Optimized analysis of organic acids in edible mushrooms from Portugal by ultra fast liquid chromatography and photodiode array detection

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Abstract

Organic acid profiles of different mushroom species were obtained by ultra-fast liquid chromatography (UFLC), by means of photodiode array detector (PDA). The chromatographic separation was achieved using a SphereClone (Phenomenex) reverse phase C_{18} column using an isocratic elution with sulphuric acid (3.6 mM) at a flow rate of 0.8 mL/min. All the compounds were separated in 8 min. The method was optimized using *Agaricus bisporus* sample and proved to be reproducible and accurate. Organic acid profiles were quite homogeneous for all mushroom samples; oxalic, malic and fumaric acids were the main organic acids; some samples also presented quinic and citric acids. *Sarcondon imbricatus* was the species that presented the highest total content (254.09 mg/g dw), while *Bovista nigrescens* presented the lowest concentration (1.33 mg/g dw). The high amounts of organic acids present in all the species may suggest that they could be related to the antioxidant activity found in these species and previously reported by us.

Keywords Edible mushrooms; UFLC-PAD; Analysis optimization; Organic acids

Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), including free radical forms, are constantly produced during the normal cellular metabolism and in excess they can damage cellular lipids, proteins and DNA (Valko et al. 2007). Protection against those species is ensured by antioxidant enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidases and glutathione redutase) and non-enzymatic molecules (e.g. glutathione, α-tocopherol, ascorbic acid and lipoic acid) (Gutteridge & Halliwell 2000; Lee et al. 2004). Nevertheless, these defences are frequently insufficient to totally prevent the damage, resulting in diseases and accelerated aging. Natural products with antioxidant activity may help the endogenous defence system, assuming a major importance as possible protector agents reducing oxidative damage.

Mushrooms are a source of antioxidant compounds such as tocopherols (Barros et al. 2008a; Heleno et al. 2010), ascorbic acid, carotenoids (Ferreira et al. 2009), phenolic compounds (Barros et al. 2009; Vaz et al. 2011a) and organic acids (Ribeiro et al. 2006; Valentão et al. 2005). Particularly, organic acids play a determinant role in maintaining fruit and vegetable quality and organoleptic characteristics and have also been used in their quality control (Cámara et al. 1994). The nature and concentration of these compounds are also important factors in mushrooms flavor (Ribeiro et al. 2006; Valentão et al. 2005). Acids have a lower susceptibility to change during processing and storage than other components such as pigments and flavor compounds (Cámara et al. 1994). Most important, organic acids may have a protective role against various diseases due to their antioxidant activity (such as the case of tartaric, malic, citric or succinic acids), being able to chelate metals or to delocalize the electronic charge coming from free radicals (López-Bucio et al. 2000; Seabra et al. 2006).

Some available studies report the organic acids profile of mushrooms, namely fruiting bodies of *Amanita rubescens*, *Boletus edulis*, *Hygrophorus agathosmus*, *Russula cyanoxantha*, *Suillus bellini*, *Suillus luteus*, *Suillus granulatus*, *Tricholoma equestre*, *Tricholomopsis rutilans* (Ribeiro et al. 2006), *Amanita caesarea*, *Gyroporus castaneus*, *Lactarius deliciosus*, *Suillus collinitus*, *Xerocomus chrysenteron* (Valentão et al. 2005), *Fistulina hepatica* (Ribeiro et al. 2007) and *Morchella deliciosa* (Rotzoll et al. 2006), or mycelium of *Agaricus blazei* (Carvajal et al. 2012) and *Leucopaxillus giganteus* (Ribeiro et al. 2008a). Moreover, Ribeiro et al. stated that organic acids are preferably fixed in the cap (Ribeiro et al. 2008b) and that their production by mushroom mycelium is affected by the nitrogen source in the culture medium (Ribeiro et al. 2008a).

Nevertheless, there is a lack of data about organic acids profile in wild edible mushrooms and corresponding efficient analysis techniques. In the present work, a methodology for organic acids extraction was applied and an analysis using ultra fast liquid chromatography and photodiode array detection (UFLC-PAD) was optimized and validated. Afterwards, the methodology was applied to 58 different species.

Materials and methods

Mushroom species

Forty eight species of wild edible mushrooms were collected in Bragança (Northeast Portugal) and ten commercial species were obtained in local supermarkets. Information about the analysed species is provided in **Table 1**. Taxonomical identification of sporocarps was made and representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança. All the samples

were lyophilised (Ly-8-FM-ULE, Snijders, Holland), reduced to a fine dried powder (20 mesh) and mixed to obtain a homogenate sample.

Standards and reagents

The standards of organic acids (L(+)-ascorbic acid; citric acid; malic acid; oxalic acid; shikinic acid; succinic acid; fumaric acid; quinic acid) were purchased from Sigma (St. Louis, MO, USA). 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).

Organic acids extraction and analysis

Samples (~2 g) were extracted by stirring with 25 mL of meta-phosphoric acid (25ºC at 150 rpm) for 45 min and subsequently filtered through Whatman No. 4 paper (Vazquez et al. 1994). Before analysis by ultra fast liquid chromatograph (UFLC) coupled to photodiode array detector (PDA), the sample was filtered through 0.2 µm nylon filters. The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Coperation). Separation was achieved on a SphereClone (Phenomenex) reverse phase C_{18} column (5 µm, 250 mm × 4.6 mm i.d) thermostatted at 35 ºC. The elution was performed with sulphuric acid 3.6 mM using a flow rate of 0.8 mL/min. Detection was carried out in a PDA, using 215 nm and 245 nm (for ascorbic acid) as preferred wavelengths. The organic acids found were quantified by 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 mg per g of dry weight.

Validation assays

Linearity and sensitivity of the UFLC analysis were determined and the method was validated by the instrumental repeatability, precision and accuracy, using *Agaricus bisporus*.

The repeatability was accomplished by analyzing the mushroom sample, *Agaricus bisporus*, seven times in the same day. Precision was accessed after three extractions of the same sample being each one analyzed three times in the same day. The accuracy of the method was evaluated by the standard addition procedure (percentage of recovery), with three addition levels (25, 50 and 100% of the peak/area concentration) each one in triplicate. The standards mixture (oxalic, quinic, malic, citric and fumaric acids) was added to the sample and the extraction procedure was carried out.

Statistical analysis

Organic acids extraction was performed in duplicate and each sample was injected three times in UFLC-PAD. The results are expressed as mean values ± standard deviation (SD). The differences between mushroom species were 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 and discussion

The analytical characteristics of the method for organic acids analysis were evaluated by the linearity and determination of limits of detection and quantification (**Table 2**). After

studying the linearity for each compound (13 levels), a 7-level calibration curve was made using the peak/area ratio *versus* concentration of the standard (μg/mL). The average of triplicate determinations for each level was used. The method validation was performed using oxalic, quinic, malic, citric and fumaric acids (**Figure 1a**), because these were the main organic acids present in the analysed samples. The correlation coefficients were higher than 0.999 for all the compounds. The limits of detection (LOD), calculated as the concentration corresponding to three times the standard error of the calibration curve divided by the slope, ranged from 0.080 to 36 μg/mL. The limits of quantification (LOQ) were calculated using the concentration corresponding to ten times the calibration error divided by the slope, and ranged from 0.26 to 1.2×10^2 μg/mL.

In order to evaluate the instrumental precision, the sample (*Agaricus bisporus*) was injected seven times. The chromatographic method proved to be precise (CV% between 0.040 and 1.4%, **Table 3**). Repeatability was evaluated by applying the whole extraction procedure three times to the same sample. All the obtained CV values were low (ranging from 0.50 and 1.7%, **Table 3**). The method accuracy was evaluated by the standard addition procedure (percentage of recovery). The standards mixture was added to the samples in three concentration levels (25, 50 and 100% of the peak/area concentration, each one in triplicate) before the extraction. The method showed good recovery values, with mean percentages ranging between 91 and 99%. **Figure 1b** shows the organic acids profile of *Agaricus bisporus*.

All the mushroom samples presented oxalic, malic and fumaric acids; some samples also revealed the presence of quinic and citric acids (**Table 4**).

The main organic acid found in most of the studied species was malic acid, which is a dicarboxylic acid made by all living organisms, occurring naturally in all fruits and many vegetables. It contributes to the pleasantly sour taste of fruits, and it is used as a food

additive. *Sarcodon imbricatus* presented the highest content of this particular acid (240.65 mg/g dw), but also of total organic acids (254.09 mg/g dw). Otherwise, *Bovista nigrescens*, *Bovista aestivales* and *Hygrophorus chrysodon* presented the lowest malic acid concentration (0.51, traces and 0.68 mg/g dw, respectively).

Oxalic acid was also found in all the samples; it is present in many plants, including black tea, and occurs naturally in animals. It should be stated that calcium oxalate is the most common component of kidney stones and can be directly absorbed by the gut in spite of its insolubility (Ribeiro et al. 2008a). Although oxalic acid was one of the main organic acids present in the studied samples, some species showed low concentrations, such as *Amanita spissa*, *Fistulina hepatica* and *Bovista nigrescens* (traces, 0.16 and 0.82 mg/g dw, respectively).

Fumaric acid was also present in all the studied species. This organic acid is important because of its antioxidant, antimicrobial and acidifying properties (Ribeiro et al. 2008a). *Cortinarius praestans* presented the highest concentration (12.31 mg/g dw) of this organic acid, while *Bovista nigrescens* and *Bovista aestivales* presented the lowest ones (traces and 0.07 mg/g dw, respectively). *Bovista nigrescens* also presented the lowest content of total organic acids (1.33 mg/g dw).

Quinic and citric acids were found in some species. Quinic acid is a crystalline acid normally obtained from plant products; it is a versatile chiral starting material for the synthesis of new pharmaceuticals. *Clitocybe odora* presented the highest content of quinic acid (198.17 mg/g dw) which contributed to the high content of total organic acids obtained in this species (217.69 mg/g dw). *Lactarius volemus* presented the lowest content of quinic acid (1.17 mg/g dw). The main organic was acid found in *Lentinus edodes* was citric acid. This compound is known to be very important in the prevention of mushrooms browning and to extend its shelf life; this is because of its antibacterial and antioxidant

properties (Ribeiro et al. 2008a). Nevertheless, *Cortinarius violaceus* presented the lowest concentration of this acid (5.33 mg/g dw).

As far as we know, there is no information on the organic composition of the studied species, with exception of *Boletus edulis* (Ribeiro et al. 2006; Ribeiro et al. 2008b; Valentão et al. 2005), *Fistulina hepatica* (Ribeiro et al. 2007) and *Lactarius deliciosus* (Valentão et al. 2005). Some differences were found in the results reported herein and the ones described by those authors. This could be due to numerous factors such as the different extraction methodology applied, as also environmental conditions related to samples collection, the year of collection and location (Manzi et al. 2004).

The studied mushroom samples reveal interesting antioxidant properties (Barros et al. 2007b; Barros et al. 2008b; Barros et al. 2008c; Barros et al. 2008d; Grangeia et al. 2011; Heleno et al. 2011; Pereira et al. 2012; Reis et al. 2011; Reis et al. 2012; Vaz et al. 2011b), and the organic acids present in those species might be related to the mentioned properties.

Conclusion

The organic acid profiles of 58 mushroom species were obtained by UFLC-PDA, using an optimized methodology, which proved to be reproducible and accurate and allowed compounds separation in 8 min. Oxalic, malic, fumaric, quinic and citric acids were identified and quantified. *Sarcondon imbricatus* was the species with highest total content, while *Bovista nigrescens* presented the lowest concentration.

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Table 1. Information about the analysed edible species.

Scientific name	Collection year	Local of collection	Reference*
Agaricus bisporus	2011	Commercial	Reis et al. 2012
Agaricus bisporus portobello	2011	Commercial	Reis et al. 2012
Agaricus campestris	2010	Fields	Pereira et al. 2012
Agaricus comtulus	2010	Fields	Pereira et al. 2012
Agaricus lutosus	2010	Fields	Pereira et al. 2012
Agaricus silvaticus	2010	*Pinus sp.*	Barros et al. 2008c
Amanita caesarea	2010	*Castanea sativa*	Reis et al. 2011
Amanita spissa	2010	*Pinus sp.*	n.a.
Armillaria mellea	2009	*Pinus sp.*	Vaz et al. 2011b
Boletus aereus	2009	Mixed stands	Heleno et al. 2011
Boletus armeniacus	2010	*Castanea sativa*	Pereira et al. 2012
Boletus citrinoporus	2010	*Quercus sp.*	n.a.
Boletus edulis	2007	Commercial	Barros et al. 2008b
Boletus edulis	2010	*Quercus pyrenaica*	Heleno et al. 2011
Boletus fragrans	2010	*Castanea sativa*	Grangeia et al. 2011
Boletus impolitus	2010	*Quercus sp.*	Pereira et al. 2012
Boletus reticulatus	2009	*Castanea sativa*	Heleno et al. 2011
Bovista aestivalis	2010	Mixed stands	Pereira et al. 2012
Bovista nigrescens	2010	Mixed stands	Pereira et al. 2012
Calocybe gambosa	2009	Mixed stands	Vaz et al. 2011b
Cantarellus cibarius	2007	Commercial	Barros et al. 2008b
Cantarellus cibarius	2007	*Quercus pyrenaica*	Barros et al. 2008d
Clavariadelphus pistillaris	2010	*Quercus sp.*	Pereira et al. 2012
Clavariadelphus truncatus	2010	Mixed stands	Pereira et al. 2012
Clitocybe costata	2010	Mixed stands	Pereira et al. 2012
Clitocybe gibba	2010	*Pinus sp.*	Pereira et al. 2012
Clitocybe odora	2009	*Pinus sp.*	Vaz et al. 2011b
Clorophyllum rhacodes	2010	Mixed stands	Pereira et al. 2012
Coprinus comatus	2007	Fields	Vaz et al. 2011b
Cortinarius anomalus	2009	Mixed stands	Reis et al. 2011
Cortinarius praestans	2010	Mixed stands	Pereira et al. 2012
Cortinarius violaceus	2009	*Quercus pyrenaica*	Reis et al. 2011
Craterellus cornucopioides	2007	Commercial	Barros et al. 2008b
Fistulina hepatica	2009	*Quercus pyrenaica*	Heleno et al. 2009
Flammulina velutipes	2011	Commercial	Pereira et al. 2012
Flammulina velutipes	2010	Mixed stands	Reis et al. 2012
Hygrophoropsis aurantiaca	2009	Mixed stands	Heleno et al. 2009

Hygrophorus chrysodon	2010	*Pinus sp.*	Pereira et al. 2012
Lacaria amethystina	2010	*Quercus pyrenaica*	Heleno et al. 2010
Lactarius deliciosus	2006	*Pinus sp.*	Barros et al. 2007a
Lactarius volemus	2009	*Quercus pyrenaica*	Reis et al. 2011
Lentinula edodes	2011	Commercial	Reis et al. 2012
Lepista nuda	2007	*Pinus pinaster*	Barros et al. 2008d
Leucoagaricus leucothites	2010	Fields	Pereira et al. 2012
Leucopaxillus giganteus	2010	*Pinus sp.*	Barros et al. 2007a
Lycoperdon imbrinum	2010	*Pinus sp.*	Pereira et al. 2012
Macrolepiota excoriata	2009	Mixed stands	Grangeia et al. 2011
Macrolepiota procera	2010	*Pinus sp.*	Barros et al. 2007b
Marasmius oreades	2007	Commercial	Barros et al. 2008b
Pleurotus eryngii	2011	Commercial	Reis et al. 2012
Pleurotus ostreatus	2011	Commercial	Reis et al. 2012
Ramaria aurea	2010	*Quercus sp.*	Pereira et al. 2012
Russula delica	2009	Mixed stands	Heleno et al. 2009
Russula olivacea	2010	*Quercus sp.*	Grangeia et al. 2011
Sarcodon imbricatus	2010	*Pinus sp.*	Barros et al. 2007a
Suillus variegatus	2010	*Pinus sp.*	Pereira et al. 2012
Tricholoma imbricatum	2009	Mixed stands	Heleno et al. 2009
Tricholoma portentosum	2007	*Pinus sp.*	Barros et al. 2007a

*These references provide information about nutritional composition and/or antioxidant properties of the mushroom species, and report the first time in which they were collected and studied by us. n.a.- not available.

Table 2. Analytical characteristics of the method for organic acids analysis**.**

	R_t (retention time)		Correlation coefficient (r^2)	Linearity range (μg/mL)	Limit	
	min	CV, % (n=13)			LOD (μg/mL)	LOQ (μg/mL)
Oxalic acid	3.0	0.31	0.9990	$0.097 – 3.1\times10^{2}$	12.6	42
Quinic acid	3.3	0.14	1.000	$0.78 – 5.0\times10^{-3}$	24	81
Malic acid	3.8	0.76	0.9998	$0.78 – 5.0\times10^{-3}$	36	1.2×10^{2}
Citric acid	6.0	0.75	1.000	$2.0 – 2.5\times10^{-3}$	10	35
Fumaric acid	6.9	0.51	0.9996	0.016 – 25	0.080	0.26

CV- Coefficient of variation; LOD- limit of detection; LOQ- limit of quantification.

Table 3. Validation of the method parameters using *Agaricus bisporus*.

	Precision CV, % (n=6)	Repeatability CV, % (n=6)	Accuracy (Recovery, %)
Oxalic acid	1.4	1.1	99
Quinic acid	0.77	0.36	95
Malic acid	0.53	0.71	91
Citric acid	0.59	1.7	92
Fumaric acid	0.040	0.50	93

CV- Coefficient of variation.

Table 4. Organic acids composition (mg/g of dry weight) of the studied edible mushrooms (mean ± SD; n=6).

	Oxalic acid	Quinic acid	Malic acid	Citric acid	Fumaric acid	Total identified organic acids
Agaricus bisporus	19.61 ± 0.44	6.44 ± 0.92	29.51 ± 0.43	43.23 ± 0.52	1.14 ± 0.00	99.93 ± 2.30^{h}
Agaricus bisporus portobello	15.33 ± 1.35	nd	30.05 ± 1.23	34.62 ± 1.40	2.57 ± 0.03	82.57 ± 1.49^{kj}
Agaricus campestris	11.30 ± 0.06	nd	17.81 ± 0.34	nd	2.98 ± 0.01	32.09 ± 0.40^{xayz}
Agaricus comtulus	9.59 ± 0.32	78.80 ± 1.04	11.28 ± 0.61	26.55 ± 0.22	1.99 ± 0.00	128.21 ± 2.20^{f}
Agaricus lutosus	5.93 ± 0.37	nd	11.63 ± 0.64	58.29 ± 0.13	3.46 ± 0.00	79.31 ± 0.40^{kl}
Agaricus silvaticus	4.86 ± 0.22	nd	23.88 ± 0.38	43.00 ± 0.04	3.77 ± 0.12	75.51 ± 0.32^{l}
Amanita caesarea	3.45 ± 0.10	nd	16.23 ± 0.33	nd	4.97 ± 0.48	24.65 ± 0.71^{bdc}
Amanita spissa	tr	nd	26.17 ± 0.39	18.90 ± 0.10	5.11 ± 0.01	50.18 ± 0.49^{qsr}
Armillaria mellea	1.40 ± 0.22	8.24 ± 1.08	13.77 ± 0.29	nd	2.71 ± 0.08	26.12 ± 1.67^{bacz}
Boletus aereus	20.77 ± 4.87	nd	85.69 ± 6.57	nd	0.30 ± 0.02	106.76 ± 1.72^{g}
Boletus armeniacus	62.20 ± 0.17	nd	118.33 ± 10.98	nd	0.63 ± 0.29	181.16 ± 10.52^{d}
Boletus citrinoporus	5.56 ± 0.49	nd	8.33 ± 0.25	nd	1.34 ± 0.02	15.23 ± 0.72^{fe}
Boletus edulis (commercial)	22.61 ± 0.98	nd	16.98 ± 0.13	nd	0.15 ± 0.01	39.74 ± 0.85^{wvu}
Boletus edulis (wild)	6.02 ± 0.12	nd	17.34 ± 0.92	nd	2.21 ± 0.08	25.57 ± 0.89^{bdac}
Boletus fragrans	1.86 ± 0.02	23.01 ± 0.27	17.11 ± 1.03	30.60 ± 0.21	0.86 ± 0.04	73.44 ± 1.07^{ml}
Boletus impolitus	4.38 ± 0.17	nd	7.61 ± 0.69	nd	2.42 ± 0.11	14.41 ± 0.98^{fe}
Boletus reticulatus	38.90 ± 4.09	nd	4.63 ± 0.57	nd	0.34 ± 0.03	43.87 ± 3.55^{tsu}
Bovista aestivalis	10.57 ± 2.83	nd	tr	nd	0.07 ± 0.03	10.64 ± 2.86^{gf}
Bovista nigrescens	0.82 ± 0.40	nd	0.51 ± 0.04	nd	tr	1.33 ± 0.44^{h}

Cantarellus cibarius (wild)	1.31 ± 0.05	nd	38.72 ± 2.15	12.02 ± 1.10	1.63 ± 0.14	53.68 ± 1.13^{qp}
Clavariadelphus pistillaris	0.98 ± 0.01	nd	21.20 ± 0.54	nd	9.06 ± 0.06	31.24 ± 0.61^{xayz}
Clavariadelphus truncatus	3.91 ± 0.79	nd	2.73 ± 0.36	7.84 ± 0.96	1.20 ± 0.20	15.68 ± 1.18^{fe}
Clitocybe costata	8.09 ± 0.02	nd	24.91 ± 0.14	26.72 ± 0.10	3.30 ± 0.00	63.02 ± 0.26^{on}
Clitocybe gibba	12.56 ± 2.87	nd	3.31 ± 0.60	nd	3.32 ± 0.29	19.19 ± 3.76^{de}
Clitocybe odora	14.08 ± 0.24	198.17 ± 1.96	4.25 ± 0.70	nd	1.19 ± 0.04	217.69 ± 2.46^{b}
Clorophyllum rhacodes	10.22 ± 0.91	nd	5.58 ± 0.74	34.74 ± 0.90	6.26 ± 0.04	56.80 ± 2.51^{op}
Coprinus comatus	4.92 ± 0.29	nd	20.34 ± 1.03	nd	8.48 ± 0.88	33.74 ± 1.62^{xwy}
Cortinarius anomalus	6.15 ± 0.11	nd	15.04 ± 0.22	nd	10.58 ± 0.01	31.77 ± 0.11^{xayz}
Cortinarius praestans	1.53 ± 0.11	nd	19.33 ± 0.07	13.38 ± 1.68	12.31 ± 0.56	46.55 ± 0.94^{tsr}
Cortinarius violaceus	1.76 ± 0.23	4.03 ± 0.55	8.68 ± 0.11	5.33 ± 0.07	8.68 ± 0.08	28.48 ± 0.88^{bayz}
Craterellus cornucopioides	3.29 ± 0.36	nd	27.84 ± 1.53	nd	2.59 ± 0.18	33.72 ± 1.35^{xwy}
Fistulina hepatica	0.16 ± 0.03	nd	33.43 ± 0.61	29.69 ± 1.26	3.77 ± 0.89	67.05 ± 2.81^{mn}
Flammulina velutipes (commercial)	5.11 ± 0.70	nd	18.48 ± 0.64	60.47 ± 0.25	2.05 ± 0.17	86.11 ± 0.48^{j}
Flammulina velutipes (wild)	14.09 ± 0.57	nd	32.81 ± 0.41	nd	1.62 ± 0.06	48.52 ± 0.92^{qsr}
Hygrophoropsis aurantiaca	5.17 ± 0.30	nd	14.62 ± 0.03	nd	1.00 ± 0.09	20.79 ± 0.36^{dce}
Hygrophorus chrysodon	4.88 ± 0.89	nd	0.68 ± 0.44	nd	0.22 ± 0.07	5.78 ± 1.41^{gh}
Lacaria amethystine	2.00 ± 0.00	nd	8.03 ± 0.35	14.28 ± 1.51	6.64 ± 0.23	30.95 ± 1.39^{bxayz}
Lactarius deliciosus	5.11 ± 0.49	nd	23.32 ± 0.53	nd	1.14 ± 0.05	29.57 ± 1.07^{bayz}
Lactarius volemus	6.60 ± 0.04	1.17 ± 0.11	29.81 ± 0.40	nd	2.51 ± 0.00	40.09 ± 0.55^{twvu}
Lentinus edodes	10.06 ± 0.14	nd	28.87 ± 0.41	165.58 ± 6.10	5.02 ± 0.07	209.53 ± 5.48^{c}
Lepista nuda	43.44 ± 3.98	125.27 ± 3.79	8.69 ± 1.93	nd	0.68 ± 0.20	178.08 ± 9.90^{d}
Leucoagaricus leucothites	3.26 ± 0.08	nd	17.42 ± 0.07	nd	5.87 ± 0.06	26.55 ± 0.21^{bacz}

Leucopaxillus giganteus	2.09 ± 0.21	nd	60.25 ± 5.47	nd	2.30 ± 0.30	64.64 ± 5.56^{n}
Lycoperdon imbrinum	1.38 ± 0.21	nd	tr	nd	0.24 ± 0.06	1.62 ± 0.27^{h}
Macrolepiota excoriata	6.35 ± 0.15	nd	23.72 ± 0.88	nd	2.44 ± 0.01	32.51 ± 1.04^{xyz}
Macrolepiota procera	13.29 ± 0.02	nd	9.69 ± 0.73	26.38 ± 0.29	0.41 ± 0.01	49.77 ± 0.41^{qsr}
Marasmius oreades	17.97 ± 1.32	nd	78.60 ± 3.08	43.61 ± 1.12	0.40 ± 0.00	140.58 ± 3.29^{e}
Pleurotus eryngii	2.02 ± 0.03	nd	18.48 ± 0.07	28.73 ± 0.57	2.50 ± 0.05	51.73 ± 0.59^{qpr}
Pleurotus ostreatus	4.35 ± 0.37	nd	15.11 ± 1.56	21.37 ± 2.47	3.40 ± 0.44	44.23 ± 4.09^{tsu}
Ramaria aurea	1.40 ± 0.09	nd	4.59 ± 0.19	4.39 ± 0.01	4.77 ± 0.01	15.15 ± 0.10^{fe}
Russula delica	10.11 ± 0.39	nd	29.45 ± 2.07	nd	2.29 ± 0.18	41.85 ± 2.64^{tvu}
Russula olivacea	3.71 ± 0.18	nd	11.70 ± 0.87	nd	2.19 ± 0.00	17.60 ± 0.69^{e}
Sarcodon imbricatus	12.66 ± 0.22	nd	240.65 ± 2.35	nd	0.78 ± 0.06	254.09 ± 2.63^{a}
Suillus variegates	24.58 ± 0.24	nd	3.83 ± 0.07	nd	0.22 ± 0.00	28.63 ± 0.31^{bayz}
Tricholoma imbricatum	3.32 ± 0.21	nd	44.26 ± 0.11	nd	6.30 ± 0.06	53.88 ± 0.04^{qp}
Tricholoma portentosum	4.26 ± 0.02	nd	64.91 ± 5.93	19.02 ± 1.92	5.02 ± 0.34	93.21± 4.33^{i}

In each column, different letters mean significant differences (p<0.05); nd- not detected; tr- traces.

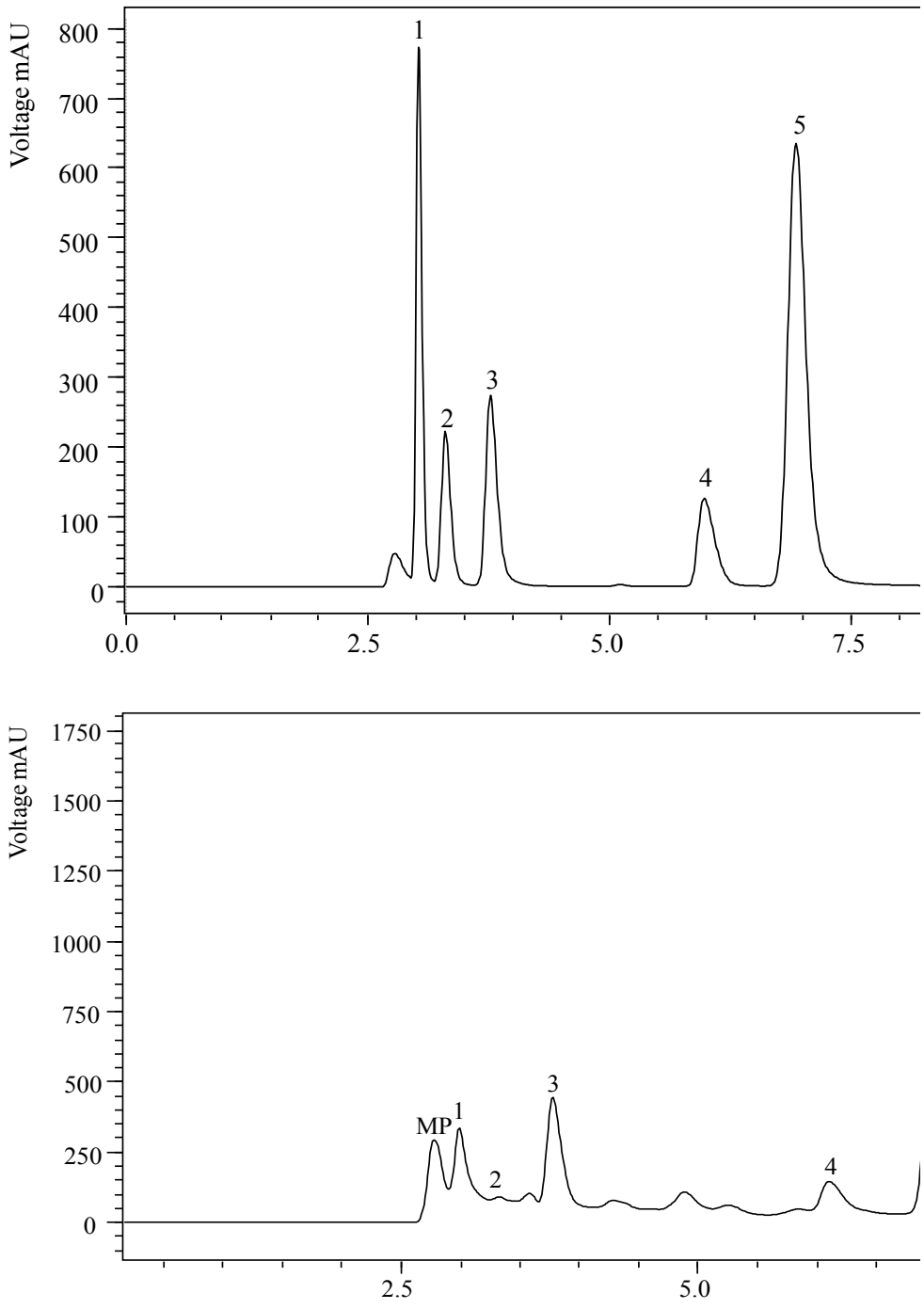

Figure 1. UFLC organic acids profile recorded at 215 nm: (**a**) organic acid (**b**) *Agaricus bisporus*. MP- mobile phase; 1- oxalic acid; 2-quinic acid; 3-citric acid and 5- fumaric acid.