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Quantitation of Nine Organic Acids in Wild Mushrooms

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The organic acids composition of six wild edible mushroom species (*Amanita caesarea*, *Boletus edulis*, *Gyroporus castaneus*, *Lactarius deliciosus*, *Suillus collinitus*, and *Xerocomus chrysenteron*) was determined by an HPLC-UV detector method. The results showed that all of the samples presented a profile composed of at least five organic acids: citric, ketoglutaric, malic, succinic, and fumaric acids. Several samples also contained oxalic, ascorbic, quinic, and shikimic acids. In a general way, the quantitation of the identified compounds indicated that malic acid, followed by the pair citric plus ketoglutaric acids, were the main compounds in the analyzed species, with the exception of *A. caesarea*, in which malic and ascorbic acids were the most abundant compounds. The relative amounts and the presence/absence of each identified compound may be useful for the differentiation of the species.

KEYWORDS: Organic acids; wild edible mushrooms; *Amanita caesarea*; *Boletus edulis*; *Gyroporus castaneus*; *Lactarius deliciosus*; *Suillus collinitus*; *Xerocomus chrysenteron*

INTRODUCTION

Wild-growing mushrooms have a worldwide distribution and have been a popular delicacy in many countries. In fact, since ancient times mushrooms have been consumed by humans as a part of the normal diet and, in recent times, they constitute an increasing share in the world diet (*1*−*4*). They have a highly desirable taste and aroma, being also consumed for their texture: they add flavor and texture to a meal (*1*, *5*, *6*).

From a nutritional point of view, mushrooms are considered to be healthy foods. They are high in protein (19−35% dry basis, including all of the essential amino acids) and have a good balance of vitamins, namely, thiamin, riboflavin, ascorbic acid, and vitamin D_2, as well as minerals. They are poor in calories and fat and contain appreciable amounts of dietary fiber (*1*, *4*, *7*, *8*). However, the cultivation, watering, fruiting, and storage conditions can influence the chemical composition and, as a consequence, the nutritional value of the mushrooms (*4*, *8*). In addition to their nutritional value, antitumor, antiviral, hypolipidemic, and antioxidant activities have been reported (*1*, *2*, *6*, *9*).

Because of increasing mushroom consumption, data on their nutritional value are needed. *Amanita caesarea*, *Boletus edulis*, *Gyroporus castaneus*, *Lactarius deliciosus*, *Suillus collinitus*, and *Xerocomus chrysenteron* are six species of wild edible mushrooms, commonly growing in the Northeast and Beira Interior regions of Portugal, fruiting mainly in the spring and

Table 1. Characterization of Mushroom Samples

sample	species	origin[a]	date of collection
1	*Lactarius deliciosus*	Bragança 1	November 2003
2	*L. deliciosus*	Bragança 2	November 2003
3	*L. deliciosus*	Bragança 3	November 2003
4	*L. deliciosus*	Bragança 4	November 2003
5	*L. deliciosus*	Bragança 5	November 2003
6	*L. deliciosus*	Covilhã 1	November 2003
7	*L. deliciosus*	Covilhã 2	November 2003
8	*L. deliciosus*	Covilhã 3	November 2003
9	*L. deliciosus*	Covilhã 4	November 2003
10	*L. deliciosus*	Fundão 1	November 2003
11	*L. deliciosus*	Fundão 2	November 2003
12	*L. deliciosus*	Fundão 3	November 2003
13	*L. deliciosus*	Fundão 4	November 2003
14	*Boletus edulis*	Bragança	June 2003
15	*B. edulis*	Bragança1	September 2003
16	*B. edulis*	Bragança2	September 2003
17	*B. edulis*	Bragança	October 2003
18	*Suillus collinitus*	Bragança 1	December 2003
19	*S. collinitus*	Bragança 2	December 2003
20	*S. collinitus*	Bragança 3	December 2003
21	*S. collinitus*	Bragança 4	December 2003
22	*S. collinitus*	Bragança 5	December 2003
23	*Xerocomus chrysenteron*	Bragança	October 2003
24	*Amanita caesarea*	Bragança	October 2003
25	*Gyroporus castaneus*	Bragança	October 2003

[a] Each number represents a different pine tree or chestnut from the same geographical origin.

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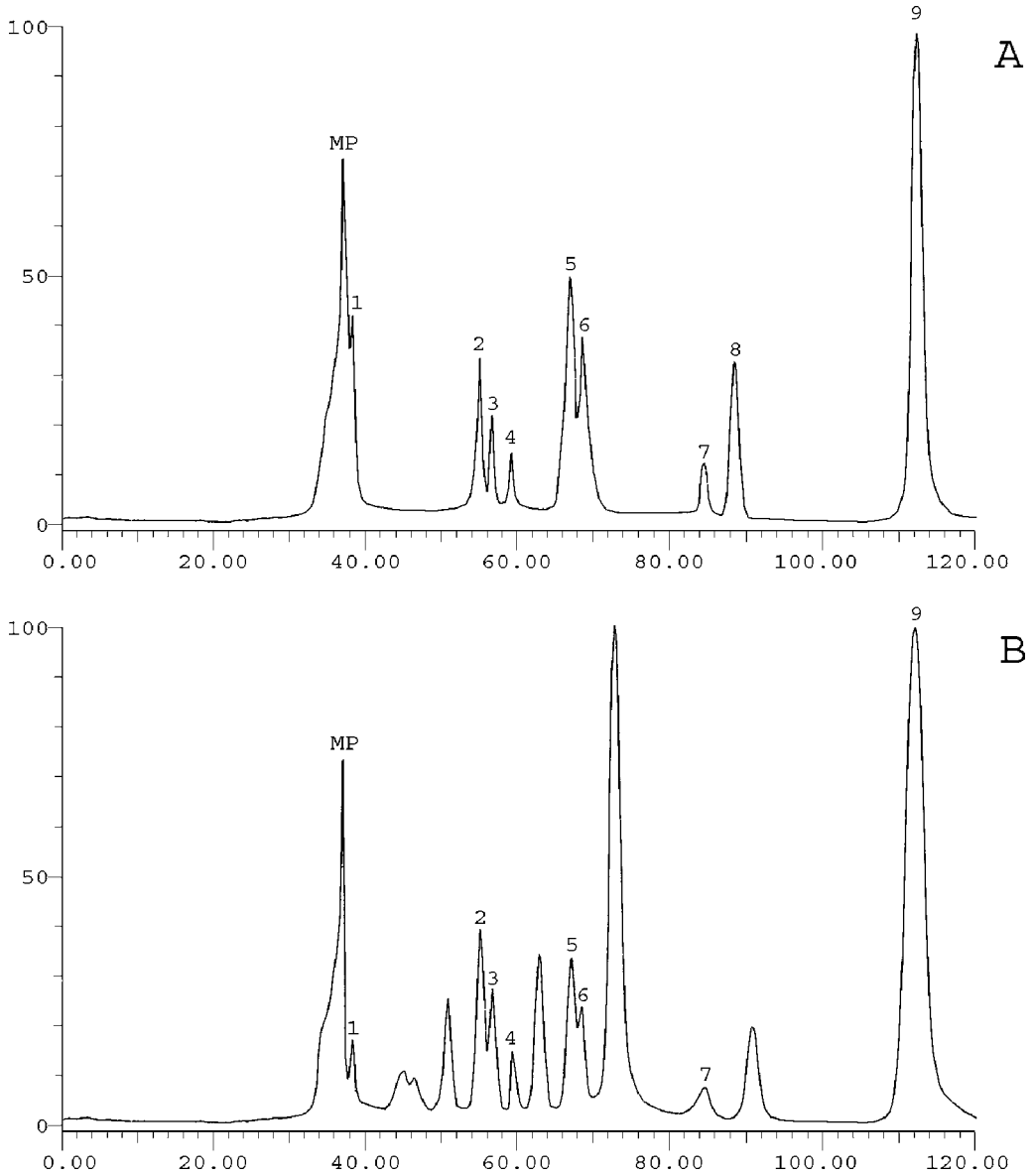

Figure 1. HPLC-UV chromatogram of (**A**) standard solution and (**B**) *S. collinitus* sample (sample 18). Detection was at 214 nm. Peaks: (MP) mobile phase; (1) oxalic acid; (2) citric acid; (3) ketoglutaric acid; (4) ascorbic acid; (5) malic acid; (6) quinic acid; (7) succinic acid; (8) shikimic acid; (9) fumaric acid.

autumn. These species are of great economical and gastronomical importance. Several works have previously involved these species. In the case of *A. caesarea* its sterol constituents were determined (*10*). *B. edulis* was studied for its trace element composition (*2*, *11*, *12*), vitamin D (*13*), dietary fiber, chitin, β-glucan, and total phenols contents (*7*), flavor constituents (*14*), and insecticidal properties (*15*). Earlier works on *L. deliciosus* reported the characterization of a lectin (*16*), sesquiterpenes (*17*), and a fulvene hydrocarbon (*18*), trace element (*11*) and sodium (*19*) concentrations, insecticidal capacity (*15*), and definition of cultivation conditions for improving the production of the ectomycorrhizal fungus mycelium (*20*). Sodium (*19*) and trace element contents (*11*), insecticidal properties (*15*), and identification of a lectin (*21*) from *X. chrysenteron* were also described before. As far as we know, nothing as been reported about *G. castaneus* and *S. collinitus*.

The nature and concentration of organic acids are important factors influencing the organoleptic characteristics of fruits and vegetables, namely, their flavor (*22*). Acids are known to have a lower susceptibility to change during processing and storage than other components such as pigments and flavor compounds (*23*). In addition, organic acids may have a protective role against various diseases due to their antioxidant activity (*24*). To our knowledge, there is no study about organic acids composition of any of the six previously mentioned wild edible mushroom species.

The aim of the present work was to establish the organic acids composition of *A. caesarea*, *B. edulis*, *G. castaneus*, *L. deliciosus*, *S. collinitus*, and *X. chrysenteron*. With this purpose, samples of these species from Bragança (northeastern Portugal) were analyzed. In addition, to check the influence of the geographical origin on the organic acids profile, samples from *L. deliciosus* were collected in three different places: Bragança (northeastern Portugal) and Covilhã and Fundão (Beira Interior). To evaluate the effect of the time of collection on the organic acids composition, samples from *B. edulis* were collected in late spring and autumn in the Bragança region.

MATERIALS AND METHODS

Samples. Samples of *L. deliciosus* and *S. collinitus* were collected under live pine trees (*Pinus* sp.), in Bragança, Covilhã, and Fundão for the former and in Bragança for the latter in autumn 2003. *A.*

Table 2. Organic Acids Content in Mushroom Samples (Milligrams per Kilogram)[a]

	compound							
sample	oxalic acid (RT 38.2 min)	citric acid + ketoglutaric acid (RTs 55.6, 57.2 min)	ascorbic acid (RT 59.4 min)	malic acid + quinic acid (RTs 67.4, 68.9 min)	succinic acid (RT 85.5 min)	shikimic acid (RT 87.8 min)	fumaric acid (RT 114.1)	total
1	4.62 (0.05)	92.97 (8.88)		1346.97 (1.72)[b]	0.24 (0.00)		13.18 (0.02)	1457.97
2	116.44 (2.40)	237.60 (9.05)		3390.10 (68.31)[b]	0.28 (0.01)		205.12 (0.25)	3949.53
3	67.12 (2.20)	410.17 (11.29)	nq	1516.91 (13.10)[b]	0.25 (0.00)		88.45 (0.20)	2082.90
4	118.37 (1.57)	316.93 (8.13)	328.23 (2.31)	1858.38 (9.11)[b]	0.27 (0.02)		213.19 (2.60)	2835.37
5	201.00 (10.84)	6112.72 (21.51)		405.57 (23.79)[b]	0.16 (0.01)		29.54 (2.18)	6749.44
6	19.62 (1.14)	1352.81 (71.26)		2153.44 (48.8)[b]	0.54 (0.01)		74.11 (3.07)	3600.54
7	22.54 (3.38)	111.80 (8.71)	362.65 (35.29)	1624.07 (26.04)[b]	0.20 (0.00)		105.88 (1.56)	2227.13
8	47.40 (3.79)	294.90 (2.75)	353.58 (30.68)	2318.89 (166.71)[b]	0.10 (0.00)		115.62 (7.73)	3130.46
9	76.59 (0.42)	327.05 (32.75)		1870.27 (27.47)[b]	0.39 (0.00)		237.56 (0.96)	2511.86
10	121.66 (0.17)	309.18 (1.16)	343.12 (1.08)	3778.97 (4.28)[b]	0.56 (0.02)		201.65 (0.04)	4755.14
11	70.83 (0.49)	1168.62 (6.14)		1565.01 (9.78)[b]	0.30 (0.02)		103.28 (1.60)	2908.04
12		271.09 (42.26)	972.81 (5.09)	3885.92 (39.37)[b]	1.28 (0.05)		67.79 (6.13)	5198.90
13	90.11 (6.60)	271.21 (30.43)	187.55 (27.00)	3349.64 (18.55)[b]	0.50 (0.00)		217.65 (4.16)	4116.67
14	136.00 (0.74)	432.89 (40.61)		2267.06 (60.25)	0.07 (0.01)		11.64 (0.38)	2847.66
15	536.12 (0.13)	785.45 (4.92)		1393.17 (55.46)	0.33 (0.02)		75.16 (1.53)	2790.23
16	307.09 (0.84)	348.77 (68.58)		3383.41 (7.72)	0.31 (0.01)		54.90 (2.78)	4094.48
17		378.65 (41.17)		4126.68 (255.13)	0.04 (0.00)		22.38 (1.14)	4527.75
18	27.67 (1.68)	362.73 (5.40)	952.10 (114.39)	1784.45 (24.54)	0.45 (0.05)		198.09 (1.97)	3325.49
19	123.47 (3.17)	748.62 (6.75)	514.15 (16.02)	1540.72 (12.52)	4.82 (0.02)		372.79 (2.75)	3304.57
20	41.43 (2.96)	913.74 (0.82)	92.40 (12.02)	465.67 (5.79)	0.69 (0.05)	1.46 (0.09)	49.49 (1.37)	1564.88
21	125.86 (3.59)	2903.02 (460.82)	3788.04 (128.73)	2654.77 (196.98)	8.67 (0.02)		359.53 (1.47)	9839.88
22	70.77 (0.68)	1040.97 (17.51)		1897.94 (42.17)	5.68 (0.12)		397.16 (0.89)	3412.51
23		377.97 (50.46)		3040.97 (455.44)[b]	0.19 (0.21)		12.77 (2.98)	3431.89
24		452.07 (13.71)	2071.46 (9.51)	3333.03 (25.73)[b]	0.07 (0.01)	1.62 (0.07)	43.19 (0.13)	5901.44
25		1282.65 (48.76)		3812.78 (86.55)[b]	0.23 (0.00)		74.65 (1.14)	5170.31

[a] Results are expressed as mean (standard deviation) of three determinations. nq, not quantified. [b] Values represent only malic acid.

caesarea, *B. edulis*, *G. castaneus*, and *X. chrysenteron* were collected from a chestnut orchard (*Castanea sativa* Mill.). All of these species were picked in October 2003 except for *B. edulis*, which was collected during three different periods in 2003. In **Table 1**, the geographical origin and date of collection of the different samples are given.

After collection, the mushrooms were grouped by taxon and dried at 30 °C for 72 h. Taxonomic identification was made according to guidelines provided by several authors (*25*−*30*), and representative voucher specimens were deposited at the herbarium of the Escola Superior Agrária of Instituto Politécnico de Bragança.

Standards and Reagents. The standards (citric, ketoglutaric, malic, succinic, fumaric, oxalic, ascorbic, quinic, shikimic, aconitic, pyruvic, malonic, and tartaric acids) were from Sigma (St. Louis, MO) and from Extrasynthése (Genay, France). Methanol and hydrochloric and formic acids were obtained from Merck (Darmstadt, Germany), and sulfuric acid was from Pronalab (Lisboa, Portugal). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

Solid-Phase Extraction (SPE) Columns. The Isolute C18 non-end-capped (NEC) SPE columns (50 μm particle size, 60 Å porosity; 10 g of sorbent mass/70 mL of reservoir volume) were purchased from International Sorbent Technology Ltd. (Mid Glamorgan, U.K.).

Extraction of Organic Acids from Mushrooms. Organic acids extraction was performed according to a described procedure (*31*). Each mushroom powdered sample (∼1 g) was thoroughly mixed with methanol (5 × 50 mL), at 40 °C. The methanolic extract was filtered, concentrated to dryness under reduced pressure (40 °C), and redissolved in acid water (pH 2 with HCl). The aqueous solution was then passed through an Isolute C18 (NEC) column, previously conditioned with 30 mL of methanol and 70 mL of acid water (pH 2 with HCl). The nonpolar compounds were retained, and the polar ones, such as organic acids, are eluted with aqueous solution. This aqueous extract was evaporated to dryness under reduced pressure (40 °C) and redissolved in 0.01 N sulfuric acid (1 mL), and 20 μL was analyzed by HPLC-UV.

A standard mixture was prepared by dissolving the compounds in 0.01 N sulfuric acid, and 20 μL was analyzed by HPLC-UV.

HPLC Analysis of Organic Acids. The separation of organic acids was achieved with an analytical HPLC unit (Gilson), using an ion exclusion column (Nucleogel Ion 300 OA; 300 × 7.7 mm), in conjunction with a column heating device at 30 °C. Elution was carried out isocratically with 0.01 N sulfuric acid as the mobile phase, at a flow rate of 0.1 mL/min, for 120 min. Detection was performed with a UV detector set at 214 nm.

Organic acids quantitation was achieved by the absorbance recorded in the chromatograms relative to external standards, and the peaks in the chromatograms were integrated using a default baseline construction technique. Citric acid and ketoglutaric acid were quantified together as citric acid. Malic acid and quinic acid were quantified together as quinic acid.

RESULTS AND DISCUSSION

A. caesarea, *B. edulis*, *G. castaneus*, *L. deliciosus*, *S. collinitus*, and *X. chrysenteron* are six edible mushroom species greatly appreciated in many European countries, where they are consumed in several forms: raw, stewed, fried, roasted, grilled, or in sauces. Their high consumption demands a better knowledge of their chemical composition. Therefore, this work was developed for the identification and quantitation of the organic acids of these species.

The HPLC-UV analysis showed that all of the samples presented a profile composed of at least five organic acids: citric, ketoglutaric, malic, succinic, and fumaric acids. Several samples also contained oxalic, ascorbic, quinic, and shikimic acids (**Figure 1**). None of the samples presented aconitic, pyruvic, malonic, or tartaric acids. To test the recovery of the identified compounds, known quantities of each organic acid were added to a weighed portion of mushroom. The sample was analyzed in triplicate before and after the additions. The recovery rate was ∼97% for all of the identified organic acids. Generally, the quantitation of the identified compounds indicated that malic acid and the pair citric plus ketoglutaric acids were the main compounds in the analyzed species with the exception of *A. caesarea*, in which malic and ascorbic acids were the most

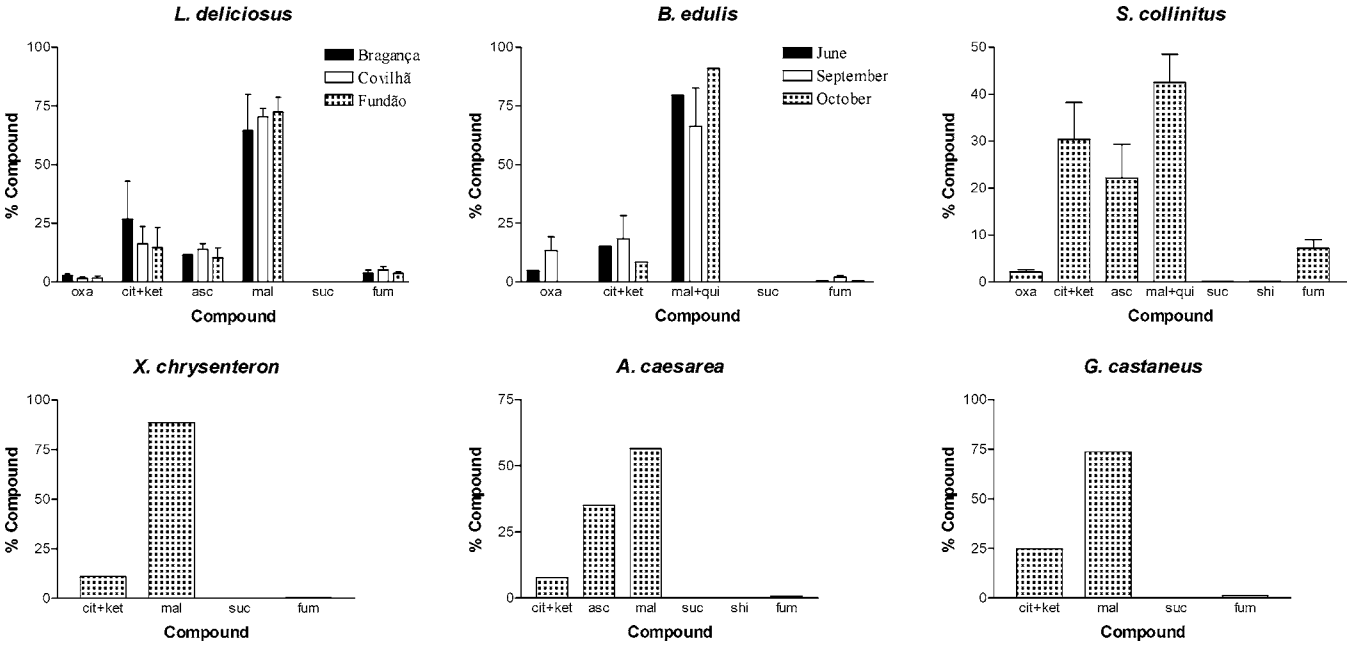

Figure 2. Organic acids profile of *A. caesarea*, *B. edulis*, *G. castoneus*, *L. deliciosus*, *S. collinitus*, and *X. chrysenteron.* Values represent mean, and standard error bars are on the top of each column. Abbreviations: oxa, oxalic acid; cit, citric acid; ket, ketoglutaric acid; asc, ascorbic acid; mal, malic acid; qui, quinic acid; suc, succinic acid; shi, shikimic acid; fum, fumaric acid.

abundant compounds. Succinic acid was always present in very small amounts, below 0.2% of the total quantified compounds (**Table 2**; **Figure 2**). Each species exhibited a distinct organic acids profile.

In *L. deliciosus* samples malic acid represented 65−72% of the total organic acids content (**Figure 2**), followed by the pair citric plus ketoglutaric acids, which corresponded to 15−27% of total compounds. Oxalic acid and ascorbic acid were also present, and succinic acid was the minor compound. No differences were found among the three geographical origins, indicating that this factor may not interfere with the organic acids composition.

The sum of all quantified acids in *B. edulis* samples ranged from ∼3 to 4 g/kg (**Table 2**), with malic plus quinic acids corresponding to 66−91% (**Figure 2**). One striking characteristic of this species is the presence of quinic acid and the absence of ascorbic and shikimic acids. When the results obtained with samples collected during the three months were compared, no differences could be seen in the profile from late spring (June) to autumn (September and October), which means that the collection date does not seem to have an influence on the organic acids content.

S. collinitus was the only species exhibiting all nine identified organic acids (**Figure 1**), although shikimic acid was present in only one sample and in very small amounts (0.1% of total identified compounds) (**Table 2**; **Figure 2**). The pairs malic plus quinic acids and citric plus ketoglutaric acids were the compounds present in higher amounts, representing 42 and 30% of total acids, respectively.

In *X. chrysenteron* samples only five organic acids were identified: citric, ketoglutaric, malic, succinic, and fumaric acids. The sum of these acids corresponded to ∼3 g/kg, of which 89% was represented by malic acid and 11% by the pair citric plus ketoglutaric acids (**Table 2**; **Figure 2**).

The qualitative composition of *G. castaneus* was identical to that of *X. chrysenteron*. Differences were noted at a quantitative level: in *G. castaneus* the sum of the identified acids was higher (∼5 g/kg), with malic acid as the major compound, although it represented only 74% of total identified acids. In addition, the amounts of the pair citric plus ketoglutaric acids and of fumaric acid were noticeably higher, corresponding to 25 and 1%, respectively (**Table 2**; **Figure 2**).

A. caesarea had one of the highest organic acids content (∼6 g/kg) (**Table 2**). Oxalic and quinic acids were not found, and malic acid was the main compound (56% of total acids). The most remarkable characteristic of its profile may be the presence of ascorbic acid in the highest relative amount (35%) when compared with the other analyzed species (**Figure 2**).

As far as we know, this is the first report concerning the organic acids composition of *A. caesarea*, *B. edulis*, *G. castaneus*, *L. deliciosus*, *S. collinitus*, and *X. chrysenteron*. This study shows that organic acids analysis can be useful in taxonomic studies involving these mushroom species, although further studies with a large number of samples, mainly of *X. chrysenteron*, *A. caesarea*, and *G. castoneus*, are necessary to confirm the differences observed.

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