1955) or sauerkraut juice (Kreß and Lelley, 1991) or in the form of the addition of lactic acid bacterial strains (Jabłońska-Ryś and Sławińska, 2012; Jabłońska-Ryś et al., 2005; Jabłońska-Ryś et al., 2016; Joshi et al., 1996; Milanović et al., 2010; Niksic et al., 1997; Skapska et al., 2008).

Probiotic strains are currently used in dairy products. Probiotic LAB have also been applied in non-dairy probiotic beverages (Prado et al., 2008; Yoon et al., 2004; Yoon et al., 2005; Yoon et al., 2006) and other plant-derived products, e.g. fermented oatmeal gruel (Molin, 2001). There are also reports of the probiotic strains application as starter cultures for fermented sausage (Rubio et al., 2013) or other meat products (Neffe-Skocińska et al., 2015; Stadnik and Dolatowski, 2015).

The lack of literature data on the use of probiotic strains in mushroom processing has prompted investigations into the potential application of the *Lactobacillus plantarum* 299v strain with documented probiotic properties in the process of lactic fermentation of button mushroom fruiting bodies. This research involved assessing the course of the fermentation process based on observation of the pH parameter, organoleptic assessment of the products, colour analysis, and evaluating the total phenolics content and antioxidant activity of raw material, intermediate products, and final products.

MATERIALS AND METHODS

Materials

The study material was fruiting bodies of the button mushroom *Agaricus bisporus* purchased from a producer and intended for further processing immediately after harvest (maximum after 4 h). The starter cultures were two strains of lactic acid bacteria (LAB), *Lactobacillus plantarum* Ib (obtained from the culture collection held by the Department of Biotechnology, Human Nutrition and Food Commodity Science, University of Life Sciences in Lublin, Poland), used in previous investigations (Jabłońska-Ryś et al., 2005; Jabłońska-Ryś and Sławińska, 2012; Jabłońska-Ryś et al., 2016) and strain *Lactobacillus plantarum* 299v

with documented probiotic properties (Probi AB, Lund, Sweden). LAB were propagated twice in MRS broth (Biocorp, Warsaw, Poland) and incubated (TK-2, Cabrolab, Warsaw, Poland) overnight at 30°C. After centrifugation (MPW 350-R, MPW, Warsaw, Poland) at 1400×g for 10 min, microbial cells were harvested and washed twice in sterile 0.9% NaCl (P.O.Ch., Gliwice, Poland) before inoculation.

Preparation of fermented mushrooms

Fruiting bodies with a diameter of 3.5–4.5 cm with stipes trimmed short were chosen for the fermentation process. The mushrooms were cleaned thoroughly to remove substratum debris, washed, and blanched in boiling water for 2 min. The fermentation procedure was based on a previous study (Jabłońska-Ryś et al., 2016). After cooling, 2% (w/w) NaCl and 1% (w/w) sucrose were added. Additionally, one experimental combination was supplemented with pepper, crushed bay leaves, and onion slices in the following amounts: 0.1, 0.2, and 5%, respectively. The material prepared in this way was left for 2 hours to obtain juice. Next, starter cultures (*L. plantarum* Ib or probiotic *L. plantarum* 299v) were inoculated into the mushrooms to achieve an initial level of 10⁷ cfu/g of the product. The mushrooms were mixed with inocula and placed tightly (to remove air) in 500 mL glass jars. Following this, 70 mL of a 2% salt solution was added and the jars were then closed. Lactic fermentation proceeded for 8 days at 21–22°C, after which the fermented mushrooms were stored at 5°C for 5 weeks for maturation to proceed. After 43 days of the experiment, fermented mushroom samples were collected for analysis. The fermentation process was performed in triplicate.

The following mushroom samples were analysed as part of the research: FM – fresh mushrooms, BM – blanched mushrooms, LP – mushrooms fermented using *L. plantarum* Ib, PLP – mushrooms fermented using probiotic *L. plantarum* 299v, LPS – mushrooms fermented using *L. plantarum* Ib with spices. Colour analysis was carried out immediately after the washing process (WM sample – washed mushrooms).

Determination of pH

Evolution of pH was monitored at days 0, 1, 2, 3, 4, and 8 of fermentation and after 15 and 43 days of refrigerated storage.

Microbiological analyses

Analyses were performed on the homogenized product (mushroom fruiting bodies with brine) on the first day (initial level of LAB) and final day of the experiment (the content of LAB in the final product). The count of lactic acid bacteria was determined using the plate method on MRS agar (PN-ISO 15214:2002).

Analysis of dry weight

Calculations of total phenolic contents and antioxidant activities were performed on a dry-weight (DW) basis. Mushroom samples were dried in a laboratory dryer (SML 32/250, Zalmed, Warsaw, Poland) at 105°C until a constant weight was reached.

Analysis of total phenolic contents and antioxidant activities

Mushroom sample preparation. The extraction procedure, analysis of total phenolic compounds and determination of antioxidant activities were based on a method described by Jabłońska-Ryś et al. (2016) with some modifications. Mushroom samples were frozen at –20°C for 24 h and then lyophilized in an Alpha 1–2 LD plus freeze-dryer (Martin Christ, Osterode am Harz, Germany) for 72 h. The condenser temperature was set at –60°C, the vacuum was maintained at 0.8 mbar, and the shelf temperature was set at 25°C. The lyophilized materials were ground (Społem WŻ-1 mill, Warsaw, Poland) and then 1 g of the mushroom sample was subjected to extraction using 30 mL of 80% (v/v) ethanol in a shaker (Elpan 357, Elpan, Lubawa, Poland) at 80°C and 175 rpm for 1 h. The extracts obtained were centrifuged (MPW 350-R, MPW, Warsaw, Poland) at 4800×g for 15 min and then used for analysing the total phenolic compounds and determining antioxidant activities.

Determination of total phenolic contents. Ethanol extract samples of 0.2 mL were mixed with 0.8 mL of Folin-Ciocalteu reagent (P.O.Ch., Gliwice, Poland) previously diluted with water to 1:10 (v/v). After 3 min, 1.25 mL of 7% Na₂CO₃ (P.O.Ch., Gliwice, Poland) was added. The mixture was vortexed (TK3S, Kartell, Noviglio, Italy) for 15 s and allowed to stand in the dark for 30 min. The absorbance was then measured at 765 nm using a Helios Gamma apparatus

(Thermo Fisher Scientific, Waltham, Massachusetts, USA). Results were expressed as mg of gallic acid equivalents (GAE) per 1 g of mushrooms on a dry-weight (DW) basis.

Determination of DPPH radical-scavenging activities. Each 0.2 mL ethanol extract was mixed with 0.8 mL of a 0.2 mM DPPH ethanol solution. The mixture was shaken (TK3S, Kartell, Noviglio, Italy) for 15 seconds and left to stand for 15 minutes in the dark. The absorbance was measured at 520 nm using Helios Gamma apparatus (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Antioxidant activity was expressed as μmol of Trolox equivalents (TE) per 1 g of mushrooms (DW).

Determination of ferric reducing antioxidant power (FRAP). FRAP reagent was prepared by mixing a 300 mM acetate buffer at pH 3.6 with a 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) (Sigma-Aldrich, Poznań, Poland) solution (10 mM TPTZ in 40 mM HCl), and a 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution at a 10:1:1 ratio. Each 0.1 mL ethanol extract was mixed with 1.9 mL of FRAP reagent. After 15 min of incubation (water bath W610, LABOPLAY, Bytom, Poland) at 37°C in the dark, the absorbance was measured at 593 nm using Helios Gamma apparatus (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Results were reported as μmol of Trolox equivalents (TE) per 1 g of mushrooms (DW).

Sensory analysis. The analysis of sensory parameters (fragrance, colour, taste, texture) of fermented mushrooms was scored on a 5–1 scale (5 = excellent, 4 = very good, 3 = good, 2 = bad, 1 = very bad) by a panel of seven judges.

Colour analysis. The L^* (whiteness or darkness), a^* (greenness or redness), b^* (blueness or yellowness) colour values of mushrooms were determined using a X-Rite Color® Premiere 8200 spectrophotometer (X-Rite Incorporated, USA). Measurements were performed using a D65 light source and a standard colorimetric observer with a 10° field of view. The colorimeter was calibrated with respect to the white pattern. Measurements were carried out on the top layer of the mushroom caps; colour analysis was performed in 15 replicates.

Statistical analysis. The results were recorded as mean values \pm standard deviation (SD) and compared using a one-way analysis of variance (ANOVA) at $p < 0.05$ with STATISTICA 9 (StatSoft, Kraków, Poland). Tukey's test was carried out to compare the data. Correlations between polyphenol contents and antioxidant activities were calculated using Office Excel 2007.

RESULTS AND DISCUSSION

pH and microbial quality

After a 1-week lactic fermentation process, the pH value declined to a level of 3.6 (*L. plantarum* Ib) and 3.75 (*L. plantarum* 299v); these values persisted or decreased slightly during the maturation period of the fermented samples under refrigeration storage (Fig. 1). The pH value of fermented foods is an important factor for extending product shelf life and microbial safety. Therefore, it is important to obtain a rapid and large reduction in the pH value in fermented mushrooms. At 5°C, the fermented mushrooms were stable for 5 weeks with no sign of microbial spoilage, after which the mushroom samples were collected for the final analysis. The count of lactic acid bacteria in the final products (43 days after setting up the experiment) was 5.5×10^8 cfu/g, 9.2×10^7 cfu/g, and 4.0×10^8 cfu/g in mushrooms fermented using *L. plantarum* Ib, *L. plantarum* 299v and *L. plantarum* Ib with

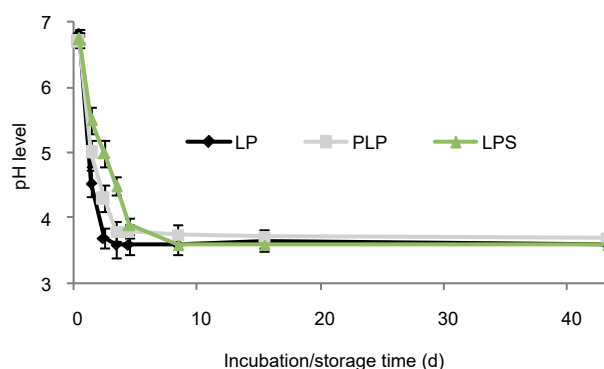


Fig. 1. Evolution of pH values of fermented mushrooms: LP – mushrooms fermented using *L. plantarum* Ib, PLP – mushrooms fermented using probiotic *L. plantarum* 299v, LPS – mushrooms fermented using *L. plantarum* Ib with spices

spices, respectively. In the investigations conducted by Skąpska et al. (2008), the count of inoculated LAB was 7.3 log jkt/g. After 4 days, the count of fermentation bacteria increased to over 9 log jkt/g, and subsequently decreased to c. 8 log jkt/g in the 7-week storage period.

Total phenolic contents and antioxidant activities

The total phenolic contents in mushrooms are shown in Figure 2. In fresh mushroom fruiting bodies, the total phenolic content was 4.87 mg of GAE/g DW. The blanching process contributed to a significant ($p < 0.05$) decrease in phenolic contents in the fruiting bodies of button mushrooms to a level of 3.47 mg of GAE/g DW. The lactic fermentation process exerted a varied effect on the content of these compounds, depending on the strain used. A significant decrease in the content of phenolic compounds was observed in the samples supplemented with the *L. plantarum* Ib strain. In turn, the sample fermented with the probiotic strain exhibited a slight, but not statistically significant, increase in this parameter, compared with the blanched mushrooms (3.55 mg of GAE/g DW).

Two methods were used for evaluation of the antioxidant activities of fresh, blanched, and fermented button mushrooms. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method is based on neutralization of a stable

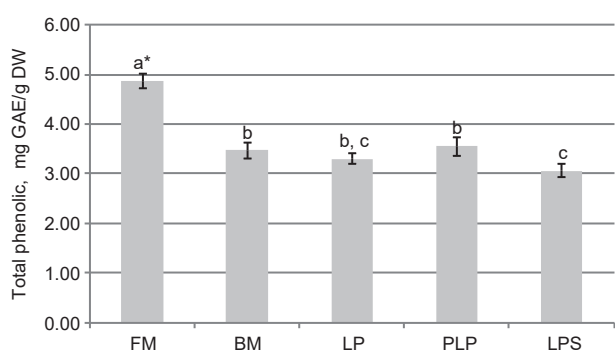


Fig. 2. Total phenolic contents: FM – fresh mushrooms, BM – blanched mushrooms, LP – mushrooms fermented using *L. plantarum* Ib, PLP – mushrooms fermented using probiotic *L. plantarum* 299v, LPS – mushrooms fermented using *L. plantarum* Ib with spices. *Different letters denote statistically significant ($p < 0.05$) differences

DPPH radical by antioxidants present in fermented mushrooms. The ferric reducing antioxidant power (FRAP) method was used for measurement of the reducing activity of the mushrooms. The results are shown in Figure 3 and 4. Fresh fruiting bodies were characterised by the highest antioxidant activity, i.e. 26 $\mu\text{mol TE/g DW}$ and 16.25 $\mu\text{mol TE/g DW}$, respectively, for the FRAP and DPPH methods. Blanching caused a significant ($p < 0.05$) decrease in antioxidant activities to the levels of 17.16 $\mu\text{mol TE/g DW}$ and

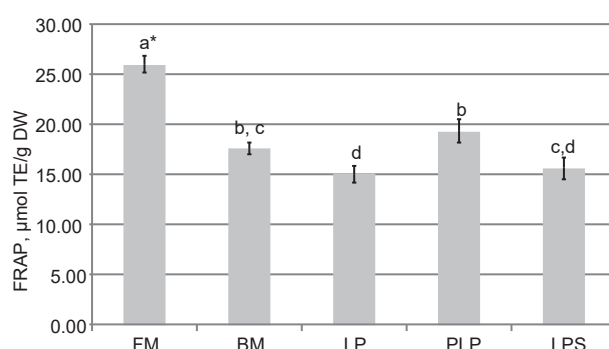


Fig. 3. The ferric-reducing antioxidant power (FRAP): FM – fresh mushrooms, BM – blanched mushrooms, LP – mushrooms fermented using *L. plantarum* Ib, PLP – mushrooms fermented using probiotic *L. plantarum* 299v, LPS – mushrooms fermented using *L. plantarum* Ib with spices. *Different letters denote statistically significant ($p < 0.05$) differences

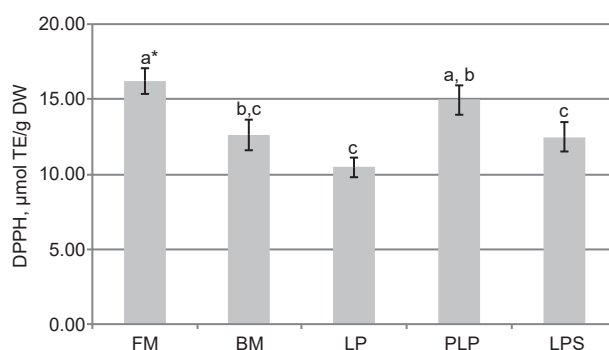


Fig. 4. Scavenging activities against DPPH radicals: FM – fresh mushrooms, BM – blanched mushrooms, LP – mushrooms fermented using *L. plantarum* Ib, PLP – mushrooms fermented using probiotic *L. plantarum* 299v, LPS – mushrooms fermented using *L. plantarum* Ib with spices. *Different letters denote statistically significant ($p < 0.05$) differences

12.64 $\mu\text{mol TE/g DW}$ for the FRAP and DPPH methods, respectively. As in the case of phenolic compound content, the lactic acid fermentation process had a varied effect on antioxidant properties. In both methods, the highest values were found for samples fermented with the probiotic strain, and the values were lower than for the blanched samples. There were no significant differences in the phenolic compound content and antioxidant activity between samples fermented with the *L. plantarum* Ib strain with or without the addition of spices.

Strong correlations were obtained between the total phenolic content and the ferric-reducing antioxidant power, and between the total phenolic content and the scavenging activity determined using the DPPH radical ($R = 0.972$ and $R = 0.783$, respectively).

The available literature provides many reports of the phenolic compound content and antioxidant activity in fresh and blanched mushrooms fruiting bodies, while there are few such data for fermented mushrooms. Similar contents of phenolic compounds to those presented in this paper were reported by Bernas et al. (2006), Palacios et al. (2011), and Jaworska et al. (2015), i.e. 2.9–5.4 mg/g DW, ca. 3.5 mg/g DW, and 4.83 mg/g DW, respectively. In their investigations of wild and cultivated white button mushrooms, Tajalli et al. (2015) showed a wide variation in the content of phenolic compounds ranging from 3.61 to 9.61 mg GAE/g DW. The values obtained in studies conducted by other authors are also substantially higher, i.e. from 8 to 12.3 mg/g DW (Dubost et al., 2007; Ghahremani-Majd and Dashti, 2015; Skąpska et al., 2008). Skąpska et al. (2008) reported that blanching button mushrooms caused a 60% decrease in total polyphenol content and a 54% decrease in antioxidant activities, with further decreases noted during ongoing fermentation. However, after 3 weeks of refrigerated storage, a rising tendency was found. The research carried out by Jabłońska-Ryś et al. (2016) concerning the assessment of selected quality parameters of fermented oyster and chanterelle mushrooms indicated that heat treatment preceding the fermentation process had the greatest influence on the level of antioxidant activity and phenolic compound content.

The decrease in the content of phenolic compounds and the reduction of antioxidant activity noted in the blanching process can be caused by the impact of

high temperature and leaching of water-soluble compounds. Heating during cooking can destroy the chemical structures of polyphenols and can cause a decrease in the antioxidant activities of mushrooms (Barros et al., 2007). In turn, the possible increase in these values observed in some fermented samples can be explained by enzymatic hydrolysis run by LAB enzymes, which may convert bound phenolic compounds to the free form, thereby increasing their extraction rate.

Sensory analysis

Among the four parameters, fermented mushroom texture received the highest scores; irrespective of the experimental combination, this characteristic was scored 5 (Fig. 5). The highest scores for aroma and taste were assigned to the experimental combination with the spices; mushrooms fermented without these additives were evaluated similarly, but slightly higher scores were given to the sample fermented with the *L. plantarum* Ib strain. In terms of colour scores, samples fermented with the probiotic strain received a higher score (4.5) than the samples with *L. plantarum* Ib (4.3). Interestingly, the addition of the spices

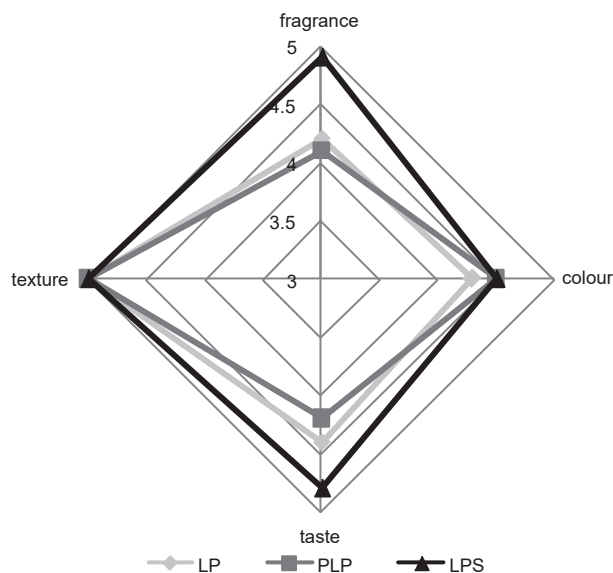


Fig. 5. Sensory analyses: LP – mushrooms fermented using *L. plantarum* Ib, PLP – mushrooms fermented using probiotic *L. plantarum* 299v, LPS – mushrooms fermented using *L. plantarum* Ib with spices

contributed to the increase in the score to 4.5, which was also reflected in the results of the instrumental colour analysis, where the value of parameter L^* , despite the lack of statistically significant differences, was highest in the combinations with the probiotic strain (PLP) and addition of the spices (LPS). This may be explained by the bleaching properties of onion. Bernaś and Jaworska (2015) reported that an aqueous onion extract inhibited enzymatic browning of *A. bisporus* fruiting bodies and had a beneficial effect on the colour of mushrooms during 8-month frozen storage.

Colour of mushrooms

The results of analysis of the colour of fresh and processed mushrooms are shown in Table 1. Lightness is the most common parameter used in the literature for assessment of mushroom colour. The full intensity range of lightness expressed as the L^* value is 0–100 units, where 0 (darkness) represents total darkness and 100 (whiteness) represents bright white. The L^* value was highest for the fresh samples of button mushrooms (93.23). Compared with literature data, the value is high. Similar results in the range of 90 units are only reported by Xiangyou et al. (2014). Gałązka-Czarnecka and Krala (2009) report a value of the L^* parameter of 81.7 for fresh mushrooms, and

Bernaś et al. (2006) describe values in the range of 83–87. It should be emphasised that our mushrooms were purchased directly from the manufacturer, which allowed rapid processing and analyses of fresh material immediately after harvest. Colour is a very important parameter of mushroom quality, indicating their freshness. At room temperature, browning of fruiting bodies takes place within a few hours, as a result of enzymatic degradation of phenols to quinones, which condense to dark melanins (Damięcka and Szudyga, 2006).

The process of washing contributed to a significant decrease in lightness (88.74), and blanching resulted in a further statistically significant decrease in the value of the L^* parameter to the level of 76.06. The deterioration in colour lightness in mushrooms caused by washing and blanching may reach values between 9 to even 26% (Gałązka-Czarnecka and Krala, 2009; Jaworska et al., 2008).

In the fermented samples, a slight increase in the value of the L^* parameter was observed; however, this increase was not statistically significant. Similarly, no significant differences between the products obtained with the application of the different strains were observed.

The fresh mushrooms were characterised by the lowest value of the a^* parameter, i.e. redness (0.57), which was highest in the washed (3.12) and blanched (2.22) mushrooms. The fermentation process contributed to a significant reduction, to the level of 1.74–2.03, in the proportion of redness in the colour structure. The type of the strain used had no statistically significant effect on the differences in the values of the parameter. Fresh mushrooms were also characterised by the lowest value of the b^* parameter, i.e. yellowness (12.75). This value increased to 16.16 after the washing process and 18.46 after blanching. The fermentation process caused a further significant increase in the value of this parameter to the level of 23.24–25.5. Again, the type of the strain applied had no statistically significant effect on the differences in the value of the parameter.

Table 1. Colour analysis

Samples	Value of colour parameters		
	L^*	a^*	b^*
FM	93.23 ±1.05 ^a	0.57 ±0.48 ^c	12.75 ±1.41 ^c
WM	88.74 ±3.40 ^b	3.12 ±1.37 ^a	16.16 ±2.42 ^b
BM	76.06 ±1.76 ^c	2.22 ±1.23 ^{ab}	18.46 ±1.15 ^b
LP	76.89 ±2.37 ^c	2.03 ±0.94 ^{ab}	23.75 ±2.85 ^a
PLP	77.43 ±1.26 ^c	1.74 ±0.99 ^{bc}	25.50 ±1.55 ^a
LPS	77.55 ±1.97 ^c	1.85 ±0.58 ^b	23.24 ±2.45 ^a

FM – fresh mushrooms, WM – washed mushrooms, BM – blanched mushrooms, LP – mushrooms fermented using *L. plantarum* Ib, PLP – mushrooms fermented using probiotic *L. plantarum* 299v, LPS – mushrooms fermented using *L. plantarum* Ib with spices.

In each column, different letters denote statistically significant ($p < 0.05$) differences.

CONCLUSION

In summary, our results showed that the *L. plantarum* 299v strain with documented probiotic properties

can be used in the process of fermentation of button mushroom fruiting bodies. The pH in the final product reached a level below 4, which ensured long shelf life and microbiological quality as well as a LAB count of 9.2×10^7 cfu/g. Mushrooms fermented with the probiotic strain exhibited significantly higher contents of phenolic compounds and higher antioxidant activity in comparison with mushrooms fermented with the *L. plantarum* Ib strain. The product was also characterised by good sensory qualities.

REFERENCES

- Barros, L., Baptista, P., Correia, D. M., Morais, J. S., Ferreira, I. C. F. R. (2007). Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. *J. Agric. Food Chem.*, 55, 4781–4788.
- Bernaś, E., Jaworska, G. (2015). Use of onion extract to prevent enzymatic browning of frozen *Agaricus bisporus* mushrooms. *Int. J. Refrig.*, 57, 257–264.
- Bernaś, E., Jaworska, G., Kmiecik, W. (2006). Storage and processing of edible mushrooms. *Acta Sci. Pol., Technol. Aliment.*, 5, 2, 5–26.
- Damięcka, J., Szudyga, K. (2006). Jakość owocników pieczarki dla przetwórstwa a sposób uprawy [Quality of mushroom (*Agaricus bisporus*) fruit bodies for processing and the way of its cultivation]. *Przem. Spoż.*, 12, 38–40 [in Polish].
- Dubost, N. J., Ou, B., Beelman, R. B. (2007). Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chem.*, 105, 727–735.
- Gałązka-Czarnecka, I., Krala, L. (2009). Wpływ blanszowania na jakość mrożonych pieczarek *Agaricus bisporus* Sing. [The influence of blanching on white mushrooms *Agaricus bisporus* Sing. quality]. *Chłodnictwo*, 44, 6, 56–59 [in Polish].
- Ghahremani-Majd, H., Dashti, F. (2015). Chemical composition and antioxidant properties of cultivated button mushrooms (*Agaricus bisporus*). *Hort. Environ. Biotechnol.*, 56, 3, 376–382.
- Hedberg, M., Hasslöf, P., Sjöström, I., Twetman, S., Stecksén-Blicks, C. (2008). Sugar fermentation in probiotic bacteria – an in vitro study. *Oral Microbiol. Immunol.*, 23, 482–485.
- Jabłońska-Ryś, E., Kalbarczyk, J., Sztaba, A. (2005). Zastosowanie kultur starterowych bakterii mlekowych i propionowych w procesie kwaszenia owocników pieczarki [The use of starter cultures of lactic and propionic acid bacteria in the process of button mushrooms fermentation]. Abstracts: 5th Jubilee Scientific Conference, Food quality and safety: determinants of raw materials, technology, manufacturing and legal. 17–18 November, Białobrzegi, Poland, 23–24 [in Polish].
- Jabłońska-Ryś, E., Sławińska, A. (2012). Possibilities of use of lactic fermentation in bio conservation of edible mushrooms fruit bodies. Abstracts: 2th International Conference and Workshop, Plant – the source of research material. 18–20 October, Lublin, Poland.
- Jabłońska-Ryś, E., Sławińska, A., Szwajgier, D. (2016). Effect of lactic acid fermentation on antioxidant properties and phenolic acid contents of oyster (*Pleurotus ostreatus*) and chanterelle (*Cantharellus cibarius*) mushrooms. *Food Sci. Biotechnol.*, 25, 2, 439–444.
- Jaworska, G., Bernaś, E., Cichoń, Z., Possinger, P. (2008). Establishing the optimal period of storage for frozen *Agaricus bisporus*, depending on the preliminary processing applied. *Int. J. Refrig.*, 31, 1042–1050.
- Jaworska, G., Pogoń, K., Bernaś, E., Duda-Chodak, A. (2015). Nutraceuticals and antioxidant activity of prepared for consumption commercial mushrooms *Agaricus bisporus* and *Pleurotus ostreatus*. *J. Food Quality* 38, 111–122.
- Joshi, V. K., Kaur, M., Thakur, N. S. (1996). Lactic acid fermentation of mushroom (*Agaricus bisporus*) for preservation and preparation of sauce. *Acta Aliment.*, 25, 1–11.
- Kreß, M., Lelley, J. (1991). Preservation of oyster mushrooms by lactic acid fermentation. In M. J. Maher (Ed.), *Science and cultivation of edible fungi* (pp. 665–671). Rotterdam, Netherlands: Taylor and Francis.
- Mering, A. (1955). *Przetwory grzybowe* [Processed mushrooms]. Warszawa: Wydawnictwo Przemysłu Lekkiego i Spożywczego [in Polish].
- Milanović, N., Davidović, A., Savič, A. (2010). Lactic acid fermentation of mushroom (*Agaricus bisporus*) with *Lactobacillus plantarum*. In: 9th Savjetovanje hemičara i tehnologa Republike Srpske (pp. 338–345). 12–13 November, Banja Luka, Bosnia and Herzegovina.
- Molin, G. (2001). Probiotics in foods not containing milk or milk constituents, with special reference to *Lactobacillus plantarum* 299v. *Am. J. Clin. Nutr.*, 73(suppl.), 380S–385S.
- Neffe-Skocińska, K., Jaworska, D., Kołożyn-Krajewska, D., Dolatowski, Z., Jachacz-Jówo, L. (2015). The effect of LAB as probiotic starter culture and green tea extract addition on dry fermented pork loins quality. *BioMed Research International*. Article ID 452757.

- Niksic M., Stojanovic M., Zivanovic S., Veljic S. (1997). Ecological approach in preservation of edible mushrooms by lactic acid fermentation. In J. Baras (Ed.), *Ecology in food industry and biotechnology* (pp. 217–223). Poslovna zajednica “Vrenje”, Belgrade, Serbia.
- PN-ISO 15214:2002. Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby mezofilnych bakterii fermentacji mlekowej. Metoda płytkowa w temperaturze 30°C [Microbiology of food and feed. Horizontal method for determination of the count of mesophilic lactic fermentation bacteria. Plate method at a temperature of 30°C] [in Polish].
- Palacios, I., Lozano, M., Moro, C., D’Arrigo, M., Rostagno, M. A., Martinez, J. A., ..., Villares, A. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chem.*, 128, 674–678.
- Prado, F. C., Parada, J. L., Pandey, A., Soccol, C. R. (2008). Trends in non-dairy probiotic beverages. *Food Res. Int.*, 41, 111–123.
- Rajewska, J., Bałasińska, B. (2004). Związki biologicznie aktywne zawarte w grzybach jadalnych i ich korzystny wpływ na zdrowie [Biologically active compounds of edible mushrooms and their beneficial impact on health]. *Post. Hig. Med. Dośw.*, 58, 352–357.
- Rubio, R., Aymerich, T., Bover-Cid, S., Guàrdia, M. D., Arnau, J., Garriga, M. (2013). Probiotic strains *Lactobacillus plantarum* 299V and *Lactobacillus rhamnosus* GG as starter cultures for fermented sausages. *LWT – Food Sci. Technol.*, 54, 51–56.
- Skąpska, S., Owczarek, L., Jasińska, U., Hasińska, A., Danielczuk J., Sokołowska, B. (2008). Zmiany pojemności przeciwutleniającej grzybów jadalnych w procesie kiszenia [Changes in the antioxidant capacity of edible mushrooms during lactic acid fermentation]. *Food. Sci. Techn. Qual.*, 59, 243–250 [in Polish].
- Stadnik, J., Dolatowski, Z. J. (2015). Free amino acids and biogenic amines content during ageing of dry-cured pork loins inoculated with *Lactobacillus casei* LOCK 0900 probiotic strain. *Food Sci. Technol. Res.*, 21(2), 167–174.
- Stojanovic, M., Niksic, M., Veres, M., Petrovic, L. (1994). Preservation of edible mushrooms (*Agaricus bisporus*) by lactic acid fermentation. *Microbiologia*, 21, 16–21.
- Tajalli, F., Malekzadeh, K., Soltanian, H., Janpoor, J., Rezaeian, S., Pourianfar, H. R. (2015). Antioxidant capacity of several Iranian, wild and cultivated strains of the button mushroom. *Braz. J. Microbiol.*, 46, 3, 769–776.
- Tsai, S.-Y., Wu, T.-P., Huang, S.-J., Mau, J.-L. (2007). Non-volatile taste components of *Agaricus bisporus* harvested at different stages of maturity. *Food Chem.*, 103, 1457–1464.
- Xiangyou, W., Jincui, T., Juan, W. (2014). Effect of pre-cooling temperature on physiological quality of cold stored *Agaricus bisporus*. *Int. J. Agric. Biol. Eng.*, 7, 2, 108–114.
- Yoon, K. Y., Woodams, E. E., Hang, Y. D. (2004). Probiotication of tomato juice by lactic acid bacteria. *J. Microbiol.*, 42, 315–318.
- Yoon, K. Y., Woodams, E. E., Hang, Y. D. (2005). Fermentation of beet juice by beneficial lactic acid bacteria. *Lebensm. Wiss. Technol.*, 38, 73–75.
- Yoon, K. Y., Woodams, E. E., Hang, Y. D. (2006). Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technol.*, 97, 1427–1430.