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ORIGINAL ARTICLE

Nutritional value, chemical composition, antioxidant activity and enrichment of cream cheese with chestnut mushroom *Agrocybe aegerita* (Brig.) Sing

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Abstract A very well-known and appreciated mushroom, *Agrocybe aegerita* (Brig.) Sing, was the subject of chemical profiling, antioxidant assays and sensory evaluation test in cream cheese. Methanolic extract obtained from a wild sample of *A. aegerita* fruiting body was fully chemically identified. Sample was found to be rich in carbohydrates (84.51 g/100 g dw), ash and proteins (6.69 g/100 g dw and 6.68 g/100 g dw, respectively). Trehalose was the main free sugar while malic acid was the most abundant organic acid. Four isoforms of tocopherols were identified; γ- tocopherol was the dominant isoform with 86.08 μg/100 g dw, followed by β-tocopherol, δ-tocopherol and α-tocopherol (8.80 μg/100 g dw, 3.40 μg/100 g dw and 2.10 μg/100 g dw, respectively). Polyunsaturated fatty acids were predominant, with linoleic acid as the most prominent one (78.40 %). Methanolic extract of chestnut mushroom exhibited high antioxidant activity. Sensory evaluation test included grading by panelists and comparing the overall acceptability of cream cheese alone and enriched cream cheese with dry powder of *A. aegerita*. General conclusion of the participants was that the newly developed product was more likeable in comparison to cream cheese alone. Due to the health-beneficial effects of antioxidants and wealth of chemically identified nutrients, *A. aegerita* is a promising starting material for incorporation on larger scale products.

Keywords *Agrocybe aegerita* · Chemical profile · Antioxidant potential · Cream cheese · Sensory evaluation test

Research highlights
- *Agrocybe aegerita* proved to be a rich source of linoleic acid.
- Analysis of the extract showed high content of carbohydrates and proteins.
- Malic acid was the most abundant organic acid and γ-tocopherol was the most abundant isoform.
- Methanolic extract exhibited high antioxidant activity.
- Dried fruiting body proved to be a great enhancer of flavor when incorporated in cream cheese, based on the results of sensory evaluation.

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Introduction

Mushrooms are generally known as a valuable source of nutrients, some of which have medicinal properties. Due to their unique aroma and flavor, they are widely used as culinary delicacy in many countries (Ribeiro et al. 2007). In recent decades, due to the rising trend of a number of diseases (including auto-immune diseases, as well as cancer, hypertension, diabetes etc.) there is a public pressure to explore the possibilities of alternative therapeutic agents (Ergönül et al. 2013). Wild-growing mushrooms accumulate a number of compounds: carbohydrates, fibers, vitamins, minerals, proteins, fats and different secondary metabolites with proven antimicrobial, antitumor, antifungal, antioxidant activity (Ribeiro et al. 2007; Ergönül et al. 2013; Wasser and Weiss 1999; Diyabalange et al. 2008). Medicinal mushrooms should not be claimed to cure disease since the mechanism of their

action is not revealed, but recent studies strongly indicate that mushrooms have a role in disease prevention, and suppression or remission of a diseased state (Chang and Miles 2004). Some common edible mushrooms like *G. lucidum* or *L. edodes* (Popović et al. 2013; Ferreira et al. 2014; Stojković et al. 2014) are subject of intense studies for quite some time, so the current focus is on isolated substances or crude extracts derived from lesser-known edible mushrooms (Boh et al. 2007; Altobelli 2011). They are the potential source of diverse biomolecules with nutritional and/or medicinal properties (Alves et al. 2012). This fact made them promising candidates for the development of medicines and food supplements. Furthermore, wild mushrooms have also emerged as a source of antioxidant compounds which are important in the process of eliminating free radicals and other reactive radical species produced as a part of the normal process of aerobic metabolism. Free radicals are responsible for the structural damage of cells, which is correlated with various types of cancer, cardiovascular diseases or diabetes (Lo and Cheung 2005; Lindequist et al. 2005).

Agrocybe aegerita (Brig.) Sing is a white rot basidiomycete, commercially cultivated in Italy and highly appreciated as a delicacy (so called Pioppino mushroom) (Ullrich et al. 2004). The common name for the mushroom is black poplar mushroom or chestnut mushroom, since it is often found on poplar wood-logs (Diyabalange et al. 2008). It is found in North America, Europe and Asia (Ullrich et al. 2004).

To the best of our knowledge, scientific data on the detailed chemical profile as well as on the biological activity (beyond the scope of enzymes) are very scarce; therefore a study covering these fields was conducted. In the present study, a methanolic extract obtained from a wild sample of *A. aegerita,* collected in Serbia, was explored for its antioxidant potential. Furthermore, being an edible species, the mushroom was fully characterized regarding nutritional properties, hydrophilic and lipophilic compounds, and was the subject of sensory evaluation test after incorporation in cream cheese.

Material and methods

Mushroom species

Agrocybe aegerita was collected from the wood logs of poplar trees at Jabučki rit (Northern Serbia) during April 2012 and authenticated by Dr Jasmina Glamočlija (Institute for Biological Research "Siniša Stanković"). Voucher specimen has been deposited at the Fungal Collection Unit of the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia, under number Aa-001-2012. Fresh fruiting bodies were randomly divided to smaller samples and freeze-dried by lyophilization (LH Leybold, Lyovac GT2, Frenkendorf). When reaching constant mass, specimens were milled to a fine powder, mixed to obtain an homogenate sample, and kept at +4 °C untill further analysis.

Chemical characterization

Nutritional value

The samples were analysed for their chemical composition (moisture, proteins, fat, carbohydrates and ash) through AOAC procedures (AOAC 1995). 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).

Sugars

Following the extraction procedure described by Reis et al. (2012a), 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 autosampler (AS-2057 Jasco), coupled to a refraction index detector (RIdetector Knauer Smartline 2300). Sugars identification was made by comparing the relative retention times of sample peaks with standards. Data were analyzed using Clarity 2.4 Software (DataApex). Quantification was based on the RI signal response of each standard, using the internal standard (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. (Barros et al. 2013), 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.

Fatty acids

Following the extraction and trans esterification procedures described by Reis et al. (2012a), fatty acids were determined using a gas chromatographer (DANI 1000) equipped with a

split/splitless injector and a flame ionization detector (GC-FID). Fatty acids identification was made by comparing the relative retention times of the fatty acid methyl esters (FAME) standards (standard 47885-U, Sigma, St. Louis, MO, USA), with the samples. 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. (2010), tocopherols were determined by 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.

Preparation of the extract

Mushroom powder (10 g) was extracted with 240 mL of methanol overnight at −20 °C. Extract was sonicated for 15 min, then centrifuged at 4000*g* for 10 min at +4 °C and subsequently filtered through Whatman No. 4 paper (Vaz et al. 2010). The residue was then re-extracted with three additional portions of methanol (3 × 100 mL) following the same procedure (ultrasonic bath and filter paper). The combined extract was evaporated at 40 °C (rotary evaporator Büchi R-210) to dryness. Prior to analyses, extract was dissolved in appropriate solvent.

Antioxidant activity

Successive dilutions were made from the stock solution and antioxidant activity of the samples was evaluated by different in vitro assays already described by Stojković et al. (2013) to evaluate the antioxidant activity of the samples. The sample concentrations (mg/mL) 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 a positive control.

Folin–Ciocalteu assay

One of the extract solutions (5 mg/mL; 1 mL) was mixed with Folin–Ciocalteu reagent (5 mL, previously diluted with water 1:10, *v*/*v*) and sodium carbonate (75 g/L, 4 mL). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40 °C for color 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 the Folin–Ciocalteu reagent by the samples was expressed as mg of gallic acid equivalents (GAE) per g of extract.

Ferricyanide/Prussian blue assay

The extract solutions with different concentrations (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, 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 deionized water (0.8 mL) and ferric chloride (0.1 %*w*/*v*, 0.16 mL), and the absorbance was measured at 690 nm in 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). The mixture was left to stand for 30 min in the dark, and the absorbance was measured at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: % RSA = $[(A_{DPPH}\text{-}A_S)/A_{DPPH}] \times 100$, 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 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: Inhibition ratio (%) = [(A–B)/A] × 100 %, where A and B were the absorbance of the control and the sample solution, respectively.

Enrichment of cream cheese with *A. aegerita* powder

Cream cheese

Full-fat cream cheese "a la Kajmak", produced from cow milk by Mlekara Šabac was purchased from a local supermarket and kept in refrigerator at +4 °C until further analysis. All the samples were used before expiry date of the product. Composition of the cream cheese stated on the packaging was- energy: 242 kcal; fat: 23.5 g; proteins: 6.1 g and carbohydrates: 1.53 g including lactose: 1.1 g, all values expressed by 100 g.

The packaging showed the presence of no artificial preservatives. Experiments of inoculating Malt Agar (MA) and Muller–Hinton Agar (MHA) plates with cheese diluted 1 in 10 with phosphate buffered saline (PBS) and kept at 25°C and 37 °C, for 48 h, showed no bacterial nor fungal contamination of the product.

Sensorial evaluation of new functional product

The test was attended by 75 untrained participants, all staff members of Department of Plant Physiology of Institute for Biological Research "Siniša Stanković". The overall acceptance, smell and taste of the product were evaluated using the method described by Reis et al. (2012b). A full-fat cream cheese, product of Šabac Dairy (Serbia) was used. Cream cheese was used as a control in relation to a new product-cream cheese enriched with dry powder of mushroom *Agrocybe aegerita* and was evaluated by the participants. The product was made simply by mixing the dry powder of *Agrocybe aegerita* (3 g/100 g of cream cheese) with cream cheese allowing the mixture to stand for 24 h (in order to allow cream cheese to fully absorb the taste and smell of mushroom). Participants were asked to evaluate overall acceptance of cream cheese alone and cream cheese enriched with mushroom powder on a scale 1–5 (1-extremely dislike, 2-dislike, 3-neither like nor dislike, 4-like, 5-extremely like). Results were averaged by number of participants.

Furthermore, nutritional value of the enriched cream cheese was calculated again by taking into account the nutritional value of the cream cheese stated on the label of the product, and the content of fat, proteins and carbohydrates from chemical analysis of *A. aegerita*.

Results and discussion

Chemical composition

Results regarding the nutritional value of *A. aegerita* are presented in Table 1. Carbohydrates and proteins are the most abundant compounds (84.51 g/100 g dw and 6.68 g/100 g dw, respectively). Mushrooms are generally considered to be a good source of digestable proteins, and are reported to contain all the essential amino acids needed in the human diet (Mshandete and Cuff 2007). Ash content was low (6.69 g/100 g dw); *A. aegerita* was also poor in fat (2.13 g/100 g dw) and had low caloric value (383.91 kcal/100 g dw), which makes this mushroom a good candidate for low-caloric diets (Table 1). Trehalose was the dominant sugar (12.49 g/100 g dw), while sugar alcohol mannitol was present at 0.93 g/100 g dw (Table 1). This information is consistent with previously published data related to the sugar profile in mushrooms (Bernas et al. 2006). Organic acids are amongst the many antioxidant compounds determined in mushrooms (Ribeiro et al. 2007; Cámara et al. 1994). They play a decisive role in determining organoleptic properties of mushrooms, giving them a distinctive taste and smell (Ribeiro et al. 2007; Cámara et al. 1994; Valentão et al. 2005). Organic acids are known for their chemical stability and slow changes during storage. Also, there is a possibility of a protective role against various diseases (Bernas et al. 2006). As for the chemical profiles of organic acids, the presence of four organic acids was determined. Malic acid was the most abundant organic acid (1.82 g/100 g dw), followed by citric acid (0.88 g/100 g), then fumaric acid (0.26 g/100 g) and oxalic acid (0.09 g/100 g) (Table 1). Total content was 3.06 g/100 g dw.

As for the fatty acid composition, the most dominant fatty acid in *A. aegerita* is linoleic acid (78.40 %), (Table 2), followed by palmitic (13.07 %), oleic and stearic acids (3.03 % and 2.13 %, respectively). Other fatty acids were represented with a share less than 1.00 %. The main fatty acids found by Shuai et al. (2012) in *A. aegerita* were linoleic acid (C18:2n6c) >

Table 1 Nutritional value and hydrophilic compounds of *Agrocybe aegerita* (mean±SD)

Ash (g/100 g dw)	Proteins (g/100 g dw)	Fat (g/100 g dw)	Carbohydrates (g/100 g dw)	Energy (kcal/100 g dw)
6.69±0.33	6.68±0.26	2.13±0.02	84.50±0.24	383.90±1.02
Mannitol (g/100 g dw)	Trehalose (g/100 g dw)	Total (g/100 g dw)		
0.93±0.01	12.49±0.09	13.42±0.08		
Oxalic acid (g/100 g dw)	Malic acid (g/100 g dw)	Citric Acid (g/100 g dw)	Fumaric Acid (g/100 g dw)	Total (g/100 g dw)
0.09±0.01	1.82±2.21	0.88±0.01	0.26±0.01	3.06±2.23

dw dry weight

palmitic acid (C16:0) > oleic acid (18:1n9c). A total FA value of 33.13 mg/10 g was reported in literature for *A. aegerita*, while ratio between unsaturated fatty acids and saturated fatty acids was 3.80. The prevalence of PUFA over MUFA (in case of *A. aegerita* 78.60 % over 3.47 %), and the determination of high amount of linoleic acid is significant factor in defining the mushroom as healthy food (Chang and Miles 2004). Linoleic acid (omega-6 fatty acid) which is essential for human organism is the precursor of mushroom aromatic compounds, giving them their specific taste (Ribeiro et al. 2007). The intake of dietary fats through food is necessary for optimal functioning and balance of fats in the organism (Ribeiro et al. 2007; Ergönül et al. 2013). Unsaturated fatty acids are essential for our health, having a strong beneficial effect in the prevention and management of cardiovascular diseases, triglyceride level, blood pressure etc., whereas saturated fatty acids, which are present in higher amounts in food of animal origin, are associated with increased levels of triglycerides in the blood and commonly are associated with hypertension etc. (Simpoulos 1999). Previously published data, including information obtained in this study indicate that mushrooms have high share of dietary fats. The proportion of lipids goes from 1.75 % in fresh per 100 g fruiting bodies to 5.5 % in dried mushrooms, where they have important role in biochemical processes (Barros et al. 2008). Nearly 75 % of total fatty acids have been determined to be unsaturated in selected mushrooms (*Volvariella volvacea*, *Lentinula edodes*, *Agaricus bisporus*, *Auricularia auricula*, *Tremella fuciformis*). The high content of unsaturated fatty acids is due to the linoleic acid which accounts for 76 % in *L. edodes*, 70 % in *V. volvacea*, and 69 % in *A. bisporus* (Chang and Miles 2004). Our chemical analysis revealed the presence of a group of related compounds with similar chemical structure called tocopherols. Tocopherols are a highly represented group of compounds in wild mushrooms having the antioxidant role. They play a role in preventing degenerative damage caused by oxidative stress. The usual tocopherol concentration in mushrooms is rather lower than those in plants and is 40–600 μg/100 g dw. It is also determined that cultivated species (588.24 μg/100 g dw) have lower tocopherol concentration than the wild-growing (45.01 μg/100 g dw), (Stojković et al. 2013). In the studied mushroom, γ-tocopherol was the most abundant isoform (86.08 μg/100 g dw), followed by β-tocopherol (8.80 μg/100 g dw), δ-tocopherol (3.40 μg/100 g dw) and α-tocopherol (2.10 μg/100 g dw) (Table 2).

Table 2 Lipophilic compounds of *Agrocybe aegerita* (mean±SD)

C6:0	0.10±0.01
C8:0	0.13±0.01
C10:0	0.08±0.01
C12:0	0.06±0.01
C14:0	0.23±0.01
C14:1	0.01±0.00
C15:0	0.43±0.01
C16:0	13.07±0.11
C16:1	0.29±0.01
C17:0	0.25±0.01
C18:0	2.13±0.02
C18:1n9	3.03±0.01
C18:2n6	78.40±0.10
C18:3n3	0.07±0.01
C20:0	0.49±0.01
C20:1	0.05±0.01
C20:2	0.03±0.00
C20:3n3+C21:0	0.08±0.01
C20:5n3	0.02±0.00
C22:0	0.40±0.01
C22:1n9	0.02±0.01
C23:0	0.11±0.01
C24:0	0.45±0.01
C24:1	0.07±0.02
Total SFA (% of total FA)	17.93±0.07
Total MUFA (% of total FA)	3.47±0.03
Total PUFA (% of total FA)	78.60±0.10
α-Tocopherol	2.10±1.10
β-Tocopherol	8.80±2.20
γ-Tocopherol	86.08±12.90
δ-Tocopherol	3.40±0.20
Total (μg/100 g dw)	100.38±13.81

dw dry weight, *FA* Fatty acids, *SFA* Saturated fatty acids, *MUFA* Monounsaturated fatty acids, *PUFA* Polyunsaturated fatty acids

From the obtained results, it can be concluded that tocopherols are highly represented group of compounds in wild growing mushroom. For a long time, α-tocopherol was considered to be the most active form of vitamin E and was reported to have the highest biological activity. However, recent studies have shown that the other forms are also active (Heleno et al. 2010).

To our knowledge no chemical profiling of fatty acids, organic acids and tocopherols has been performed on *A. aegerita*. Instead, accessible data revealed a number of compounds of different chemical nature that are determined in various extracts of genus *Agrocybe*. Diyabalange et al. (2008) described the presence of ceramide, methyl-β-D-glucopyranoside and α-D-glucopyranoside, along with linoleic acid and its methyl ester. Our studies are in accordance with previous studies reporting the presence of palmitic acid, linoleic acid, mannitol and trehalose in the fruiting body of *A. aegerita* (Valentão et al. 2005). A novel lectin was isolated from aqueous extract of *A. aegerita* from China by affinity chromatography (Zhao et al. 2003). *A. aegerita* was also reported to contain several bioactive metabolites, such as indole derivatives with free radical-scavenging ability (Kim et al. 1997), polysaccharides with hypoglycemic activity (Tadashi et al. 1994) and agrocybin, a peptide with anti-fungal activity (Ngai et al. 2005). Previously, Gao et al. (Gao et al. 2010) reported that the protein components from *A. aegerita* showed tumor rejection activity. Also two antitumor lectins, AAL and AAL-2, were identified from the protein components of *A. aegerita* (Zhao et al. 2003; Feng et al. 2010).

Antioxidant activity

Antioxidant activity of the methanolic extract was measured by four different methods (Table 3). These assays measured free radical scavenging activity, reducing power and lipid peroxidation inhibition. Concerning the Folin-Ciocalteu assay, higher values mean higher reducing power; for the other assays, the results are presented in EC_{50} values, what means that higher values correspond to lower reducing power or antioxidant potential. The extract gave 17.36 mg GAE/g extract in the Folin-Ciocalteu assay, and revealed high DPPH radical-scavenging activity assay (EC_{50}=7.23 mg/mL). Slightly higher effect was observed in the β-carotene/linoleate assay (EC_{50}=6.11 mg/mL), while Ferricyanide/Prussian blue assay and TBARS assays showed even higher effects (EC_{50}=2.66 mg/ml; EC_{50}=0.39 mg/mL, respectively). The same behavior was previously reported for other mushroom species. The observed antioxidant activity may be the consequence of the presence of different antioxidant compounds described in the previous section such as tocopherols (mainly α-tocopherol) and organic acids and due to the phenolic acids (Petrovic et al. 2014) presented in *Agrocybe aegerita*. Lo and Cheung (2005) reported antioxidant activity of the methanol crude extract of *A. aegerita* and its fractions, isolated by liquid–liquid partition, using scavenging activity of 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation (ABTS) and inhibition of lipid peroxidation of rat brain homogenate. The ethyl acetate (EA) fraction, which showed the most potent antioxidant activity in the mentioned two assays (0.254 mM Trolox per mg of sample and 0.0502 mg/mL, respectively), was further fractionated by a Sephadex LH-20 column into four subfractions (EA1–EA4). EA3 exhibited the strongest radical-scavenging activity in the ABTS (0.934 mM Trolox per mg of sample) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (0.139 mg/mL), and showed a similar extent of in vitro inhibition of human LDL oxidation to caffeic acid. Significant correlation was found between the total phenolic content and the antioxidant activity (p<0:01) in the EA fraction and its subfractions.

Sensory evaluation test

Our attempt to combine science, the food industry and consumers interest resulted in creation of new product that has been chemically defined and tested among participants for its acceptability. Cream cheese enriched with dry powder of *A. aegerita* is a unique product with an increased nutritional value and praised by majority of the participants who are a part of sensory evaluation test. The results of sensorial evaluation are presented in the Table 4. From the above table it is evident that panelists liked cream cheese enriched with *A. aegerita* more in comparison to cream cheese alone. The average grade of cream cheese alone was 3.86 while of cream cheese enriched with *A. aegerita* powder was 4.39. Overall, acceptance of cream cheese with addition of the mushroom powder was graded higher and was more acceptable to majority of panelists in comparison to cream cheese alone. This can be considered a pilot experiment in which it can

Table 3 Antioxidant activity of *Agrocybe aegerita* methanolic extract (mean±SD)

Reducing power		Scavenging activity	Lipid peroxidation inhibition	
Folin-Ciocalteu (mg GAE/g extract)	Ferricyanide/Prussian blue (EC_{50}; mg/mL)	DPPH scavenging activity (EC_{50}; mg/mL)	B-carotene/linoleate (EC_{50}; mg/mL)	TBARS (EC_{50}; g/mL)
17.36±0.88	2.66±0.10	7.23±0.18	6.11±1.60	0.39±0.06

EC_{50} Extract concentration corresponding to 50 % of antioxidant activity or 0.5 of absorbance for the Ferricyanide/Prussian blue assay, *GAE* Gallic acid equivalents

Table 4 Sensorial evaluation and nutritional value of the cream cheese and cream cheese enriched with *A. aegerita* powder

Overall acceptability[a]	Cream cheese 3.86±0.68	Cream cheese + *A. aegerita* 4.39±0.48
Proteins (g/100 g product)	6.1	6.3
Fat (g/100 g product)	23.5	23.7
Carbohydrates (g/100 g product)	1.53	4.07
Energy (kcal/100 g product)	242	255

1 = extremely dislike, 2 = dislike, 3 = neither like nor dislike, 4 = like; 5 = extremely like

[a] The results are expressed as the average of all grades

be determined how the addition of mushroom powder enhanced the overall nutritional value, taste and smell of cream cheese (Table 4). Energetic value of cream cheese alone was 242 kcal/100 g and of cream cheese enriched with *A. aegerita* powder was 255 kcal/100 g of product. Also, our sensory evaluation test revealed the general mood of participants to accept such a product, as it is cost-effective, which is the important factor for production on a larger scale. It should be noted that the enriched product benefits the consumers in nutritional sense, along with the fact that results of our sensory evaluation test showed that this product was highly acceptable for the consumers. Namely, in the 2012, Brennan and the associates (Brennan et al. 2012) used *A. aegerita's* spent compost (hyphae and the base of the mushroom), a food waste from its production, and incorporated it in the form of flour in ready-to-eat extruded cereal snack product. The improvement of nutritional value of snack bars, as well as other products widely used (including dairy, meat products etc.) is a necessity due to public demand for healthy food. Low fat and high carbohydrate content make mushrooms great candidates for healthier processed food.

Conclusion

One of the reasons why mushroom extracts/preparations are not available to broad masses is perhaps inconsistence in amount of fruiting bodies and chemical similarities. More precisely, the amount of available wild mushrooms varies depending on a number of factors (humidity, the availability of hosts etc.). Also, if they are harvested from different locations, it may result in discrepancies in chemical profile, and subsequently in different activity. Since *A. aegerita* is part of the mushroom cultivation, and the fungal material is primarily available, attention should definitely be dedicated to it. According to chemical profile and bioactivities presented herein, *A. aegerita* was explored regarding antioxidant purposes. We have shown that edible *A. aegerita* possessed functional compounds with regards to the identified organic acids, fatty acids and tocopherols. The new product enriched with *A. aegerita* might be a functional food due to the health-beneficial effects of incorporated mushroom powder.

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