



## An insight into antidiabetic properties of six medicinal and edible mushrooms: Inhibition of $\alpha$ -amylase and $\alpha$ -glucosidase linked to type-2 diabetes

D. Stojkovic<sup>a</sup>, M. Smiljkovic<sup>a</sup>, A. Ciric<sup>a</sup>, J. Glamoclija<sup>a</sup>, L. Van Griensven<sup>b</sup>, I.C.F.R. Ferreira<sup>c</sup>, M. Sokovic<sup>a,\*</sup>

<sup>a</sup> Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia

<sup>b</sup> Plant Research International, Wageningen University and Research, P.O. Box 16, 6700 AA Wageningen, Netherlands

<sup>c</sup> Mountain Research Centre (CIMO), School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, apartado 1172, 5301-855 Bragança, Portugal

### ARTICLE INFO

#### Article history:

Received 24 October 2017

Received in revised form 15 December 2017

Accepted 6 January 2018

#### Keywords:

Diabetes mellitus

$\alpha$ -Amylase

$\alpha$ -Glucosidase

Mushroom

Edible and medicinal

### ABSTRACT

As a continuation of our search for biologically active mushroom species the present study investigates *in vitro* antidiabetic properties of six edible and medicinal mushroom species: *Agaricus blazei* Murrill, *Coprinus comatus* (O.F.Müll.) Pers., *Cordyceps militaris* (L.) Fr., *Inonotus obliquus* (Ach. ex Pers.) Pilát, *Morchella conica* Pers. and *Phellinus linteus* Berk. & M.A. Curtis. *In vitro* assays on  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme inhibition were performed with methanolic extracts of the selected mushrooms. Furthermore, we calculated the necessary daily intake of mushroom extracts and dry mushroom powders based on the equivalent doses of therapeutic drug acarbose given to diabetic patients per day. Our comparative study on enzyme inhibition showed that the most promising potential is ascribed to *I. obliquus* extract, while no inhibition of  $\alpha$ -amylase was recorded with *M. conica* and *C. militaris* methanolic extract at the tested concentration. This comparative study is the first highlighting *in vitro* antidiabetic potential by inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase with methanolic extracts; which makes the investigated species more promising for the diabetes type-2 treatment by an additional and different mechanism of action.

© 2018 Published by Elsevier B.V. on behalf of SAAB.

### 1. Introduction

Mushrooms are important dietary components in some cultures, consumed in daily diet as supplementary food since olden times (Soković et al., 2016). Mushroom extracts are increasingly consumed because of their health beneficial effects, including the enhancement of immune function and antitumor activity (Popović et al., 2013). It is confirmed that mushroom extracts contain diverse compounds such as alkaloids, fibers, lectins, protein, polyphenols and polysaccharides, which are contributing to their biological activities (Popović et al., 2013). Mushrooms are thought to exert many pharmacological functions such as antitumor, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolemic, antihypertensive, antiplatelet-aggregating, antihyperglycemic, antimicrobial and other activities (Lindequist, 2013; Paterson and Lima, 2014). Mushrooms as functional food and dietary supplements can help in the intervention of sub-health states and may prevent the full-blown consequences of life-threatening diseases. An equilibrated diet linked with the mushroom consumption has an advantage of the nutritional/medicinal features of mushrooms (Soković et al., 2016) and their pharmacological

applications. The term "mushroom" is used here for a medicinal/edible fruiting body of higher fungi.

*Diabetes mellitus* is a chronic disorder of metabolism followed by abnormal rise in plasma glucose levels, as a consequence of unequilibrated insulin production and/or insensitivity to the effect of this hormone in signal transduction of cellular receptors. These metabolic changes are accompanied by modifications in carbohydrate, lipid and protein metabolism. Most of the diabetes type 2 complications in patients are due to hyperglycemia as their main cause (Ortiz et al., 2007; Shobana et al., 2009). One of the effective strategies for diabetes type-2 management is the inhibition of complex polysaccharide hydrolysis by pancreatic  $\alpha$ -amylase and absorption limitation of glucose by inhibiting intestinal  $\alpha$ -glucosidase enzyme. Acarbose, miglitol, voglibose are commercial drugs used for  $\alpha$ -glucosidase inhibition in the control of the diabetic disease (Saito et al., 1998). Chronic  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitions may be useful in the management of type-2 diabetes and obesity (Melo et al., 1999; Koike, 2005). The drugs currently used as reversible inhibitors of  $\alpha$ -amylase and  $\alpha$ -glucosidase for the treatment of diabetic patients exhibit side effects such as abdominal distension, meteorism, bloating, flatulence and possibly diarrhea (Fujisawa et al., 2005).

To continue our investigations (Stojković et al., 2013; Reis et al., 2014; Stojković et al., 2014; Bizarro et al., 2015; Glamoclija et al.,

\* Corresponding author.

E-mail address: [mriss@ibiss.bg.ac.rs](mailto:mriss@ibiss.bg.ac.rs) (M. Sokovic).

2015; Vieira et al., 2016) on biological activities of mushroom species, we designed this study to explore the antidiabetic properties of six edible and medicinal mushrooms: *Agaricus blazei* Murrill, *Coprinus comatus* (O.F.Müll.) Pers., *Cordyceps militaris* (L.) Fr., *Inonotus obliquus* (Ach. ex Pers.) Pilát, *Morchella conica* Pers. and *Phellinus linteus* Berk. & M.A. Curtis. *In vitro* assays on  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme inhibition were performed with methanolic extracts of the selected mushrooms.

## 2. Material and methods

### 2.1. Mushroom samples

Six edible and medicinal mushroom species were used in this study: *A. blazei* Murrill (commercial sample), *C. comatus* (O.F.Müll.) Pers. (collected - wild growing in Serbia), *C. militaris* (L.) Fr. (commercial sample), *I. obliquus* (Ach. ex Pers.) Pilát (commercial sample), *M. conica* Pers. (collected - wild growing in Serbia) and *P. linteus* Berk. & M.A. Curtis (commercial sample). The origin of samples, identification and preparation of dried powder were described previously (Stojković et al., 2013; Reis et al., 2014; Stojković et al., 2014; Bizarro et al., 2015; Glamočlija et al., 2015; Vieira et al., 2016).

### 2.2. Extraction procedures

Mushroom samples (~5 g) were extracted by stirring with 150 mL of methanol (–20 °C at 150 rpm) for 24 h and subsequently filtered through Whatman No. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland) to dryness.

### 2.3. $\alpha$ -Amylase and $\alpha$ -glucosidase inhibition assays

Mushroom extracts at different concentrations dissolved to 500  $\mu$ L and 500  $\mu$ L of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing Porcine pancreatic  $\alpha$ -amylase (Sigma Aldrich, Germany) (0.5 mg/mL) were incubated at 25 °C for 10 min. Then, 500  $\mu$ L of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixtures were incubated at 25 °C for 10 min and stopped with 1.0 mL of dinitrosalicylic acid color reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water, and absorbance measured at 540 nm using the spectrophotometer (Agilent 8453, Agilent Technologies, Waldbronn, Germany).

Mushroom extracts at different concentrations dissolved to 50  $\mu$ L and 100  $\mu$ L of  $\alpha$ -glucosidase (Sigma Aldrich, Germany) solution (1.0 U/mL) in 0.1 M phosphate buffer (pH 6.9) was incubated at 25 °C for 10 min. Then, 50  $\mu$ L of 5 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures

were incubated at 25 °C for 5 min. Then, 2 mL of Na<sub>2</sub>CO<sub>3</sub> was added to terminate the reaction before reading the absorbance at 405 nm in the spectrophotometer (Agilent 8453, Agilent Technologies, Waldbronn, Germany).

The  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities were expressed as percentage of inhibition (IC<sub>50</sub>). Acarbose was used as a positive control (Worthington, 1993; Apostolidis et al., 2007).

## 3. Results and discussion

The results of  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition linked to diabetes type-2 therapy are summarized in Table 1. *C. comatus* methanolic extract had the most prominent inhibitory activity on  $\alpha$ -amylase with IC<sub>50</sub> 714.45  $\mu$ g/mL, while *C. militaris* and *M. conica* did not have inhibitory potential on this enzyme. All of the investigated mushroom species inhibited  $\alpha$ -glucosidase with *I. obliquus* as the most potent inhibitor among the studied species (IC<sub>50</sub> 220.31  $\mu$ g/mL).

Table 1 also summarizes calculations of the daily intake of mushroom methanolic extracts and dry mushroom powders necessary to achieve the same effect as commercial therapeutic drug acarbose on the daily basis. Acarbose is given to diabetic patients in an initial dose of 3  $\times$  25 mg per day. Calculations were done on the basis of  $\alpha$ -glucosidase inhibition by acarbose. The lowest daily intake of mushroom powder was predicted for *I. obliquus* with the dose of 3  $\times$  1.148 g/day, while the highest was predicted for *P. linteus* 3  $\times$  2.215 g/day (Table 1).

Kim et al. (2005) demonstrated that  $\beta$ -glucans and their enzymatically hydrolyzed oligosaccharides from *Agaricus brasiliensis* (syn. *A. blazei*) show the activities of antihypercholesterolemic, antihypertriglyceridemic, antiarteriosclerotic and antihyperglycemic, indicating antidiabetic activity in diabetic rats. The data suggested that both  $\beta$ -glucans and oligosaccharides might enhance insulin secretion from pancreatic islets as well as proliferation of islets in diabetic or normal rats (Kim et al., 2005). The results of our study showed that *A. blazei* could also have an inhibitory influence on key enzymes involved in the degradation of complex sugars (Table 1), thus delaying the process of glucose uptake. Our previous study (Stojković et al., 2014) on chemical composition of *A. blazei* revealed that this species possessed oxalic, malic and fumaric organic acids. The total content of organic acids was 1.87 g per 100 g dw. The phenolic acids found in the studied mushroom were p-coumaric acid (0.28 mg per 100 g dw) and cinnamic acid (0.12 mg per 100 g dw), (Stojković et al., 2014). Different compounds present in methanolic extracts and their different combinations are probably linked to *A. blazei* enzyme inhibitory potential.

Recent studies described *in vivo* antidiabetic activity of different extracts and fractions of *C. comatus* (Yamaç et al., 2009; Ding et al., 2010; Zhou et al., 2015). According to our knowledge, no previous records were published on  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme inhibition with *C. comatus* methanolic extract. Methanolic extract of *C. comatus* studied here had inhibitory potential on both enzymes (Table 1). Regarding *C. comatus*, our previous study (Stojković et al., 2013) revealed organic acid profile, with five different compounds namely oxalic,

**Table 1**  
Inhibitory potential of mushroom species on  $\alpha$ -amylase and  $\alpha$ -glucosidase linked to type-2 diabetes treatment.

Mushroom species	Yield of extracts (%)	$\alpha$ -Amylase inhibition; IC <sub>50</sub> ( $\mu$ g/mL)	$\alpha$ -Glucosidase inhibition; IC <sub>50</sub> ( $\mu$ g/mL)	mg of extracts per day equivalent to acarbose 3 $\times$ 25 mg tablets given to a diabetic patient daily <sup>a</sup>	g of dry mushroom powder per day equivalent to acarbose 3 $\times$ 25 mg tablets given to a diabetic patient daily <sup>a</sup>
<i>Agaricus blazei</i>	14.24	1719.20 $\pm$ 89.53	357.23 $\pm$ 20.11	3 $\times$ 227.65	3 $\times$ 1.598
<i>Coprinus comatus</i>	10.57	714.45 $\pm$ 44.44	322.74 $\pm$ 11.21	3 $\times$ 205.67	3 $\times$ 1.945
<i>Cordyceps militaris</i>	13.25	na <sup>b</sup>	415.66 $\pm$ 36.12	3 $\times$ 264.88	3 $\times$ 1.999
<i>Inonotus obliquus</i>	12.23	830.32 $\pm$ 28.24	220.31 $\pm$ 7.37	3 $\times$ 140.40	3 $\times$ 1.148
<i>Morchella conica</i>	15.41	na	521.12 $\pm$ 16.45	3 $\times$ 332.09	3 $\times$ 2.155
<i>Phellinus linteus</i>	13.73	1479.46 $\pm$ 92.86	477.33 $\pm$ 17.55	3 $\times$ 304.19	3 $\times$ 2.215
Acarbose	–	87.15 $\pm$ 2.93	39.23 $\pm$ 5.41	–	–

<sup>a</sup> Calculated on the basis of  $\alpha$ -glucosidase inhibition.

<sup>b</sup> na – not active at the tested concentrations.

quinic, malic, citric and fumaric acids. Concerning phenolic acids, the studied sample revealed the presence of dominant *p*-hydroxybenzoic acid (0.09 mg/100 g) and presence of *p*-coumaric acid, as also the related compound cinnamic acid (Stojković et al., 2013). Phenolic compounds and organic acids are probably related to the anti-diabetic activity investigated herein.

Water and polysaccharidic extracts of *C. militaris* previously showed antidiabetic properties in diabetes induced rats (Liu et al., 2016). Our study showed that methanolic extract of *C. militaris* did not inhibit the activity of  $\alpha$ -amylase (Table 1), while it had inhibitory potential on  $\alpha$ -glucosidase. A recent study showed that water extract of *C. militaris* had inhibitory activities on  $\alpha$ -glucosidase with values of 45.77% with 0.1 mg/mL, 54.76% with 0.25 mg/mL, 58.74% with 0.5 mg/mL, and 60.5% with 1.0 mg/mL (Kim et al., 2017). The organic acid we found previously in higher amounts in *C. militaris* was citric acid 7.97 g/100 g (Bizarro et al., 2015). Regarding phenolic acids, *p*-hydroxybenzoic acid was the only compound found in the studied species (0.02 mg/100 g dw). However, cinnamic acid was also found (0.11 mg/100 g dw), (Bizarro et al., 2015). The link between chemical composition of methanolic extract of *C. militaris* and the enzyme inhibitory activity certainly exists.

Water extracts and polysaccharidic fractions of *I. obliquus* are recorded to possess *in vivo* antidiabetic properties (Qin et al., 2015). Extract of *I. obliquus* tested in our study possessed inhibitory potential of both enzymes investigated (Table 1). A previous study by Ying et al. (2014) revealed terpenoids from this species as inhibitors of glucosidase. In our previous study (Glamočlija et al., 2015) oxalic acid was the only organic acid detected in the extract of *I. obliquus* (72 mg/g extract). The phenolic acids found were gallic, protocatechuic and *p*-hydroxybenzoic acids, as also the related compound cinnamic acid (Glamočlija et al., 2015). Relation between organic, phenolic acids, their combination in the extract and antidiabetic activity explored herein certainly exists.

According to our knowledge, no previous studies were published on *M. conica* antidiabetic activity. Our results indicate that methanolic extract of this species did not inhibit  $\alpha$ -amylase, while  $\alpha$ -glucosidase was inhibited (Table 1). Regarding *M. conica* organic acid profile, previously (Vieira et al., 2016) we have quantified three different organic acids: oxalic, quinic, and fumaric acids. The phenolic compounds gallic and *p*-hydroxybenzoic acids were only present in the wild growing sample, as well as cinnamic acid.

A polysaccharide isolated from *P. linteus* has previously shown hypoglycemic properties in alloxan-induced diabetic mice (Zhao et al., 2014). *P. linteus* methanolic extract exhibited enzyme inhibitory potential in this study (Table 1). The hydrophilic compounds recorded in *P. linteus* earlier (Reis et al., 2014) were only organic acids — oxalic acid was the only detected molecule (0.295 g/100 g dw).

The investigated mushroom species possessed antidiabetic properties regarding the inhibition of the enzymes investigated. *C. militaris* and *M. conica* failed to inhibit the activity of  $\alpha$ -amylase, while inhibiting the activity of  $\alpha$ -glucosidase. *I. obliquus* methanolic extract was the most potent inhibitor of  $\alpha$ -glucosidase, while *C. comatus* has the highest potential in inhibition of  $\alpha$ -amylase. The lowest antidiabetically effective daily intake of mushroom powder was predicted for *I. obliquus* with the dose of  $3 \times 1.148$  g/day, while the highest was predicted for *P. linteus*  $3 \times 2.215$  g/day. The activity of the extracts investigated herein could be linked to their chemical compositions previously published by our research groups. Organic acids, phenolic acids, fatty acids and their specific combination in each species contributed to unique *in vitro* antidiabetic activity of investigated mushrooms.

Although majority of previous studies showed *in vivo* antidiabetic potential of water and polysaccharidic mushroom extracts by different experimental approaches, our study is the first highlighting *in vitro* antidiabetic potential by inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase with methanolic extracts; which makes the investigated species more promising for the diabetes type-2 treatment by another additional and different mechanism of action.

## Acknowledgment

Conflict of interest: None. The authors thank Serbian Ministry of Education, Science and Technological Development for the financial support (grant number 173032).

## References

- Apostolidis, E., Kwon, Y.I., Shetty, K., 2007. Inhibitory potential of herb, fruit, and fungal enriched cheese against key enzymes linked to type 2 diabetes and hypertension. *Innovative Food Science and Emerging Technologies* 8, 46–54.
- Bizarro, A., Ferreira, I.C.F.R., Soković, M., Van Griensven, L.J.L.D., Sousa, D., Vasconcelos, M.H., Lima, R.T., 2015. *Cordyceps militaris* (L.) link fruiting body reduces the growth of a non-small cell lung cancer cell line by increasing cellular levels of p53 and p21. *Molecules* 20.
- Ding, Z., Lu, Y., Lu, Z., Lv, F., Wang, Y., Bie, X., Wang, F., Zhang, K., 2010. Hypoglycaemic effect of comatin, an antidiabetic substance separated from *Coprinus comatus* broth, on alloxan-induced-diabetic rats. *Food Chemistry* 121, 39–43.
- Fujisawa, T., Ikegami, H., Inoue, K., Kawabata, Y., Ogihara, T., 2005. Effect of two  $\alpha$ -glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. *Metabolism: Clinical and Experimental* 54, 387–390.
- Glamočlija, J., Ćirić, A., Nikolić, M., Fernandes, A., Barros, L., Calhella, R.C., Ferreira, I.C.F.R., Soković, M., Van Griensven, L.J.L.D., 2015. Chemical characterization and biological activity of Chaga (*Inonotus obliquus*), a medicinal "mushroom". *Journal of Ethnopharmacology* 162, 232–232.
- Kim, D.J., Kang, Y.H., Kim, K.K., Kim, T.W., Park, J.B., Choe, M., 2017. Increased glucose metabolism and alpha-glucosidase inhibition in *Cordyceps militaris* water extract-treated HepG2 cells. *Nutrition Research and Practice* 11, 180–189.
- Kim, Y.-W., Kim, K.-H., Choi, H.-J., Lee, D.-S., 2005. Anti-diabetic activity of  $\beta$ -glucans and their enzymatically hydrolyzed oligosaccharides from *Agaricus blazei*. *Biotechnology Letters* 27, 483–487.
- Koike, D., 2005. Effect of a wheat amylase inhibitor on canine carbohydrate digestion, gastrointestinal function and pancreatic growth. *Gastroenterology* 108, 1221–1229.
- Lindequist, U., 2013. The merit of medicinal mushrooms from a pharmaceutical point of view. *International Journal of Medicinal Mushrooms* 15, 517–523.
- Liu, C., Song, J., Teng, M., Zheng, X., Li, X., Tian, Y., Pan, M., Li, Y., Lee, R.J., Wang, D., 2016. Antidiabetic and antinephritic activities of aqueous extract of *Cordyceps militaris* fruit body in diet-streptozotocin-induced diabetic Sprague Dawley rats. *Oxidative Medicine and Cellular Longevity* 2016, 9685257.
- Melo, F.R., Sales, M.P., Pereira, L.S., Bloch, C.J.R., Franco, O.L., Ary, M.B., 1999.  $\alpha$ -Amylase inhibitors from cowpea seeds. *Protein and Peptide Letters* 6, 385–390.
- Ortiz, A.R.R., Garcia, J.S., Castillo, E.P., Ramirez, A.G., Villalobos, M.R., Estrada, S.S., 2007. Alpha-glucosidase inhibitory activity of the methanolic extract from *Tournefortia hartwegiana*: an anti-hyperglycemic agent. *Journal of Ethnopharmacology* 109, 48–53.
- Paterson, R.R., Lima, N., 2014. Biomedical effects of mushrooms with emphasis on pure compounds. *Biomedical Journal* 37, 357–368.
- Popović, V., Živković, J., Davidović, S., Stevanović, M., Stojković, D., 2013. Mycotherapy of cancer: an update on cytotoxic and antitumor activities of mushrooms, bioactive principles and molecular mechanisms of their action. *Current Topics in Medicinal Chemistry* 13, 2791–2806.
- Qin, D.-W., Gu, Z., Guo, J.-Y., 2015. Medicinal mushroom for prevention of disease of modern civilization. *Evidence-based Complementary and Alternative Medicine* 2015, 812725.
- Reis, F.S., Barreira, J.C.M., Calhella, R.C., Van Griensven, L.J.L.D., Ćirić, A., Glamočlija, J., Soković, M., Ferreira, I.C.F.R., 2014. Chemical characterization of the medicinal mushroom *Phellinus linteus* (Berkeley & Curtis) Teng and contribution of different fractions to its bioactivity. *LWT - Food Science and Technology* 58, 478–485.
- Saito, N., Sakai, H., Sekihara, H., Yajima, Y., 1998. Effect of an  $\alpha$ -glucosidase inhibitor (Voglibose), in combination with sulphonyl urea, on glycemic control in type 2 diabetes patients. *Journal of International Medical Research* 26, 219–232.
- Shobana, S., Sreerama, Y.N., Mallesh, N.G., 2009. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: mode of inhibition of  $\alpha$ -glucosidase and pancreatic amylase. *Food Chemistry* 115, 1268–1273.
- Soković, M., Ćirić, A., Glamočlija, J., Stojković, D., 2016. The bioactive properties of mushrooms. In: Ferreira, I.C.F.R., Morales, P., Barros, L. (Eds.), *Wild Plants, Mushrooms and Nuts: Functional Food Properties and Applications*. John Wiley & Sons, Ltd., Chichester, UK, pp. 83–122.
- Stojković, D., Reis, F.S., Barros, L., Glamočlija, J., Ćirić, A., Van Griensven, L.J.L.D., Soković, M., Ferreira, I.C.F.R., 2013. Nutrients and non-nutrients composition and bioactivity of wild and cultivated *Coprinus comatus* (O.F.Müll.) Pers. *Food and Chemical Toxicology* 59, 289–296.
- Stojković, D., Reis, F.S., Glamočlija, J., Ćirić, A., Barros, L., Van Griensven, L.J.L.D., Ferreira, I.C.F.R., Soković, M., 2014. 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. *Food and Function* 5, 1602–1612.
- Vieira, V., Fernandes, A., Barros, L., Glamočlija, J., Ćirić, A., Stojković, D., Martins, A., Soković, M., Ferreira, I.C.F.R., 2016. *Wild Morchella conica* Pers. from different origins: a comparative study of nutritional and bioactive properties. *Journal of the Science of Food and Agriculture* 96, 90–98.
- Worthington, V., 1993. Alpha amylase. In: Worthington, V. (Ed.), *Worthington Enzyme Manual*. Worthington Biochemical Corp., Freehold, NJ, pp. 36–41.

- Yamaç, M., Zeytinoglu, M., Kanbak, G., Bayramoglu, G., Senturk, H., 2009. Hypoglycemic effect of crude exopolysaccharides produced by *Cerrena unicolor*, *Coprinus comatus*, and *Lenzites betulina* isolates in streptozotocin-induced diabetic rats. *Pharmaceutical Biology* 47, 168–174.
- Ying, Y.-M., Zhang, L.-Y., Zhang, X., Bai, H.-B., Liang, D.-E., Ma, L.-F., Shan, W.-G., Zhan, Z.-J., 2014. Terpenoids with alpha-glucosidase inhibitory activity from the submerged culture of *Inonotus obliquus*. *Phytochemistry* 108, 171–176.
- Zhao, C., Liao, Z., Wu, X., Liu, Y., Liu, X., Lin, Z., Huang, Y., Liu, B., 2014. Isolation, purification, and structural features of a polysaccharide from *Phellinus linteus* and its hypoglycemic effect in alloxan-induced diabetic mice. *Journal of Food Science* 2014, H1002–H1010.
- Zhou, S., Liu, Y., Yang, Y., Tang, Q., Zhang, J., 2015. Hypoglycemic activity of polysaccharide from fruiting bodies of the shaggy ink cap medicinal mushroom, *Coprinus comatus* (Higher basidiomycetes), on mice induced by alloxan and its potential mechanism. *International Journal of Medicinal Mushrooms* 17, 957–964.