Food Chemistry 147 (2014) 252–256

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Storage temperature and UV-irradiation influence on the ergosterol content in edible mushrooms

Ana Villares *, Laura Mateo-Vivaracho, Ana García-Lafuente, Eva Guillamón

Centro para la Calidad de los Alimentos, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Campus Universitario Duques de Soria, c/José Tudela s/n, 42004 Soria, Spain

ARTICLE INFO

Article history:
Received 10 October 2012
Received in revised form 12 April 2013
Accepted 29 September 2013
Available online 9 October 2013

Keywords:
Ergosterol
Ergosteryl ester
Mushroom
Fungi
HPLC

ABSTRACT

Ergosterol (5,7,22-ergostatrien-3β-ol) and ergosteryl derivatives from different genera of edible mushrooms were separated and quantified by an isocratic reversed-phase high performance liquid chromatography (HPLC) method. The technique allowed a rapid separation of free ergosterol and two ergosteryl derivatives occurring in mushrooms. The ergosterol content varied considerably depending on the fungus. Thus, the species *Agaricus bisporus* and *Hygrophorus marzuolus* presented high quantities of ergosterol (6.4–6.8 mg/g, dry matter) followed by *Pleurotus ostreatus*, *Calocybe gambosa*, *Lentinus edodes*, and *Boletus edulis* (3.3–4.0 mg/g). In contrast, other species, such as *Cantharellus cibarius*, *Lactarius deliciosus* and *Craterellus cornucopioides*, contained significantly lower ergosterol amounts (0.2–0.4 mg/g). Two ergosteryl derivatives were found in mushrooms and also the content depended on the fungus. The stability of ergosterol, in terms of the formation of ergosterol peroxide, was evaluated under different storage temperatures and UV radiation. The lower the temperature (−20 °C) and the radiation time (10 min), the lower ergosterol oxidation was observed.

1. Introduction

Edible mushrooms can be viewed as good candidates to be included in healthy diets. Besides their unique flavour and sensory properties, mushrooms provide excellent nutritional and healthy properties (Guillamón et al., 2010). On a dry weight basis, mushrooms can be considered a good source of proteins, carbohydrates and dietary fibre (Barros, Baptista, Estevinho, & Ferreira, 2007; Manzi, Aguzzi, & Pizzoferrato, 2001), in addition to the significant amounts of bioactive metabolites involved in health promotion and disease prevention.

Advanced fungi produce ergosterol (5, 7, 22-ergostatrien-3β-ol) as the main sterol, differing from major plant sterols in having two double bonds within the sterol ring structure instead of one (Mattila, Lampi, Ronkainen, Toivo, & Piironen, 2002). Ergosterol has attracted much attention due to its healthy properties, for instance, this sterol has shown antioxidant (Shao, Hernandez, Kramer, Rinker, & Tsao, 2010) and anti-inflammatory (Kuo, Hsieh, & Lin, 2011) activities, as well as anti-hyperlipidemic (Hu et al., 2006) properties. In addition, the ergosterol peroxide has demonstrated potential anticancer effects (Russo et al., 2010).

Ergosterol is present in two main forms, which are free and esterified, and the relative abundances would depend on the fungal species. Free ergosterol plays an important role in fluidity, permeability, and integrity of the cell membrane; in addition, this molecule seems to be involved in the effects of membrane-bound proteins associated with nutrient transport and chitin synthesis (Sturley, 2000). Differently, the ergosteryl esters are stored in the hydrophobic core of cytosolic lipid particles and play a role in sterol homeostasis (Shobayashi et al., 2005). The relative percentage of free ergosterol and ergosteryl esters in the cell is regulated by several factors, including biosynthesis, exogenous uptake, transport and storage. Under certain conditions, ergosteryl esters may be hydrolyzed to free ergosterol, therefore, they can be viewed as a source of free ergosterol.

Sterols are commonly quantified by means of gas chromatography coupled to mass spectrometry (Abd Malek, Kanagasabapathy, Sabaratnam, Abdullah, & Yaacob, 2012; Francavilla, Trotta, & Luque, 2010; Kanagasabapathy, Malek, Kuppusamy, & Vikineswary, 2011; Miller, Haubrich, Wang, Snell, & Nes, 2012; Parkinson, Warren, & Pawliszyn, 2010; Zhang, Tan, Hu, & Li, 2011). Reversed-phase liquid chromatography appears to be an advantageous technique for determining ergosterol content in mushrooms since it provides a rapid method capable of separating free ergosterol from the ergosteryl derivatives (Tan et al., 2013). In this work, an isocratic reversed-phase liquid chromatography method is described for the separation and analysis of free ergosterol and ergosteryl derivatives occurring in mushrooms. Ergosterol and related compounds have been extracted from the fruiting bodies of 9 different

* Corresponding author. Tel.: +34 975 233204; fax: +34 975 233205.
E-mail address: anavillares@gmail.com (A. Villares).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.09.144

mushrooms (*Agaricus bisporus*, *Boletus edulis*, *Calocybe gambosa*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Hygrophorus marzuolus*, *Lactarius deliciosus*, *Lentinus edodes* and *Pleurotus ostreatus*). The free and total ergosterol contents have been quantified and the stability of ergosteryl extracts has been evaluated under different storage temperatures and times of UV radiation.

2. Experimental

2.1. Samples

Fruiting bodies of mushrooms (*A. bisporus*, *B. edulis*, *C. cibarius*, *C. cornucopioides*, *C. gambosa*, *H. marzuolus*, *L. deliciosus*, *L. edodes*, and *P. ostreatus*) were purchased from local market. The samples were immediately lyophilized (Telstar Cryodos), and kept at 4 °C in hermetically vacuum-sealed plastic bags (Tecnotrip) up to analysis.

2.2. Standards and reagents

Chloroform (99.3%), hexane (98.2%), acetonitrile (99.9%), methanol (99.8%), hydrogen peroxide (31.2%), and sulphuric acid (99%) were purchased from Prolabo Pestinorm (VWR International). The ergosterol standard (95%) was purchased from Sigma Chemical Co. Xylenol orange and ferrous ammonium sulphate (99%) were obtained from Sigma Chemical Co.

2.3. Isolation and purification of ergosterol and esters

Mushroom samples were freeze-dried and finely milled. Mushroom powder (0.10 g) was extracted with a chloroform/methanol mixture (2:1. *v*/*v*) by stirring in an ultrasonic bath (X-tra 30 H, Elmasonic) at 4 °C for 1 h as previously described (Villares, García-Lafuente, Guillamón, & Ramos, 2012). The extracted liquid was then applied to an Oasis MAX (150 mg, Waters) column preconditioned with hexane (8 mL). Sterols and esters were separated from other lipids by passing chloroform (2 × 3 mL) (Bonzom, Nicolaou, Zloh, Baldeo, & Gibbons, 1999). The sample was evaporated to dryness under a nitrogen stream and redissolved in chloroform/methanol (2:1. *v*/*v*). Prior to HPLC analysis samples were filtered through a 0.20 μm disposable LC filter disk.

2.4. Analysis of ergosterol by liquid chromatography

The extracts were analysed using an Alliance® HPLC system 2695 (Waters) coupled to a photodiode array detector 2998 (Waters). Separation was achieved on a Symmetry reverse phase C_{18} column (Waters, 3.5 μm, 75 × 4.6 mm) thermostatted at 25 °C. A solvent system consisting of 75% acetonitrile and 25% methanol was used in an isocratic mode. The solvent flow rate was 1.5 mL/min and the injection volume was 10 μL. Ergosterol was identified on the basis of the retention time of the standard and the quantification was achieved by the absorbance recorded at 280 nm relative to the external standard. The calibration curve showed a high degree of linearity ($r^2 > 0.999$).

2.5. Ergosterol extracts stability

For the temperature stability experiments, 1 mL of mushroom extracts were placed into test tubes and stored in darkness at room temperature (25 °C), refrigerated (4 °C) and frozen (−20 °C). The irradiation with UV light was performed on mushrooms extracts (1 mL) to 240–420 nm (Vilber Lourmat Iluminator, Marne La Vallee Cedex 1, France) for 10, 30 and 60 min. The irradiation source was placed 10 cm away from samples in a dark chamber. All irradiation experiments were performed at 25 °C.

The oxidation products were detected by incubation of the treated extracts (500 μL) with FOX (200 μL). FOX reagent was prepared by mixing the appropriate volume of xylenol orange, ferrous ammonium sulphate and H_2SO_4 to give final concentrations of 100 μM, 250 μM and 25 mM, respectively, in a volume of 2 mL as previously described (Gay, Collins, & Gebicki, 1999). The presence of peroxides is detected by the oxidation of Fe (II) to Fe (III), which reacts with xylenol orange forming a coloured complex. Samples were incubated for 30 min in the dark and the absorbance was read at 595 nm with xylenol orange reagent as blank. Hydrogen peroxide was used to calculate the standard curve ($r^2 > 0.998$) for peroxide equivalents.

2.6. Statistical analysis

All analyses were performed in triplicate, and the results are expressed as the mean and the standard deviation (SD). The significant differences among samples were determined using a one-way ANOVA test. A post hoc Tukey's test was also conducted to establish the differences among mean values. All statistical analyses were performed using SPSS 15.0 and the threshold *p*-value chosen for statistical significance was $p < 0.05$.

3. Results and discussion

Ergosterol and ergosteryl derivatives in mushrooms were separated and quantified by high performance liquid chromatography (HPLC). Liquid chromatography allowed the separation and quantification of free ergosterol and ergosteryl derivatives simultaneously. Fig. 1 shows the representative chromatograms when the crude sterol fraction was injected into a column C_{18} eluted with acetonitrile:methanol (75:25 *v*/*v*).

The method allowed the separation of ergosterol from its derivatives in a short period of time and employing an isocratic eluent. The chromatograms from the studied mushrooms revealed the presence of three peaks. Ergosterol, identified by comparison of the retention time and the absorption spectrum of the standard solution, eluted at 6.5 min. The peaks at 2.9 and 8.9 min presented similar absorption features to free ergosterol; therefore, these peaks may be identified as ergosteryl derivatives. The HPLC analysis after saponification with freshly methanolic KOH solutions at 80 °C for 1 h confirmed the identification as ergosterol derivatives because the peaks disappeared and ergosterol response increased.

The analytical parameters of the method were previously determined (Villares et al., 2012). As ergosteryl derivatives showed similar absorption spectrum and were eluted under the same chromatographic conditions, the analytical parameters (calibration curve, LOD, LOQ, repeatability and reproducibility) were considered to be those from free ergosterol. Table 1 reviews the free ergosterol contents of the studied fungal species. As Table 1 shows, all mushrooms contained ergosterol and the content was significantly different depending on the fungi. *H. marzuolus* and *A. bisporus* showed the greatest contents (6.81–6.42 mg/g) followed by *B. edulis*, *L. edodes*, *C. gambosa*, and *P. ostreatus* (4.00–3.31 mg/g). In contrast, the concentration of ergosterol in *C. cibarius*, *L. deliciosus*, and *C. cornucopioides* was below 1 mg/g (dry matter). Our results agreed with previous analysis of ergosterol in mushrooms from the same genera, for instance, *A. bisporus* has been extensively studied and ergosterol content ranges from 2.04–7.80 mg/g (Jasinghe & Perera, 2005; Shao et al., 2010). *L. edodes* has been also a mushroom of research and ergosterol is present at 2.02–6.80 mg/g (Perera, Jasinghe, Ng, & Mujumdar, 2003; Yuan, Kuang, Wang, & Liu, 2008). The cultivated mushrooms from the genus *Pleurotus*

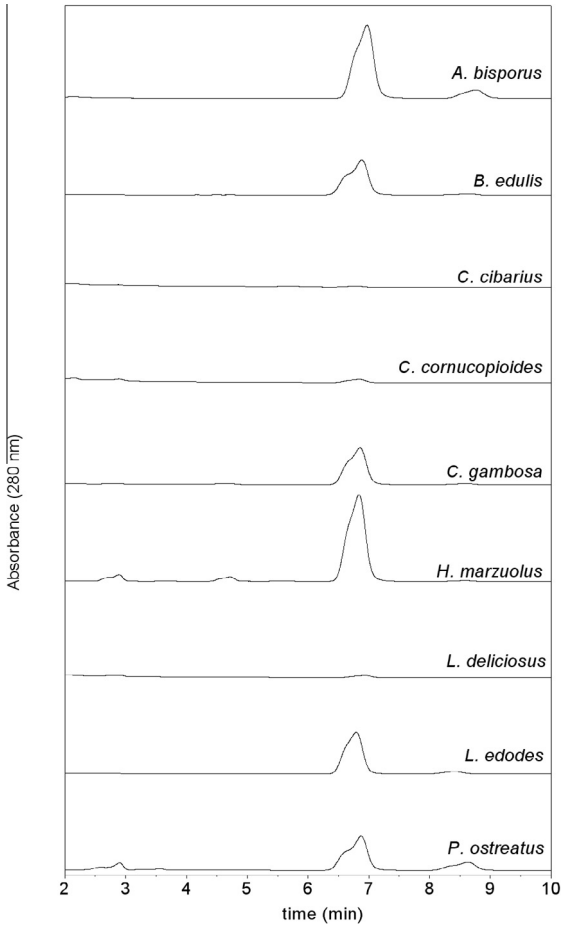

Fig. 1. HPLC chromatograms of ergosterol and derivatives isolated from nine edible mushrooms: *A. bisporus*, *B. edulis*, *C. gambosa*, *C. cibarius*, *C. cornucopioides*, *H. marzuolus*, *L. deliciosus*, *L. edodes* and *P. ostreatus*.

Table 1
Content of free ergosterol in the studied mushrooms expressed in mg of ergosterol per gram of dried mushroom. Results are expressed as mean ± standard deviation ($n = 3$). Values within columns with different letters are significantly different ($p < 0.05$).

Sample	Free ergosterol (mg/g)	
A. bisporus	6.42[a]	±0.15
B. edulis	4.00[b]	±0.53
C. cibarius	0.23[c]	±0.01
C. conucopioides	0.44[c]	±0.00
C. gambosa	3.61[b]	±0.18
H. marzuolus	6.81[a]	±0.72
L. deliciosus	0.32[c]	±0.02
L. edodes	3.64[b]	±0.02
P. ostreatus	3.31[b]	±0.17

present similar contents, for instance *P. ostreatus* and *P. cystidus* contain 4.40 and 4.35 mg/g of free ergosterol, respectively. Wild species also show significant ergosterol concentrations, for instance, *B. edulis* have 5.18 mg/g, *C. cibarius* 2.06 mg/g, *C. tubaeformis* 3.82 mg/g, and *L. trivialis* 3.03 mg/g (Mattila et al., 2002; Teichmann, Dutta, Staffas, & Jagerstad, 2007).

Apart from free ergosterol, released from the fungal cell membrane, the ergosteryl esters sequestered in cytosolic lipid particles (Shobayashi et al., 2005) contribute to the total ergosterol content determined by the above authors. The presence of ergosteryl esters is not commonly described in literature because sterols are saponified in order to quantify the total ergosterol content. Nevertheless, some mushrooms have been a matter of research and ergosteryl esters have been detected in species such as *Agrocybe aegerita*, *Cordyceps sinensis*, *Ganoderma lucidum*, *L. edodes* and *Termitomyces albuminosus* (Gonzalez, Leon, Rivera, Munoz, & Bermejo, 1999; Yuan, Wang, Liu, Kuang, & Zhao, 2007; Yuan et al., 2008; Ziegenbein, Hanssen, & Konig, 2006). Fig. 2 shows the total ergosterol content in terms of free ergosterol and ergosteryl derivatives concentrations occurring in the studied mushrooms.

Two different ergosteryl derivatives were found in the studied mushrooms and the distribution significantly depended on the fungal species. The ergosterol derivative eluting at 2.9 min (Fig. 1) seemed to be a polar derivative, such as a polyhydroxy ergosterol glucoside or ergosterol glucoside, since it eluted earlier than free ergosterol, as previously described for truffles (Villares et al., 2012). Differently, the ergosterol derivative eluting at 8.9 min was expected to be a less polar compound, probably an esterified fatty acid or an aliphatic alcohol attached to the ergosterol structure. Differences between relative abundances of free to esterified ergosterol have been previously described among various fungal species (Yuan et al., 2007). Within the studied mushrooms, the ergosteryl derivative detected at 2.9 min was found in all genera excepting *A. bisporus*. The content varied between fungal species; thus, *P. ostreatus* and *H. marzuolus* contained the highest concentrations (0.635–0.534 mg/g) followed by *C. cornucopioides* (0.295 mg/g). In contrast, *L deliciosus*, *C. gambosa*, *C. cibarius*, *B. edulis* and *L. edodes* showed lower content (0.227–0.162 mg/g). Contrary, the ergosteryl derivative eluting at 8.9 min, presumably less polar than the former, was exclusively detected in the mushroom species *A. bisporus*, *B. edulis*, *C. gambosa*, *L. edodes* and *P. ostreatus*. The highest concentrations were found in *P. ostreatus* and *A. bisporus* (1.024–0.978 mg/m) whereas the other mushrooms contained significantly lower amounts (0.334–0.287 mg/g).

Taking into account the total ergosterol content, *A. bisporus* and *H. marzuolus* showed the greatest values (7.40–7.34 mg/g) as well as free ergosterol content. The fungal species containing from 4.10 to 4.96 mg/g of total ergosterol (*C. gambosa*, *L. edodes*, *B. edulis* and *P. ostreatus*) presented both derivatives and a free ergosterol concentration from 3.31 to 4.00 mg/g. Differently, the mushrooms with low free ergosterol content (0.41–0.74 mg/g), such as *C. cibarius*, *L. deliciosus* and *C. cornucopioides*, did not contain the less polar derivative and the total ergosterol content was significantly lower than the content in other species.

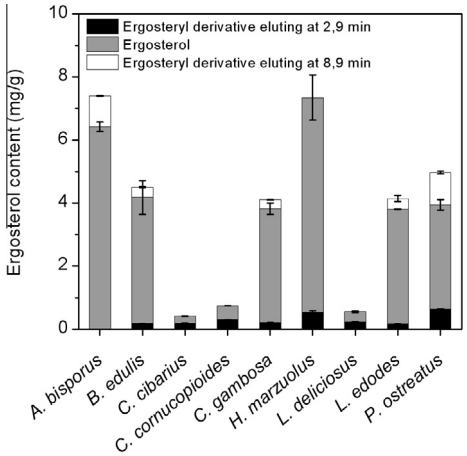

Fig. 2. Concentrations of ergosterol and ergosteryl derivatives in nine edible mushrooms: *A. bisporus*, *B. edulis*, *C. gambosa*, *C. cibarius*, *C. cornucopioides*, *H. marzuolus*, *L. deliciosus*, *L. edodes* and *P. ostreatus*, expressed as mg per gram of dried mushroom. Vertical bars indicate the standard deviation.

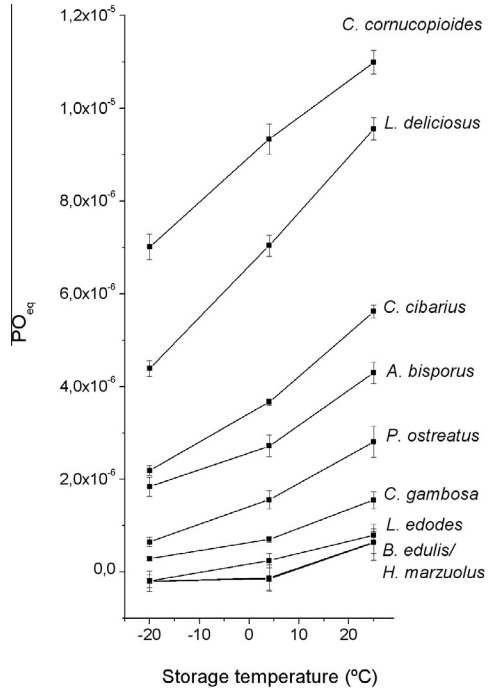

Fig. 3. Influence of the storage temperature on the stability of ergosterol extracts from mushrooms: *A. bisporus*, *B. edulis*, *C. gambosa*, *C. cibarius*, *C. cornucopioides*, *H. marzuolus*, *L. deliciosus*, *L. edodes* and *P. ostreatus*, expressed as peroxide equivalents. Vertical bars indicate the standard deviation.

Ergosterol is a rather unstable molecule and can be oxidized or photoxidized into ergosterol peroxide upon cell death (Krzyczkowski et al., 2009; Parsi & Gorecki, 2006). Despite being an oxidation product, several studies have shown that ergosterol peroxide may contribute to the potential health benefits and pharmacological activities (Russo et al., 2010; Zheng, Si, & Wong, 2009). The stability of ergosterol, in terms of the formation of ergosterol peroxide, was evaluated under different conditions. Firstly, the influence of storage temperature was studied. For that purpose, the extracts containing ergosterol and esters were stored for 4 h at three different temperatures: 25 °C, 4 °C and −20 °C. Fig. 3 shows the evolution of peroxide equivalents formation under these conditions.

All the mushrooms extracts underwent a greater oxidation as the temperature increased. At 25 °C, the formation of peroxides varied depending on the fungal species. Hence, extracts from *C. cornucopioides* and *L. deliciosus* presented the highest levels of peroxide equivalents followed by *C. cibarius*, *A. bisporus* and *P. ostreatus*. Surprisingly, the most oxidized genera were those containing the lower total and free ergosterol and those where the less polar ester was not detected. Other authors proposed that the ergosteryl esters were more stable than free ergosterol (Yuan et al., 2008), which could justify the lower oxidation of extracts containing this less polar derivative. As the storage temperature decreased, the differences between mushrooms became less marked and at −20 °C, the oxidation of the extracts from *C. gambosa*, *L. edodes*, *H. marzuolus*, and *B. edulis* was negligible.

Ergosterol undergoes photolysis when exposed to UV light (280–320 nm) to yield a variety of photoirradiation products including previtamin D_2, tachysterol and lumisterol (Mattila et al., 2002). UV exposure of ergosterol has been widely studied (Jasinghe and Perera, 2005; Teichmann et al., 2007); however, the formation of ergosterol peroxides under UV light is scarcely described (Trigos & Ortega-Regules, 2002). Peroxide formation was studied by photoirradiating the mushroom extracts with UV light. Fig. 4 shows the peroxide equivalents formed after radiation for 10, 30 and 60 min.

As Fig. 4 plots, UV radiation produced a clear oxidation of ergosterol, even after 10 min of radiation. The mushroom *C. cornucopioides* reached the maximum oxidation at 10 min, and longer times did not produce significant changes. Similarly, *L. deliciosus* was highly oxidized under UV light and radiation for 60 min did not produced changes from 30 min. This behaviour was also observed in *A. bisporus* and *H. marzuolus*, although the oxidation extent was lower. The rest of species became less oxidized and the formation of peroxides was influenced by the exposure time.

4. Conclusions

Ergosterol and ergosteryl derivatives can be separated and quantified by means of HPLC coupled to a photodiode array detector. Reversed-phase liquid chromatography provided an accurate method and allowed separation in less than 10 min, representing an almost ten-fold reduction of analysis time when compared to conventional gas chromatography methods. The ergosterol content ranged from 0.23 to 6.81 mg per gram of dried matter. Storage at −20 °C seemed to be an appropriate for ergosterol extracts from mushrooms because these conditions decreased the ergosterol oxidation.

Acknowledgements

The authors are grateful to Ministerio de Economía y Competitividad and INIA (projects RTA2009-00049 and AT07-003) for financial support of this work.

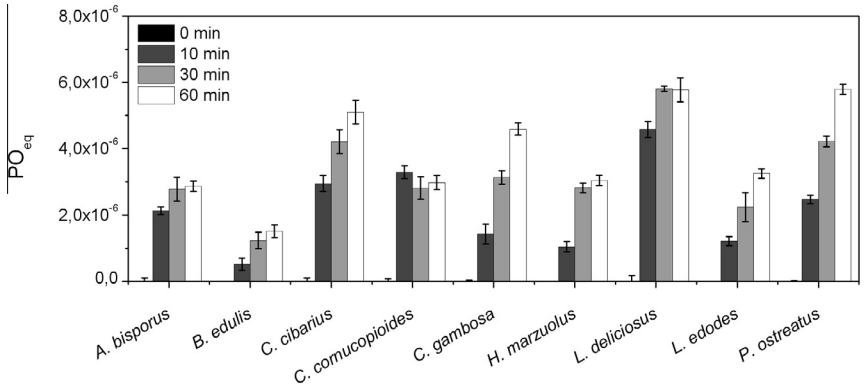

Fig. 4. Influence of the UV light radiation on the stability of ergosterol extracts from mushrooms: *A. bisporus*, *B. edulis*, *C. gambosa*, *C. cibarius*, *C. cornucopioides*, *H. marzuolus*, *L. deliciosus*, *L. edodes* and *P. ostreatus*, expressed as peroxide equivalents. Vertical bars indicate the standard deviation.

References

Abd Malek, S. N., Kanagasabapathy, G., Sabaratnam, V., Abdullah, N., & Yaacob, H. (2012). Lipid components of a Malaysian edible mushroom, *Termitomyces heimii natarajan*. *International Journal of Food Properties, 15*(4), 809–814.

Barros, L., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2007). Effect of fruiting body maturity stage on chemical composition and antimicrobial activity of *Lactarius* sp. mushrooms. *Journal of Agricultural and Food Chemistry, 55*(21), 8766–8771.

Bonzom, P. M. A., Nicolaou, A., Zloh, M., Baldeo, W., & Gibbons, W. A. (1999). NMR lipid profile of *Agaricus bisporus*. *Phytochemistry, 50*(8), 1311–1321.

Francavilla, M., Trotta, P., & Luque, R. (2010). Phytosterols from *Dunaliella tertiolecta* and *Dunaliella salina*: A potentially novel industrial application. *Bioresource Technology, 101*(11), 4144–4150.

Gay, C., Collins, J., & Gebicki, J. M. (1999). Hydroperoxide assay with the ferric-xylenol orange complex. *Analytical Biochemistry, 273*(2), 149–155.

Gonzalez, A. G., Leon, F., Rivera, A., Munoz, C. M., & Bermejo, J. (1999). Lanostanoid triterpenes from *Ganoderma lucidum*. *Journal of Natural Products, 62*(12), 1700–1701.

Guillamón, E., García-Lafuente, A., Lozano, M., D'Arrigo, M., Rostagno, M. A., Villares, A., & Martínez, J. A. (2010). Edible mushroom: Their roles in the prevention of cardiovascular diseases. *Fitoterapia, 81*, 715–723.

Hu, S. H., Liang, Z. C., Chia, Y. C., Lien, J. L., Chen, K. S., Lee, M. Y., & Wang, J. C. (2006). Antihyperlipidemic and antioxidant effects of extracts from *Pleurotus citrinopileatus*. *Journal of Agricultural and Food Chemistry, 54*(6), 2103–2110.

Jasinghe, V. J., & Perera, C. O. (2005). Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D-2 by UV irradiation. *Food Chemistry, 92*(3), 541–546.

Kanagasabapathy, G., Malek, S. N. A., Kuppusamy, U. R., & Vikineswary, S. (2011). Chemical composition and antioxidant properties of extracts of fresh fruiting bodies of *Pleurotus sajor-caju* (Fr.) singer. *Journal of Agricultural and Food Chemistry, 59*(6), 2618–2626.

Krzyczkowski, W., Malinowska, E., Suchocki, P., Kleps, J., Olejnik, M., & Herold, F. (2009). Isolation and quantitative determination of ergosterol peroxide in various edible mushroom species. *Food Chemistry, 113*(1), 351–355.

Kuo, C. F., Hsieh, C. H., & Lin, W. Y. (2011). Proteomic response of LAP-activated RAW 264.7 macrophages to the anti-inflammatory property of fungal ergosterol. *Food Chemistry, 126*(1), 207–212.

Manzi, P., Aguzzi, A., & Pizzoferrato, L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry, 73*, 321–325.

Mattila, P., Lampi, A. M., Ronkainen, R., Toivo, J., & Piironen, V. (2002). Sterol and vitamin D-2 contents in some wild and cultivated mushrooms. *Food Chemistry, 76*(3), 293–298.

Miller, M. B., Haubrich, B. A., Wang, Q., Snell, W. J., & Nes, W. D. (2012). Evolutionarily conserved Δ25(27)-olefin ergosterol biosynthesis pathway in the alga *Chlamydomonas reinhardtii*. *Journal of Lipid Research, 53*(8), 1636–1645.

Parkinson, D. R., Warren, J. M., & Pawliszyn, J. (2010). Analysis of ergosterol for the detection of mold in soils by automated on-fiber derivatization headspace extraction-SPME–GC/MS. *Analytica Chimica Acta, 661*(2), 181–187.

Parsi, Z., & Gorecki, T. (2006). Determination of ergosterol as an indicator of fungal biomass in various samples using non-discriminating flash pyrolysis. *Journal of Chromatography A, 1130*(1), 145–150.

Perera, C. O., Jasinghe, V. J., Ng, F. L., & Mujumdar, A. S. (2003). The effect of moisture content on the conversion of ergosterol to vitamin D in shiitake mushrooms. *Drying Technology, 21*(6), 1091–1099.

Russo, A., Cardile, V., Piovano, M., Caggia, S., Espinoza, C. L., & Garbarino, J. A. (2010). Pro-apoptotic activity of ergosterol peroxide and (22E)-ergosta-7,22-dien-5 alpha-hydroxy-3,6-dione in human prostate cancer cells. *Chemico-Biological Interactions, 184*(3), 352–358.

Shao, S. Q., Hernandez, M., Kramer, J. K. G., Rinker, D. L., & Tsao, R. (2010). Ergosterol profiles, fatty acid composition, and antioxidant activities of button mushrooms as affected by tissue part and developmental stage. *Journal of Agricultural and Food Chemistry, 58*(22), 11616–11625.

Shobayashi, M., Mitsueda, S., Ago, M., Fujii, T., Iwashita, K., & Iefuji, H. (2005). Effects of culture conditions on ergosterol biosynthesis by *Saccharomyces cerevisiae*. *Bioscience Biotechnology and Biochemistry, 69*(12), 2381–2388.

Sturley, S. L. (2000). Conservation of eukaryotic sterol homeostasis: New insights from studies in budding yeast. *Biochimica Et Biophysica Acta–Molecular and Cell Biology of Lipids, 1529*(1–3), 155–163.

Tan, J., Niu, X., Yang, G., Wang, L., Duan, Y., Han, Y., Zhang, K., & Zhang, C. (2013). Quantitative determination of free phytosterols in tobacco leaves by UPLC-MS/MS. *Journal of Liquid Chromatography and Related Technologies, 36*(5), 591–599.

Teichmann, A., Dutta, P. C., Staffas, A., & Jagerstad, M. (2007). Sterol and vitamin D-2 concentrations in cultivated and wild grown mushrooms: Effects of UV irradiation. *Lwt-Food Science and Technology, 40*(5), 815–822.

Trigos, A., & Ortega-Regules, A. (2002). Selective destruction of microscopic fungi through photo-oxidation of ergosterol. *Mycologia, 94*(4), 563–568.

Villares, A., García-Lafuente, A., Guillamón, E., & Ramos, A. (2012). Identification and quantification of ergosterol and phenolic compounds occurring in *Tuber* spp. truffles. *Journal of Food Composition and Analysis, 26*(1–2), 177–182.

Yuan, J. P., Kuang, H. C., Wang, J. H., & Liu, X. (2008). Evaluation of ergosterol and its esters in the pileus, gill, and stipe tissues of agaric fungi and their relative changes in the comminuted fungal tissues. *Applied Microbiology and Biotechnology, 80*(3), 459–465.

Yuan, J. P., Wang, J. H., Liu, X., Kuang, H. C., & Zhao, S. Y. (2007). Simultaneous determination of free ergosterol and ergosteryl esters in *Cordyceps sinensis* by HPLC. *Food Chemistry, 105*(4), 1755–1759.

Zhang, Z., Tan, W., Hu, Y., & Li, G. (2011). Simultaneous determination of trace sterols in complicated biological samples by gas chromatography-mass spectrometry coupled with extraction using β-sitosterol magnetic molecularly imprinted polymer beads. *Journal of Chromatography A, 1218*(28), 4275–4283.

Zheng, L., Si, J., & Wong, Y.-S. (2009). Ergosterol peroxide and 9,11-dehydroergosterol peroxide from Ling Zhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllophoromycetideae) mycelia inhibit the growth of human breast adenocarcinoma MCF-7 Cells. *International Journal of Medicinal Mushrooms, 11*(3), 249–257.

Ziegenbein, F. C., Hanssen, H. P., & Konig, W. A. (2006). Secondary metabolites from *Ganoderma lucidum* and *Spongiporus leucomallellus*. *Phytochemistry, 67*(2), 202–211.