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Cultivated strains of *Agaricus bisporus* and *A. brasiliensis*: chemical characterization and evaluation of antioxidant and antimicrobial properties for the final healthy product – natural preservatives in yoghurt

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Agaricus bisporus (J. E. Lange) Emil J. Imbach and *Agaricus brasiliensis* Wasser, M. Didukh, Amazonas & Stamets are edible mushrooms. We chemically characterized these mushrooms for nutritional value, hydrophilic and lipophilic compounds. The antioxidant and antimicrobial activities of methanolic and ethanolic extracts were assessed. Hepatotoxicity was also evaluated. The ethanolic extract of both species was tested for inhibition of *Listeria monocytogenes* growth in yoghurt. Both species proved to be a good source of bioactive compounds. *A. brasiliensis* was richer in polyunsaturated fatty acids and revealed the highest concentration of phenolic acids, and tocopherols. *A. bisporus* showed the highest monounsaturated fatty acids and ergosterol contents. *A. brasiliensis* revealed the highest antioxidant potential, and its ethanolic extract displayed the highest antibacterial potential; the methanolic extract of *A. bisporus* revealed the highest antifungal activity. *A. brasiliensis* possessed better preserving properties in yoghurt.

Introduction

About 200 edible mushroom species have been collected from various parts of the world.[1] Cultivated mushrooms are of great interest to the food industry, but can also be considered of great importance for pharmaceutical companies, due to the production of interesting compounds with bioactive properties. The most important cultivated edible mushrooms belong to the *Agaricus* genus. Among them is the "common mushroom", *Agaricus bisporus*, which is well known, and *Agaricus brasiliensis* cultivated all around the world for its medicinal properties.[2]

A. bisporus is one of the most economically important edible mushrooms. This species has been demonstrated to express diverse and valuable medicinal properties including antitumor, anti-aromatase, antimicrobial, immunomodulatory, anti-inflammatory as well as antioxidant activities.[3–8] *A. brasiliensis* is an edible fungus belonging to the Brazilian biota, and has traditionally been used to treat cancer and other diseases.[9] In the last few decades, numerous studies have reported the cytotoxic and antitumor properties of *A. brasiliensis* polysaccharides, which mainly act not only through immunomodulatory mechanisms, but also by direct cytotoxic effects on tumor cells.[10]

Besides their high nutritional value,[1] mushrooms possess a wide variety of beneficial effects to human health, with special emphasis on pharmacological properties such as antioxidant, antimicrobial, and antitumor activities.[11–15] Many food products are perishable and require protection from microbial spoilage during preparation, storage and distribution to give them acceptable shelf-life and organoleptic characteristics. Microorganisms such as *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* O157:H7, *Shigella* spp., *Listeria monocytogenes* and *Yersinia enterocolitica* were reported as the most common foodborne pathogens that are present in many foods and able to survive in milk and fermented milk products.[16,17]

The main focus of this study was to perform chemical characterization of cultivated *A. bisporus* and *A. brasiliensis*, regarding their nutritional value, hydrophilic and lipophilic compounds. Furthermore, *in vitro* antioxidant and antimicrobial (antibacterial and antifungal) potential and hepatotoxicity effects were evaluated. Although the species have been investigated by other authors, the present study proved that their chemical properties may be related to their bioactivity, since the

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species with the highest contents in phenolic acids, polyunsaturated fatty acids and tocopherols was the species with the highest antioxidant and antibacterial potential (*A. brasiliensis*). In this study the survival of *L. monocytogenes* associated with fermented milk product (yoghurt) was also investigated. Yoghurt was chosen as a model system due to its high nutritional value, and because of the rising concern of consumers in using synthetic preservatives to retard the growth of undesired microorganisms in food. Therefore, the incorporation of natural products as antimicrobial preservatives in food led us to research the potential of mushroom extracts in yoghurt. These species were used due to their high consumption worldwide.

Materials and methods

Mushroom species

Lyophilized fruiting body powder (milled mushroom) of *A. bisporus* (strain Sylvan A15) and *A. brasiliensis* (strain M7700, Mycelia) were obtained from Innerlife B. V. of Venlo, The Netherlands.

Standards and reagents

Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of the HPLC grade from Fisher Scientific (Lisbon, Portugal). The fatty acid methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers and standards of tocopherols, ergosterol, sugars, organic acids and phenolic compounds, and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Racemic tocol, 50 mg mL^{-1}, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Mueller–Hinton agar (MH) and malt agar (MA) were obtained from the Institute of Immunology and Virology, Torlak (Belgrade, Serbia). Dimethylsulfoxide (DMSO), (Merck KGaA, Germany) was used as a solvent. Phosphate buffered saline (PBS) was obtained from Sigma Chemical Co. (St. Louis, USA). Foetal bovine serum (FBS), L-glutamine, Hank's balanced salt solution (HBSS), trypsin–EDTA (ethylenediaminetetraacetic acid), nonessential amino acid solution (2 mM), penicillin/streptomycin solution (100 U mL^{-1} and 100 mg mL^{-1}, respectively), DMEM media was from Hyclone (Logan, USA). Acetic acid, ellipticine, sulforhodamine B (SRB), trypan blue, trichloroacetic acid (TCA) and Tris were from Sigma Chemical Co. (Saint Louis, USA). Methanol and all other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

Chemical characterization of *Agaricus* spp.

Nutritional value. The samples were analysed for their chemical composition (moisture, proteins, fat, carbohydrates and ash) through AOAC procedures.[18] The crude protein content (N × 4.38) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C. Total carbohydrates were calculated by difference. The energy contribution was calculated according to the following equation: energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g fat).

Hydrophilic compounds

Sugars. Following the extraction procedure described by Reis *et al.*[19] free sugars were determined by a High Performance Liquid Chromatography (HPLC) system consisting of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000) and auto-sampler (AS-2057 Jasco), coupled to a refraction index detector (RI detector Knauer Smartline 2300). Sugar identification was made by comparing the relative retention times of sample peaks with internal standards (IS). Data were analyzed using Clarity 2.4 Software (DataApex). Quantification was based on the RI signal response of each standard, using the IS (raffinose) method and by using calibration curves obtained from the commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

Organic acids. Following the extraction procedure described by Barros *et al.*[20] organic acids were determined by ultra fast liquid chromatography (UFLC, Shimadzu 20A series) coupled with a photodiode array detector (PDA). The organic acids were quantified by the comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

Phenolic compounds. Following the extraction procedure described by Barros *et al.*[21] phenolic acids and related compounds were determined using the UFLC mentioned above. Detection was carried out in a photodiode array detector (PDA), using 280 nm as the preferred wavelength. The phenolic acids and related compounds were quantified by comparison of the area of their peaks recorded at 280 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of dry weight.

Lipophilic compounds

Fatty acids. Following the extraction transesterification procedures described by Reis *et al.*[19] fatty acids were determined using a gas chromatographer (DANI 1000) equipped with a split/splitless injector and a flame ionization detector (GC-FID). Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

Tocopherols. Following the extraction procedure described by Heleno *et al.*[22] tocopherols were determined by liquid chromatography (HPLC – equipment described above, for sugars composition), and a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The compounds were identified by chromatographic comparison with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in μg per 100 g of dry weight.

Ergosterol. Following the extraction multi-step procedure described by Barreira *et al.*[23] sterols were analysed using the HPLC equipment (described above) coupled to an ultraviolet Smartline 2500 UV detector. Chromatographic separation was achieved with a BGB Analytic AG Inertsil 100A ODS-3 reverse phase column (4.6 × 150 mm, 3 μm) operating at 35 °C (7971R Grace oven). The detection was performed at 280 nm. Ergosterol was quantified by comparison of the area of its peak with the calibration curve obtained from a commercial standard. Cholecalciferol was used as the internal standard. The results were expressed in mg per 100 g of dry weight.

Extract preparation

The methanolic extract was obtained by stirring the dry fruiting body (1 g) with 40 mL of methanol for 1 h and subsequently filtered through Whatman no. 4 paper. The residue was then extracted with 20 mL of methanol for 1 h. The combined methanolic extracts were evaporated at 40 °C under reduced pressure by a rotary vacuum evaporator (Büchi R-210) to dryness. The ethanolic extract was obtained from dry fruiting bodies by stirring the sample (1 g) with 95% ethanol during 48 h at 70 °C. The extracts were filtered and centrifuged to get a clear liquid, and evaporated at 40 °C under reduced pressure by a rotary vacuum evaporator.

The extracts were re-dissolved in (a) the corresponding extraction solvent for the antioxidant activity assays (20 mg mL^{-1}), (b) 5% solution of DMSO in distilled water for the antimicrobial activity assays (100 mg mL^{-1}); and (c) distillated water for the hepatotoxicity assays (8 mg mL^{-1}).

Evaluation of the antioxidant potential of the *Agaricus* spp. extracts

General. Successive dilutions were made from the stock solution and submitted to different *in vitro* assays to evaluate the antioxidant activity of the samples. All the antioxidant assays were performed as previously described.[24] The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (ferricyanide/Prussian blue assay) against sample concentrations. Trolox was used as the standard.

Folin–Ciocalteu assay. The extract solution (1 mL) was mixed with *Folin–Ciocalteu* reagent (5 mL, previously diluted with water 1 : 10, v/v) and sodium carbonate (75 g L^{-1}, 4 mL). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm (Analytikjena spectrophotometer; Jena, Germany). Gallic acid was used to obtain the standard curve and the reduction of *Folin–Ciocalteu* reagent by the samples was expressed as mg of gallic acid equivalents (GAE) per g of extract.

Reducing power or ferricyanide/Prussian blue assay. The extract solutions with different concentrations (0.5 mL) were mixed with sodium phosphate buffer (200 mmol L^{-1}, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). The mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48 wells plate, the same with deionised water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm in a ELX800 Microplate Reader (Bio-Tek Instruments, Inc; Winooski, USA).

DPPH radical-scavenging activity assay. This methodology was performed using the microplate reader mentioned above. The reaction mixture was made in a 96 wells plate and consisted of 30 μL of a concentration range of the extract and 270 μL methanol containing DPPH radicals (6×10^{-5} mol L^{-1}). The mixture was left to stand for 30 min in the dark, and the absorption was measured at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: $\%\ RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample and A_{DPPH} is the absorbance of the DPPH solution.

Inhibition of β-carotene bleaching or β-carotene/linoleate assay. A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two millilitres of this solution were pipetted into a round-bottom flask. The chloroform was removed at 40 °C under vacuum and linoleic acid (40 mg), Tween 80 emulsifier (400 mg) and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into test tubes containing 0.2 mL of a concentration range of the extract. The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: (absorbance after 2 h of assay/initial absorbance) × 100.

Thiobarbituric acid reactive substances (TBARS) assay. Porcine (*Sus scrofa*) brains were obtained from official slaughtering of animals, dissected, and homogenized with a Polytron in ice cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1 : 2 w/v brain tissue homogenate which was centrifuged at 3000 *g* for 10 min. An aliquot (100 μL) of the supernatant was incubated with 200 μL samples of a concentration range of the extract in the presence of $FeSO_4$ (10 mM; 100 μL) and ascorbic acid (0.1 mM; 100 μL) at 37 °C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 500 μL), followed by thiobarbituric acid (TBA, 2%, w/v, 380 μL), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000 *g* for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)–TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: $\text{inhibition ratio}\ (\%) = [(A - B)/A] \times 100\%$, where A and B were the absorbance of the control and the sample solution, respectively.

Evaluation of the antimicrobial activity of the *Agaricus* spp. extracts

Antibacterial activity. The Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240) and *Listeria monocytogenes* (NCTC 7973), and the Gram-negative bacteria *Pseudomonas*

aeruginosa (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210), and *Enterobacter cloacae* (human isolate) were used. The antibacterial assay was carried out by a microdilution method.[25,26] The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU mL^{-1}. Mushroom extracts were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (10 mg mL^{-1}) and added in tryptic soy broth (TSB) medium (100 μL) with bacterial inoculum (1.0×10^4 CFU per well). The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MICs obtained from the susceptibility testing of various bacteria to tested extracts were determined also by a colorimetric microbial viability assay based on the reduction of an INT ((*p*-iodonitrotetrazolium violet) [2-(4-iodophenyl)-3-(4-nitrphenyl)-5-phenyltetrazolium chloride; Sigma]) color and compared with positive control for each bacterial strains. The minimum bactericidal concentrations (MBCs) were determined by serial sub-cultivation of 2 μL into microtitre plates containing 100 μL of broth per well and further incubation for 24 h. The lowest concentration with no visible growth was defined as the MBC, indicating more than 99.5% reduction in viable cells compared to the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank (broth medium plus diluted extracts) and the positive control. Streptomycin (Sigma P 7794) and ampicillin (Panfarma, Belgrade, Serbia) were used as positive controls (1 mg mL^{-1} in sterile physiological saline). Five percent DMSO was used as a negative control.

Antifungal activity. *Aspergillus fumigatus* (human isolate), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Trichoderma viride* (IAM 5061), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Penicillium verrucosum* var. *cyclopium* (food isolate) were used. In order to investigate the antifungal activity of the mushroom extract, a modified microdilution technique was used.[27,28] The fungal spores were washed from the surface of malt agar plates (after incubation period at 28 °C for 21 days) with sterile 0.85% saline containing 0.1% Tween 80 (v/v) and spore suspension was adjusted with sterile saline to a concentration of 1.0×10^5 per mL. Extracts were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (10 mg mL^{-1}, stock concentration) and added in broth malt medium with inoculum (0.005–3 mg mL^{-1} for extracts). The microplates were then incubated for 72 h at 28 °C. After the incubation period, the lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 μL of tested compounds dissolved in medium and incubated for 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. DMSO was used as a negative control, and commercial fungicides, bifonazole (Srbolek, Belgrade, Serbia) and ketoconazole (Zorkapharma, Šabac, Serbia) were used as positive controls (1–3000 μg mL^{-1}). Five percent DMSO was used as a negative control.

Evaluation of the toxicity of the *Agaricus* spp. extracts towards primary liver cells

A cell culture was prepared from a freshly harvested porcine liver obtained from a local slaughter house. It was designed as PLP2. Briefly, the liver tissues were rinsed in Hank's balanced salt solution containing 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin and divided into 1×1 mm^3 explants. Some of these explants were placed in 25 cm^2 tissue flasks in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM nonessential amino acids and 100 U mL^{-1} penicillin, 100 mg mL^{-1} streptomycin and incubated at 37 °C with a humidified atmosphere containing 5% CO_2. The medium was changed every 2 days. Cultivation of the cells was continued with direct monitoring every 2–3 days using a phase contrast microscope. Before confluence, cells were subcultured and plated in 96-well plates at a density of 1.0×10^4 cells per well, and cultivated in DMEM medium with 10% FBS, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin.[29] Cells were treated for 48 h with different diluted sample solutions and the same procedure described in the previous section for sulforhodamine B colorimetric (SRB) assay was followed. The results were expressed in GI_{50} values (sample concentration that inhibited 50% of the net cell growth). Ellipticine (potent cytostatic antineoplastic agent) was used as the positive control.

Capacity of *Agaricus* spp. ethanolic extracts to inhibit growth of *L. mocytogenes* in yoghurt infusion

Yoghurt. Low fat yoghurt (2.8 g), produced from cow milk was purchased from a local supermarket and kept in refrigerator at 4 °C until further analysis. All the samples were used before the expiration date of the product. The composition of the yoghurt stated on the packaging was: energy 54 kcal; fat 2.8 g; proteins minimum 3.0 g and lactose minimum 3.6 g; expressed by 100 g. On packaging it was labeled that there is yoghurt culture of *Streptococcus thermophilus*. Since this species does not produce spores, the yoghurt infusion was filtered through a membrane filter (pore size 2 μm, Sigma) to remove the bacterium. The pH value of yoghurt was 4.6, and we have adjusted it as well to 4.6 in yoghurt infusion with 0.1 N HCl. Experiments of inoculating Malt Agar (MA) and Mueller–Hinton Agar (MHA) plates with yoghurt diluted 1 in 10 with phosphate buffered saline (PBS) and kept at 25 °C and 37 °C, for 48 hours, showed no bacterial nor fungal contamination of the product.

***Agaricus* spp. ethanolic extract in yoghurt against *Listeria monocytogenes*.** Briefly, 10 mL of yoghurt was added to 90 mL of PBS in sterile flasks and homogenized for 2 min. Mushroom ethanolic extract was added (1.17–18.75 mg mL^{-1}) to the yoghurt mixture by using serial dilution technique. Prior to inoculation, overnight culture of *Listeria monocytogenes* was cultivated in TSB medium and incubated for 24 h at 37 °C. Appropriate dilutions of culture were made after the incubation period. In order to investigate *L. monocytogenes* (NCTC 7973) growth, control samples were used and contained the same serial dilutions of bacterium in yoghurt infusion without mushroom extracts.

The yoghurt was inoculated with ~10^3, 10^5, 10^7 colony-forming units (CFU) of *L. monocytogenes* in order to explore the activity of the extracts using different initial inocula. The experimental plate was incubated at 25 °C. MIC and MBC were determined in yoghurt after 72 h. The MIC of the samples was detected following the addition of 40 μL of iodonitrotetrazolium chloride (INT) (0.2 mg mL^{-1}) and incubation at 37 °C for 30 min. MBC was determined by serial sub-cultivation of 10 μL into microplates containing 100 μL of TSB. The lowest concentration that showed no growth after this sub-culturing was read as the MBC.

Statistical analysis

For each species, three samples were used and all the assays were carried out in triplicate. The results were expressed as mean values and standard errors, and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This analysis was carried out using SPSS v. 18.0 program.

Results

Chemical characterization of *Agaricus* spp

The results of the chemical characterization of both *Agaricus* species are shown in Tables 1 and 2. Carbohydrates were the most abundant macronutrients present in the studied mushrooms (75.74–89.13 g per 100 g dw; Table 1). Ash and fat contents of the *Agaricus* species were low (1.78–2.75 g per 100 g for fat and 6.04–8.16 g per 100 g for ash) and their energy contribution was very similar, (384.74 kcal per 100 g dw for *A. bisporus* and 381.10 kcal per 100 g dw for *A. brasiliensis*). The main sugar was mannitol (13.81–14.91 g per 100 g dw) and trehalose was only detected in *A. brasiliensis* (0.49 g per 100 g dw; Table 1). Regarding organic acids, the main molecules found in the studied species were oxalic, malic and fumaric acids. The contents found in both species were very similar (1.88 g per 100 g dw for *A. bisporus* and 1.87 g per 100 g dw for *A. brasiliensis*; Table 1). The phenolic acids found in the studied mushrooms were gallic and *p*-coumaric acids, as also the related compound cinnamic acid (Table 1). Gallic acid was only found in *A. bisporus* (0.10 mg per 100 g dw) and *p*-coumaric acid and the related compound cinnamic acid were only detected in *A. brasiliensis* (0.28 mg per 100 g dw and 0.12 mg per 100 g dw, respectively). Concerning the fatty acid composition of the studied species (Table 2), polyunsaturated fatty acids (PUFA) predominated over saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). *A. brasiliensis* presented the highest content in PUFA (76.41% of total FA), while *A. bisporus* gave the highest percentage of MUFA (30.31% of total FA). Thus, both species seem to be an excellent option as food; *A. brasiliensis* may be a better choice since it has a lower percentage of MUFA (1.47% of total FA; Table 2). The fatty acids found in higher amounts were palmitic acid (C16:0), a SFA; and linoleic acid (C18:2n6), a PUFA.

Regarding tocopherols, α-, γ- and δ-isoforms were quantified in the studied mushrooms (Table 2). Although the last isoform (δ-tocopherol) was only found in *A. bisporus* (35.23 μg per100 g dw), *A. brasiliensis* presented a higher concentration of tocopherols (572.28 μg per 100 g dw) due to the contribution of γ-tocopherol (562.14 μg per 100 g dw). Ergosterol was detected in both *Agaricus* species (88.93–138.74 mg per 100 g dw).

Table 1 Nutritional value of the studied *Agaricus* species (mean ± SD)[a]

	Agaricus bisporus	*Agaricus brasiliensis*
Ash (g per 100 g dw)	6.04 ± 0.17[b]	8.16 ± 0.61[a]
Proteins (g per 100 g dw)	3.05 ± 0.34[b]	13.35 ± 0.15[a]
Fat (g per 100 g dw)	1.78 ± 0.06[b]	2.75 ± 0.13[a]
Carbohydrates (g per 100 g dw)	89.13 ± 0.31[a]	75.74 ± 0.30[b]
Energy (kcal per 100 g dw)	384.74 ± 0.70[a]	381.10 ± 2.16[a]
Hydrophilic compounds		
Mannitol (g per 100 g dw)	14.91 ± 0.13[a]	13.81 ± 0.01[b]
Trehalose (g per 100 g dw)	nd	0.49 ± 0.05
Total sugars (g per 100 g dw)	14.91 ± 0.13[a]	14.30 ± 0.05[b]
Oxalic acid (g per 100 g dw)	0.58 ± 0.01[b]	1.64 ± 0.02[a]
Malic acid (g per 100 g dw)	1.30 ± 0.27	nd
Fumaric acid (g per 100 g dw)	Trace	0.23 ± 0.00
Total organic acids (g per 100 g dw)	1.88 ± 0.25[a]	1.87 ± 0.02[a]
Gallic acid (mg per 100 g dw)	0.10 ± 0.00	nd
p-Coumaric acid (mg per 100 g dw)	nd	0.28 ± 0.00
Total phenolic acids (mg per 100 g dw)	0.10 ± 0.00[b]	0.28 ± 0.00[a]
Cinnamic acid (mg per 100 g dw)	nd	0.12 ± 0.00

[a] dw – dry weight; nd – not detected. In each line different letters mean significant differences between the species ($p < 0.05$).

Antioxidant and antimicrobial activities of *Agaricus* spp. extracts

Regarding the antioxidant properties of the studied species, *A. brasiliensis* revealed higher antioxidant potential (Table 3). Its methanolic and ethanolic extracts presenting the highest total phenolics content (41.72 and 37.93 mg GAE per g extract) revealed the lowest EC_{50} values for the ferricyanide/Prussian blue assay (0.79 mg mL^{-1}), DPPH radical scavenging activity assay (1.18 mg mL^{-1}) and β-carotene/linoleate assay (0.22 mg mL^{-1}).

The results of antibacterial and antifungal activities of *A. bisporus* and *A. brasiliensis* methanolic and ethanolic extracts are presented in Tables 4 and 5, respectively. Regarding the antibacterial action, ethanolic extracts were more effective than methanolic ones. No significant difference in extract activity was observed on Gram positive and Gram negative bacteria. The activity of extracts was dependent on the microorganism species that was used. Methanolic extracts exhibited better antifungal potential. The bacteria were generally more sensitive than the studied moulds. The most sensitive bacteria to the *A. bisporus* ethanolic extract were *S. aureus* and *B. cereus*. *A. versicolor* was the most sensitive to the effect of the methanolic extract of *A. brasiliensis*. The most sensitive bacteria to *A. bisporus* ethanolic extract were *S. aureus* and *B. cereus* (Table 4).

A. versicolor was the most sensitive to the effect of methanolic extract of *A. brasiliensis* (Table 5).

Table 2 Composition in lipophilic compounds of the studied *Agaricus* species (mean ± SD)[a]

	Agaricus bisporus	*Agaricus brasiliensis*
C6:0	0.06 ± 0.01[b]	0.12 ± 0.01[a]
C8:0	0.05 ± 0.00[a]	0.04 ± 0.00[b]
C10:0	0.03 ± 0.00[b]	0.02 ± 0.00[a]
C12:0	0.10 ± 0.00[a]	0.02 ± 0.00[b]
C13:0	nd	0.01 ± 0.00
C14:0	0.58 ± 0.01[a]	0.19 ± 0.00[b]
C15:0	0.67 ± 0.00[b]	0.86 ± 0.00[a]
C15:1	nd	0.04 ± 0.00
C16:0	14.02 ± 0.03[a]	11.34 ± 0.06[b]
C16:1	0.42 ± 0.00[a]	0.19 ± 0.00[b]
C17:0	1.19 ± 0.02[a]	1.01 ± 0.01[b]
C18:0	6.54 ± 0.01[a]	3.10 ± 0.00[b]
C18:1n9	20.58 ± 0.07[a]	1.04 ± 0.01[b]
C18:2n6	43.87 ± 0.08[c]	74.48 ± 0.05[a]
C18:3n3	3.25 ± 0.11[a]	0.20 ± 0.01[b]
C20:0	3.16 ± 0.02[a]	1.48 ± 0.01[b]
C20:1	0.66 ± 0.05[a]	0.02 ± 0.01[b]
C20:2	0.10 ± 0.01[b]	0.17 ± 0.01[a]
C20:3n3 + C21:0	0.63 ± 0.01[b]	0.82 ± 0.01[a]
C20:5n3	0.11 ± 0.02[b]	0.75 ± 0.01[a]
C22:0	2.01 ± 0.03[b]	2.63 ± 0.11[a]
C22:1n9	0.08 ± 0.01[a]	0.06 ± 0.00[b]
C23:0	0.31 ± 0.02[b]	0.34 ± 0.00[a]
C24:0	1.58 ± 0.09[a]	0.97 ± 0.00[b]
C24:1	nd	0.11 ± 0.01
Total SFA (% of total FA)	30.31 ± 0.08[a]	22.12 ± 0.05[b]
Total MUFA (% of total FA)	21.74 ± 0.10[a]	1.47 ± 0.00[b]
Total PUFA (% of total FA)	47.96 ± 0.03[b]	76.41 ± 0.05[a]
α-Tocopherol (μg per 100 g dw)	3.87 ± 0.30[b]	10.14 ± 0.80[a]
γ-Tocopherol (μg per 100 g dw)	9.63 ± 0.10[b]	562.14 ± 29.43[a]
δ-Tocopherol (μg per 100 g dw)	35.23 ± 0.10	nd
Total tocopherols (μg per 100 g dw)	48.73 ± 0.10[b]	572.28 ± 30.23[a]
Ergosterol (mg per 100 g dw)	138.74 ± 0.61[a]	88.93 ± 0.21[b]

[a] dw – dry weight; nd – not detected; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. In each line different letters mean significant differences between the species ($p < 0.05$).

It should be highlighted that none of the extracts of *Agaricus* species was toxic for liver primary cells (GI_{50} values against PLP2 > 400 μg mL^{-1}, data not shown) being therefore, innocuous.

L. monocytogenes growth inhibition by *Agaricus* spp. ethanolic extracts in yoghurt

The results of *Agaricus* spp. ethanolic extracts preserving properties in yoghurt are presented in Table 6. Ethanolic extracts were chosen for the experiment due to their better *in vitro* results on *L. monocytogenes*. MIC and MBC values for *L. monocytogenes* are higher in the yoghurt experiment for *L. monocytogenes* than in the *in vitro* microdilution method. This might be explained by higher complexity of yoghurt than of the conventional laboratory media. *A. brasiliensis* ethanolic extract proved to be more efficient than that of *A. bisporus* in controlling *L. monocytogenes* growth in yoghurt. The lowest inoculum quantity (10^3 CFU mL^{-1}) gave the best results on inhibition. The inhibition and bactericidal effects were the same for the inocula of 10^5 and 10^7 CFU mL^{-1}.

Discussion

In the present study a complete chemical characterization of the cultivated species *A. bisporus* and *A. brasiliensis* was performed. Methanolic, ethanolic and polysaccharidic extracts are the most used and effective extracts in the literature to determine the bioactive properties of mushrooms,[10,15,24,30–32] therefore these extracts were explored in order to reveal which type of extract is more efficient in the majority of the assays performed. *A. brasiliensis* methanolic and ethanolic extracts presented the highest total phenolics content. These results may be related to the higher contents of phenolic acids, PUFA and tocopherols detected in this species, since the mentioned properties are often attributed to some of these bioactive compounds.[4]

Data obtained for carbohydrates analyses are in agreement with the values stated by different authors who reported mushrooms as good sources of carbohydrates and proteins as well as poor in fat and low caloric food.[19,33,34] Mannitol and trehalose are very common sugars found in mushrooms as reported by several authors.[1,19,30,35,36] The use of mannitol in food, pharmaceutical, medical, and chemical industries is widely known and therefore determination of its content in mushrooms is of particular interest.[37] Regarding phenolic compounds, gallic acid was the only phenolic acid found in *A. bisporus*, while *p*-coumaric acid and the related phenolic compound cinnamic acid were detected in *A. brasiliensis*,

Table 3 Antioxidant properties of the methanolic and ethanolic extracts of the studied *Agaricus* species (mean ± SD)[a]

	A. bisporus		*A. brasiliensis*	
	Methanolic	Ethanolic	Methanolic	Ethanolic
Folin–Ciocalteu assay (mg GAE per g extract)	14.49 ± 0.10[c]	4.69 ± 0.04[d]	41.72 ± 0.49[a]	37.93 ± 0.36[b]
Ferricyanide/Prussian blue assay (EC_{50}; mg mL^{-1})	1.68 ± 0.04[d]	16.66 ± 0.27[a]	0.94 ± 0.00[e]	2.01 ± 0.04[c]
DPPH radical-scavenging activity (EC_{50}; mg mL^{-1})	3.25 ± 0.18[d]	49.44 ± 0.53[a]	1.81 ± 0.03[e]	4.17 ± 0.09[c]
β-Carotene/linoleate assay (EC_{50}; mg mL^{-1})	5.40 ± 0.07[b]	16.65 ± 0.30[a]	1.63 ± 0.24[d]	2.85 ± 0.34[c]
TBARS assay (EC_{50}; mg mL^{-1})	0.71 ± 0.02[d]	16.79 ± 0.01[a]	0.15 ± 0.03[e]	1.80 ± 0.26[b]

[a] na – not applicable. In each line different letters mean significant differences between the species ($p < 0.05$). Concerning the *Folin–Ciocalteu* assay, higher values mean higher reducing power; for the other assays, the results are presented in EC_{50} values, which means that higher values correspond to lower reducing power or antioxidant potential. EC_{50}: extract concentration corresponding to 50% of the antioxidant activity or 0.5 of absorbance for the ferricyanide/Prussian blue assay.

Table 4 Antibacterial activity of the methanolic and ethanolic extracts of the studied *Agaricus* species (mean values of MIC and MBC in mg mL^{-1})[a]

		A. bisporus		*A. brasiliensis*			
Bacteria		Methanolic	Ethanolic	Methanolic	Ethanolic	Streptomycin	Ampicillin
Staphylococcus aureus	MIC	$0.6 \pm 0.03^{b,c,d}$	$0.03 \pm 0.00^{a,c,d}$	$0.1 \pm 0.01^{a,b,d}$	$0.08 \pm 0.00^{a,b,c}$	0.04 ± 0.00^{e}	0.25 ± 0.00^{e}
	MBC	$1.15 \pm 0.00^{b,c,d}$	$0.06 \pm 0.02^{a,c,d}$	$0.7 \pm 0.02^{a,b,d}$	$0.2 \pm 0.01^{a,b,c}$	0.09 ± 0.01^{e}	0.37 ± 0.03^{e}
Bacillus cereus	MIC	$0.6 \pm 0.02^{b,c,d}$	$0.03 \pm 0.01^{a,c,d}$	$0.2 \pm 0.00^{a,b,d}$	$0.04 \pm 0.03^{a,b,c}$	0.09 ± 0.01^{e}	0.25 ± 0.03^{e}
	MBC	$1.15 \pm 0.60^{b,c,d}$	$0.06 \pm 0.01^{a,c,d}$	$0.3 \pm 0.03^{a,b,d}$	$0.08 \pm 0.02^{a,b,c}$	0.17 ± 0.02^{e}	0.37 ± 0.03^{e}
Micrococcus flavus	MIC	$0.6 \pm 0.03^{b,d}$	$1.7 \pm 0.1^{a,c,d}$	$0.5 \pm 0.01^{b,d}$	$1.15 \pm 0.010^{a,b,c}$	0.17 ± 0.01^{e}	0.25 ± 0.02^{e}
	MBC	$2.3 \pm 0.10^{c,d}$	$2.35 \pm 0.03^{c,d}$	$1.5 \pm 0.02^{a,b,d}$	$0.35 \pm 0.03^{a,b,c}$	0.34 ± 0.00^{e}	0.37 ± 0.00^{e}
Listeria monocytogenes	MIC	$0.6 \pm 0.00^{b,c,d}$	$2.35 \pm 0.03^{a,c,d}$	$1.5 \pm 0.03^{a,b,d}$	$0.08 \pm 0.012^{a,b,c}$	0.17 ± 0.01^{e}	0.37 ± 0.02^{e}
	MBC	$2.3 \pm 0.00^{b,d}$	$4.7 \pm 0.10^{a,c,d}$	$2.3 \pm 0.10^{b,d}$	$1.5 \pm 0.00^{a,b,c}$	0.34 ± 0.00^{e}	0.49 ± 0.01^{e}
Pseudomonas aeruginosa	MIC	$0.3 \pm 0.03^{b,c,d}$	$0.6 \pm 0.02^{a,c,d}$	$0.2 \pm 0.01^{a,b}$	$0.15 \pm 0.00^{a,b}$	0.17 ± 0.01^{e}	0.74 ± 0.01^{e}
	MBC	$1.15 \pm 0.10^{b,c,d}$	$2.35 \pm 0.00^{a,c,d}$	$0.3 \pm 0.02^{a,b}$	$0.35 \pm 0.01^{a,b}$	0.34 ± 0.03^{e}	1.24 ± 0.10^{e}
Salmonella typhimurium	MIC	$0.3 \pm 0.02^{b,c,d}$	$2.35 \pm 0.03^{a,c,d}$	$0.14 \pm 0.03^{a,b}$	$0.15 \pm 0.01^{a,b}$	0.17 ± 0.02^{e}	0.37 ± 0.00^{e}
	MBC	$0.6 \pm 0.02^{b,c,d}$	$4.7 \pm 1.30^{a,c,d}$	$0.5 \pm 0.03^{a,b,d}$	$0.35 \pm 0.02^{a,b,c}$	0.34 ± 0.01^{e}	0.49 ± 0.00^{e}
Escherichia coli	MIC	$1.15 \pm 0.10^{b,c,d}$	$3.6 \pm 0.03^{a,c,d}$	$2.3 \pm 0.00^{a,b,d}$	$0.35 \pm 0.00^{a,b,c}$	0.17 ± 0.00^{e}	0.25 ± 0.01^{e}
	MBC	4.7 ± 1.30^{d}	4.7 ± 0.10^{d}	4.6 ± 0.10^{d}	$1.5 \pm 0.010^{a,b,c}$	0.34 ± 0.03^{e}	0.49 ± 0.02^{e}
Enterobacter cloacae	MIC	$0.6 \pm 0.00^{b,c,d}$	$3.6 \pm 0.10^{a,c,d}$	$1.0^{a} \pm 0.00^{b,d}$	$0.08 \pm 0.01^{a,b,c}$	0.26 ± 0.06^{e}	0.37 ± 0.03^{e}
	MBC	$2.3 \pm 0.10^{b,c,d}$	$4.7 \pm 0.13^{a,c,d}$	$1.5 \pm 0.03^{a,b,d}$	$0.15 \pm 0.00^{a,b,c}$	0.52 ± 0.02^{e}	0.74 ± 0.02^{e}

[a] MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration. In each row different letters mean significant difference between the extracts at the same concentrations ($p < 0.05$). Statistically significant difference in comparison with *A. bisporus* methanol extract; statistically significant difference in comparison with *A. bisporus* ethanol extract; statistically significant difference in comparison with *A. brasiliensis* methanol extract; statistically significant difference in comparison with *A. brasiliensis* ethanol extract; and statistically significant difference in comparison with extracts.

presenting the latter one in higher amounts. Considering that phenolic compounds are secondary metabolites, their production is influenced by the conditions associated with the mushroom growth, which might explain differences among different samples and origins.[38] Mattila *et al.*[39] compared the phenolic acid profile of some cultivated mushrooms, such as *A. bisporus* (white and brown) and *Lentinus edodes*, detecting higher amounts of cinnamic acid in *A. bisporus* (white), while *p*-hydroxybenzoic and protocatechuic acids were found in higher contents in *L. edodes* (these compounds being also present in *A. bisporus*).[39] Reis *et al.*[24] studied the phenolic composition of a commercial sample of *A. bisporus*, presenting similarities with the herein study sample, since gallic acid was also the major compound found. Nevertheless, these authors also reported the

Table 5 Antifungal activity of the methanolic and ethanolic extracts of the studied *Agaricus* species (mean values of MIC and MFC in mg mL^{-1})[a]

		A. bisporus		*A. brasiliensis*			
Fungi		Methanolic	Ethanolic	Methanolic	Ethanolic	Bifonazole	Ketoconazole
Aspergillus fumigatus	MIC	$1.15 \pm 0.10^{b,d}$	$2.35 \pm 0.10^{a,b,c}$	$1.25 \pm 0.00^{b,d}$	$3.125 \pm 0.13^{a,b,c}$	0.15 ± 0.00^{e}	0.20 ± 0.00^{e}
	MFC	$9.4 \pm 1.70^{c,d}$	$9.4 \pm 1.70^{c,d}$	$2.50.03^{a,b,d}$	$6.25 \pm 1.7^{a,b,c}$	0.20 ± 0.06^{e}	0.50 ± 0.03^{e}
Aspergillus versicolor	MIC	$0.15 \pm 0.03^{c,d}$	$0.15 \pm 0.06^{c,d}$	$0.1 \pm 0.01^{a,b,d}$	$0.7 \pm 0.03^{a,b,c}$	0.10 ± 0.01^{e}	0.20 ± 0.06^{e}
	MFC	$1.15 \pm 0.10^{b,c,d}$	$4.7 \pm 0.13^{a,c,d}$	$1.25 \pm 0.03^{a,b,d}$	$3.125 \pm 0.06^{a,b,c}$	0.20 ± 0.01^{e}	0.50 ± 0.03^{e}
Aspergillus ochraceus	MIC	$0.3 \pm 0.03^{b,c,d}$	$1.125 \pm 0.01^{a,c,d}$	$0.75 \pm 0.03^{a,b,d}$	$1.5 \pm 0.03^{a,b,c}$	0.15 ± 0.06^{e}	1.50 ± 0.10^{e}
	MFC	$0.6 \pm 0.02^{b,c,d}$	$2.35 \pm 0.10^{a,c,d}$	$1.5 \pm 0.10^{a,b,d}$	$3.125 \pm 0.00^{a,b,c}$	0.20 ± 0.03^{e}	2.0 ± 0.13^{e}
Aspergillus niger	MIC	$0.6 \pm 0.06^{b,c,d}$	$2.35 \pm 0.06^{a,c,d}$	$1.25 \pm 0.01^{a,b,d}$	$3.125 \pm 0.00^{a,b,c}$	0.15 ± 0.00^{e}	0.20 ± 0.00^{e}
	MFC	$1.15 \pm 0.10^{b,c,d}$	$4.7 \pm 0.13^{a,c,d}$	$2.5 \pm 0.13^{a,b,d}$	$6.25 \pm 0.06^{a,b,c}$	0.20 ± 0.03^{e}	0.50 ± 0.00^{e}
Trichoderma viride	MIC	$0.3 \pm 0.03^{b,c,d}$	$0.15 \pm 0.03^{a,c,d}$	$0.6 \pm 0.06^{a,b,d}$	$0.15 \pm 0.03^{a,b,c}$	0.15 ± 0.01^{e}	1.0 ± 0.06^{e}
	MFC	$0.6 \pm 0.00^{b,c,d}$	$2.35 \pm 0.00^{a,c,d}$	$1.25 \pm 0.03^{a,b,d}$	$1.50 \pm 0.00^{a,b,c}$	0.20 ± 0.03^{e}	1.0 ± 0.03^{e}
Penicillium funiculosum	MIC	$0.6 \pm 0.01^{b,d}$	$1.125 \pm 0.10^{a,c,d}$	$0.6 \pm 0.00^{b,d}$	$0.15 \pm 0.01^{a,b,c}$	0.20 ± 0.02^{e}	0.20 ± 0.06^{e}
	MFC	$1.15 \pm 0.13^{b,c,d}$	$3.25 \pm 0.13^{a,c}$	$1.25 \pm 0.01^{a,b,d}$	$3.125 \pm 0.13^{a,c}$	0.25 ± 0.03^{e}	0.50 ± 0.00^{e}
Penicillium ochrochloron	MIC	0.6 ± 0.06^{d}	0.6 ± 0.06^{d}	0.6 ± 0.06^{d}	$0.78 \pm 0.00^{a,b,c}$	0.20 ± 0.06^{e}	2.5 ± 0.13^{e}
	MFC	$1.15 \pm 0.10^{b,c,d}$	$2.35 \pm 0.10^{a,c,d}$	$1.25 \pm 0.00^{a,b,d}$	$3.125 \pm 0.10^{a,b,c}$	0.25 ± 0.06^{e}	3.5 ± 0.13^{e}
Penicillium verrucosum	MIC	$0.6 \pm 0.00^{b,d}$	$2.35 \pm 0.00^{a,c,d}$	$0.6 \pm 0.03^{b,d}$	$3.125 \pm 0.00^{a,b,c}$	0.10 ± 0.00^{e}	0.20 ± 0.06^{e}
	MFC	$1.15 \pm 0.10^{b,c,d}$	$9.4 \pm 1.70^{a,c,d}$	$1.25 \pm 0.10^{a,b,d}$	$6.25 \pm 1.7^{a,b,c}$	0.20 ± 0.01^{e}	0.30 ± 0.00^{e}

[a] MIC – minimum inhibitory concentration; MFC – minimum fungicidal concentration. In each row different letters mean significant difference between the extracts at the same concentrations ($p < 0.05$). Statistically significant difference in comparison with *A. bisporus* methanol extract; statistically significant difference in comparison with *A. bisporus* ethanol extract; statistically significant difference in comparison with *A. brasiliensis* methanol extract; statistically significant difference in comparison with *A. brasiliensis* ethanol extract; and statistically significant difference in comparison with extracts.

Table 6 Growth inhibition of *L. monocytogenes* in yoghurt by the ethanolic extracts of the studied *Agaricus* species[a]

	A. bisporus			*A. brasiliensis*		
	Listeria monocytogenes					
	10^3 CFU mL^{-1}	10^5 CFU mL^{-1}	10^7 CFU mL^{-1}	10^3 CFU mL^{-1}	10^5 CFU mL^{-1}	10^7 CFU mL^{-1}
MIC (mg mL^{-1})	$4.69 \pm 0.01^{b,c}$	9.38 ± 0.02^{a}	9.38 ± 0.02^{a}	$1.17 \pm 0.01^{b,c}$	2.34 ± 0.02^{a}	2.34 ± 0.02^{a}
MBC (mg mL^{-1})	$9.38 \pm 0.01^{b,c}$	18.75 ± 0.02^{a}	18.75 ± 0.02^{a}	$2.34 \pm 0.01^{b,c}$	4.69 ± 0.02^{a}	4.69 ± 0.01^{a}

[a] CFU – colony-forming units; MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration.

presence of cinnamic and *p*-coumaric acids.[24] Therefore, and due to these apparent differences, it is crucial to perform chemical studies and compare profiles.

Both specimens seem to be an excellent option as the food concerning content of fatty acids; *A. brasiliensis* may be a better choice since it has a lower percentage of SFA. Ozturk *et al.*[40] revealed a slightly lower SFA content (20.28%) and higher levels of unsaturated fatty acids (79.72%) in a wild specimen of *A. bisporus* from Turkey. Yilmaz *et al.*[41] also studied a wild sample from Turkey revealing a similar profile to the one shown herein. Reis *et al.*[19] revealed different fatty acid compositions in a commercial sample of *A. bisporus* from Portugal, revealing higher PUFA (78.3%) and lower MUFA (1.4%) contents.

Although organic acids are a product of the primary metabolism, some of them may also have bioactive properties such as malic acid. Malic acid was the major compound found in *A. bisporus*, and the employment of this organic acid for the preparation of food additives and synthesis of various fine chemicals has been reported.[42–44]

Different isoforms of tocopherols (α-, γ- and δ-) were quantified in the studied mushrooms. Although the last isoform (δ-tocopherol) was only found in *A. bisporus*, *A. brasiliensis* presented a higher concentration of tocopherols due to the contribution of γ-tocopherol. These isoforms were also detected in other cultivated specimen and reported by other studies.[45,46] The tocopherol content in mushroom can have a high variation in the amounts found, even if the same isoforms were detected. This study proves that the profile of tocopherols is similar to other specimens, but the quantities present can have some variations, highlighting that tocopherols are compounds with a high variability probably due to their easy degradation by oxidation processes. Ergosterol, a sterol found in the cell wall of fungi is reported as having antitumor and immunomodulatory effects.[40] This compound was detected in both *Agaricus* specimens, which proves, that fungi are an excellent option as food because of their medicinal properties, namely antitumor and immunomodulating effects.[5] Ergosterol contents in *A. bisporus* have been extensively studied, ranging from 204–780 mg per 100 g of dry weight.[47,48] Villares *et al.*[49] and Barreira *et al.*[23] studied commercial samples from Spain and Portugal, respectively, both presented a higher content of ergosterol (642 and 352 mg per 100 g dw, respectively). Nevertheless, to our knowledge, there are no studies reporting the amounts of ergosterol in *A. brasiliensis*.

It must be noted that the variation in the chemical composition among samples of different origins might be related to environmental temperature, relative humidity during growth and the relative amount of water produced or used during storage, as well as to the industrial processes to which the commercial mushrooms are submitted.[34] For cultivated mushrooms, the type and amount of compost and fertilizer used can also have influence on the results.[50] Differences found in individual compounds can also be attributed to the diversity of extraction, derivatization or even quantification methods employed.[19,22,24] Therefore, it is very important to determine these compounds, even in well studied samples such as *A. bisporus*.

Antimicrobial activity of three *Agaricus* specimens was also recently published by Ozturk *et al.*[40] who described effects of methanolic extracts against six species of Gram-positive bacteria, seven species of Gram-negative bacteria and two species of yeast.

Mazzutti *et al.*,[31] have reported that different extraction techniques and solvents applied to *A. brasilensis* conducted to different activities against *B. cereus* and *S. aureus*. These activities were lower in comparison with our results and were in the range of 0.25 mg mL^{-1} to more than 2 mg mL^{-1}. For ethanolic extract, the activity on the mentioned bacteria was more than 2 mg mL^{-1}.[31] Antibacterial activity of the *A. brasilensis* ethanolic extract (50, 75 and 100%) was obtained as 0.08–0.44 mg mL^{-1} towards *S. mutans* and *S. sobrinus*.[32]

The ability of *L. monocytogenes* to survive in a wide range of adverse conditions (acidic pH, low temperatures, and high sodium chloride concentrations), makes it difficult to control this organism in food.[51] For this reason, there is a special attention of science and industry in achieving food safety by adding some natural antibacterial products, without compromising the sensory and nutritional qualities of food.[52]

The results for antimicrobial activity against *L. monocytogenes* are better *in vitro* tested by the microdilution method than in the yoghurt experiment. This might be explained by higher complexity of yoghurt than of the conventional laboratory media. *A. brasiliensis* ethanolic extract proved to be more efficient than that of *A. bisporus* in controlling *L. monocytogenes* growth in yoghurt.

Overall, these specimens were found to be good sources of different bioactive compounds that may be considered nutraceuticals (*e.g.* phenolic acids, unsaturated fatty acids, tocopherols and ergosterol). This work also intended to prove that we

could take advantage of the beneficial properties of mushrooms and use them as food preservatives in different products such as yoghurts. Thus, this study brings additional biochemical characteristics for these cultivated mushroom species, which can be applied in the food industry as natural preservatives.

Conflict of interest

The authors declare that they have no conflict of interest.

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