Effect of the Conservation Procedure on the Contents of Phenolic Compounds and Organic Acids in Chanterelle (*Cantharellus cibarius*) Mushroom

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To check the influence of the conservation procedure in the chemical composition of chanterelle mushroom, phenolic compounds and organic acids of samples preserved under four different conditions (drying, freezing, conservation in olive oil and in vinegar) were determined. Phenolics and organic acids were analyzed by HPLC-DAD and HPLC-UV, respectively. The results showed that chanterelle is characterized by the presence of six phenolic compounds (3-, 4-, and 5-*O*-caffeoylquinic acid, caffeic acid, *p*-coumaric acid, and rutin) and five organic acids (citric, ascorbic, malic, shikimic, and fumaric acids). Samples preserved in olive oil also exhibited hydroxytyrosol, tyrosol, luteolin, and apigenin, whereas conservation in vinegar led to the detection of hydroxytyrosol, tyrosol, and tartaric acid in the analyzed samples. The conservation procedures to which chanterelle samples were subjected seem to affect the qualitative and quantitative phenolics and organic acids profiles.

KEYWORDS: Chanterelle; *Cantharellus cibarius*; conservation; phenolic compounds; organic acids

INTRODUCTION

In recent years an increase in the consumption of wild edible mushrooms has been observed, even in the developed world, although they do not constitute a significant portion of the human diet. In fact, wild-growing mushrooms have been a popular delicacy in many countries, mainly in central and eastern Europe (*1–3*), as they add flavor and texture to a meal (*4*).

Previous studies have indicated that edible mushroom species are highly nutritious, their nutritional value comparing favorably with that of meat, eggs, and milk (*5*). They present a good balance of vitamins, namely, thiamin, riboflavin, ascorbic acid, and vitamin D_2 and a good content of proteins as well as a high content of trace minerals. They are poor in calories and fat and contain appreciable amounts of dietary fiber (*3, 6–8*). However, the nutritional characteristics can be changed by the cultivation, watering, fruiting, and storage conditions, which influence their chemical composition (*7, 8*).

Edible mushrooms are characterized by a short shelf life (1–3 days at room temperature), linked to the occurrence of post-harvest changes. These changes are due to the high moisture content of the carpophores and the high activity of enzymes, such as protease or polyphenol oxidase, responsible for protein and sugar decrease and for browning reactions during storage (*9*).

The chanterelle (*Cantharellus cibarius*) is an edible mushroom with a worldwide distribution, fruiting from fall to early spring. It is highly appreciated for its wonderful fruity, apricot-like aroma, being particularly prized for cooking throughout Europe. Besides the flavor and smell, its texture is also nice. The chanterelle is of great economical interest for northeastern Portugal, because its collection constitutes a way of subsistence for the residents, apart from becoming an important commercial source.

Previous studies on the chanterelle concerned its amino acids (*5*), vitamin D (*10, 11*), protein (*3, 12*), fat (*3*), fiber (*3*), ash (*3, 12*), minerals (*2, 3, 12, 13*), ascorbic acid (*3*), and trace elements contents (*1*), the characterization of basidiolipids (*14*), a ubiquitin-like peptide (*15*), and a homodimeric laccase (*16*), the use of different nitrogen sources (*17*), the production of bioactive secondary metabolites (*18*), and the biological activities of two fatty acid derivatives formed as a response to injury (*19*), and its mutagenic (*20*) and insecticidal properties (*21*). As far as we know, nothing as been reported about the phenolics and organic acids profile of the chanterelle.

Phenolic compounds and organic acids are known to contribute to the organoleptic characteristics of fruits and vegetables (*4*). Additionally, these compounds may have a protective role against various diseases due to their antioxidant activity (*22*).

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Table 1. Chanterelle Sample Treatment

sample	conservation procedure	sample	conservation procedure
1	drying at 30 °C for 96 h	7	in vinegar
2	drying at 30 °C for 96 h	8	in vinegar
3	drying at 30 °C for 96 h	9	in vinegar
4	freezing	10	in olive oil
5	freezing	11	in olive oil
6	freezing		

The main purposes of this study were to determine the phenolic compounds and organic acids composition of chanterelle and to investigate the influence of the conservation process in these chemical parameters. With this aim, chanterelle samples subjected to four different preservation procedures (drying, freezing, and conservation in olive oil and in vinegar) were analyzed.

MATERIALS AND METHODS

Samples. Samples of chanterelle were collected from a chestnut orchard (*Castanea sativa* Mill.) in the Bragança region (northeastern Portugal), in the spring of 2004. After collection, the mushrooms were immediately transferred to the laboratory. Taxonomic identification followed that of several authors (*23−28*). Representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança.

Samples were preserved under four distinct methods commonly used for the commercialization of the chanterelle (**Table 1**). Samples 1−3 were dehydrated in a ventilated stove at 30 °C for 4 days. They were kept in the dark, in hermetically sealed plastic bags. Frozen samples (samples 4−6) were previously cleaned and boiled in water with salt during 2 min; after being dried with absorbent paper, they were placed in plastic bags, which were then quickly frozen in a freezer at −20 °C. Samples 7−9 were preserved in vinegar, as pickles: the mushrooms were cleaned and boiled in water with salt for 15 min; after being dried with absorbent paper, they were placed in flasks, which were filled with white wine vinegar with a little sugar and hermetically closed. Samples preserved in olive oil (samples 10 and 11) were boiled for 10 min in a mixture of 250 mL of white wine vinegar and 150 mL of water with salt; after being dried with absorbent paper, they were placed in flasks, which were filled with olive oil (extra virgin quality) and hermetically closed. All of the samples were kept for 4 months under the conditions described above until analysis.

Before analysis, samples 7−11 were cleaned with absorbent paper for the removal of vinegar and olive oil and frozen. With the exception of those subjected to the drying process (samples 1−3), all of the analyzed samples were freeze-dried in a Labconco 4.5 apparatus (Kansas City, MO), yielding 10% of dry matter.

Standards. The standards were from Sigma (St. Louis, MO) and from Extrasynthése (Genay, France). Methanol and formic and hydrochloric 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 sorbent mass/70 mL reservoir volume) were purchased from International Sorbent Technology Ltd. (Mid Glamorgan, U.K.).

Extraction of Phenolics and Organic Acids. Each mushroom sample (~1 g) was thoroughly mixed with methanol (5 × 50 mL), at 40 °C. The methanolic extract was filtered and concentrated to dryness under reduced pressure (40 °C).

The organic acids fractions were obtained according to a described procedure (*29*). The residue of the methanolic extract was redissolved in acid water (pH 2 with HCl) (~50 mL), and 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 aqueous extract, containing the organic acids, 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.

After the elution of organic acids and other polar compounds with the aqueous solvent, the retained phenolic fraction was eluted with methanol (~150 mL). The extracts were concentrated to dryness under reduced pressure (40 °C) and redissolved in methanol (1 mL), and 20 μL was analyzed by HPLC-DAD.

HPLC Analysis of Organic Acids. The separation was carried out as previously reported (*29*) with an analytical HPLC unit (Gilson), using an ion exclusion Nucleogel Ion 300 OA (300 × 7.7 mm) column, in conjunction with a column heating device at 30 °C. Elution was carried out at a solvent flow rate of 0.1 mL/min, isocratically, with 0.01 N sulfuric acid as the mobile phase. Detection was performed with a UV detector set at 214 nm.

Organic acids quantification was achieved by the absorbance recorded in the chromatograms relative to external standards.

HPLC Analysis of Phenolics. The extracts were analyzed on an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0 × 0.46 cm; 5 μm, particle size) column (*30−34*). The solvent system used was a gradient of water/formic acid (19:1) (A) and methanol (B), starting with 5% methanol and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 50% B at 44 min, 55% B at 47 min, 70% B at 50 min, 75% B at 56 min, and 80% B at 60 min, at a solvent flow rate of 0.9 mL/min. Detection was achieved with a Gilson diode array detector. The compounds in each sample were identified by comparing their retention times and UV−vis spectra in the 200−400 nm range with the library of spectra previously compiled by the authors. Peak purity was checked by means of the Gilson 160 SpectraViewer Software Contrast Facilities.

Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards, at 280 nm for hydroxytyrosol and tyrosol, at 320 nm for phenolic acids, and at 350 nm for flavonoids. 3- and 4-*O*-caffeoylquinic acids were quantified as 5-*O*-caffeoylquinic acid, and hydroxytyrosol was quantified as tyrosol. The other compounds were quantified as themselves.

Statistical Analysis. The evaluation of statistical significance was determined by ANOVA, followed by Newman−Keuls test. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Mushroom browning is a major biochemical event after harvest. It is one of the main features, besides texture and cap opening, considered in the quality spectrum. Mushrooms are praised for their characteristic biting texture and pleasant flavor; however, one or both of these properties are lost during mushroom processing (*35*). In addition, it is known that the storage conditions can influence the chemical composition and, as a consequence, the nutritional value of mushrooms (*7*).

Chanterelle is an edible mushroom species very appreciated in Europe, used in several cookery preparations. Because of its great consumption, data on its nutritional value are needed. Therefore, this work was developed for the identification and quantification of the phenolic compounds and organic acids of this species and the evaluation of the influence of the conservation procedure in these chemical parameters.

Phenolic Compounds. The analysis of chanterelle frozen samples (samples 4−6) by reversed-phase HPLC-DAD allowed the identification of six phenolic compounds in this species (**Figure 1**): 3-, 4-, and 5-*O*-caffeoylquinic acids, caffeic acid, *p*-coumaric acid, and rutin. Despite these compounds, several unidentified flavonoids were also noted. On a quantitative level, chanterelle has very low amounts of phenolic compounds (~20.0 mg/kg) (**Table 2**), 3-*O*-caffeoylquinic acid being the main compound (64% of total identified phenolics) (**Figure 3**). It should be noted that the deep-freezing process represents a common way to increase storage stability and facilitate mushroom consumption without seasonal constraints (*9*).
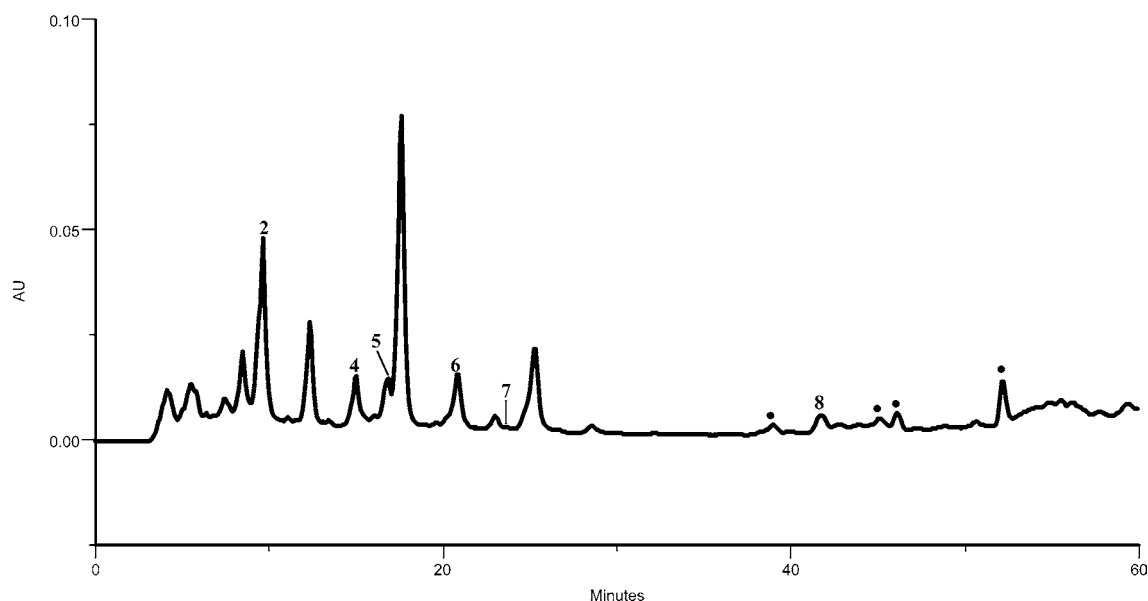
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Figure 1. HPLC-DAD chromatogram of phenolic compounds in chanterelle (frozen sample). Detection was at 320 nm. Peaks: (2) 3-*O*-caffeoylquinic acid; (4) 4-*O*-caffeoylquinic acid; (5) 5-*O*-caffeoylquinic acid; (6) caffeic acid; (7) *p*-coumaric acid; (8) rutin; (●) unidentified flavonoids.

Table 2. Phenolics Composition of Chanterelle[a]

	compound (mg/kg, dry basis)										
sample	hydroxy-tyrosol	3-*O*-caffeoyl-quinic acid	tyrosol	4-*O*-caffeoyl-quinic acid	5-*O*-caffeoyl-quinic acid	caffeic acid	*p*-coumaric acid	rutin	luteolin	apigenin	total
1		2.3 (0.2)		0.4 (0.0)		4.2 (0.0)	nq	0.8 (0.1)			7.8
2		18.2 (0.4)	nq		1.8 (0.2)	2.0 (0.1)	1.6 (0.0)	0.8 (0.1)			24.4
3		11.1 (0.3)	nq	0.7 (0.2)		1.5 (0.1)	0.5 (0.0)	0.4 (0.1)			14.2
4		11.6 (0.1)		2.6 (0.1)	2.7 (0.0)	1.9 (0.1)	0.3 (0.1)	1.8 (0.0)			21.0
5		20.0 (0.8)		1.2 (0.1)	2.3 (0.8)	1.1 (0.0)	nq	0.9 (0.1)			25.4
6		8.1 (1.5)		1.1 (0.2)	2.4 (0.2)	0.9 (0.1)	0.4 (0.3)	0.7 (0.1)			13.6
7	305.5 (8.8)		46.9 (1.8)	nq	nq	nq	nq	5.0 (0.1)			357.4
8	210.9 (3.1)		35.4 (0.6)	nq	nq	nq	nq	nq			246.3
9	359.4 (22.1)		52.8 (1.9)	nq	1.6 (0.7)	nq	nq	0.7 (0.1)			414.4
10	349.4 (22.6)		349.0 (6.6)			0.9 (0.0)	2.6 (0.1)	1.8 (0.1)	88.4 (3.9)	14.4 (1.2)	806.5
11	437.4 (37.9)		450.1 (10.0)			0.3 (0.0)	3.2 (0.3)	12.3 (0.5)	75.8 (10.3)	15.4 (1.9)	994.4

[a] Results are expressed as mean (standard deviation) of three determinations. nq, not quantified.

Table 3. Organic Acids Composition of Chanterelle[a]

	compound (mg/kg, dry basis)					
sample	citric acid	ascorbic acid	malic acid	shikimic acid	fumaric acid	total
1	696.9 (1.3)	187.3 (14.9)	4500.1 (26.8)	0.9 (0.1)	0.4 (0.1)	5385.6
2	26.3 (0.6)	19.3 (0.9)	178.5 (10.0)		5.0 (0.0)	229.1
3	881.3 (4.5)	nq	2641.3 (35.1)		36.1 (0.5)	3559.5
4	1384.1 (95.6)	nq	6999.9 (1042.1)	0.4 (0.1)	44.5 (2.8)	8428.9
5	1140.1 (27.1)	nq	4229.0 (24.8)	0.2 (0.0)	41.2 (0.6)	5410.5
6	283.7 (5.0)	33.1 (10.3)	4239.1 (7.8)	nq	1.2 (1.3)	4557.1
7	135.5 (0.6)	7865.4 (165.5)[b]	396.6 (30.0)	4.2 (0.3)	nq	8401.9
8	28.3 (0.5)	5110.6 (24.7)[b]	153.0 (1.0)	2.6 (0.0)	nq	5294.5
9	335.3 (16.9)	6104.4 (6.3)[b]	548.8 (108.8)	3.3 (0.2)	3.0 (0.8)	6994.8
10	228.7 (9.8)	1090.8 (18.5)[b]	2048.8 (66.5)	0.5 (0.1)	5.8 (0.2)	3374.7
11	149.4 (0.4)	1700.5 (18.4)[b]	3245.9 (15.9)	1.0 (0.0)	4.2 (0.0)	5101.0

[a] Results are expressed as mean (standard deviation) of three determinations. nq, not quantified. [b] Values represent the sum of ascorbic acid plus tartaric acid.

Mushroom browning results from the action of phenol oxidase on phenolic compounds, which are oxidized to quinones and form brown/black melanin pigments. In a previous work (*35*) the necessity of refrigeration to retard browning was shown. On the other hand, the loss of water also increased phenol oxidase activity. However, the drying process is the most widely used method to ensure long-term storage (*9*). As expected, dehydrated samples (samples 1−3) exhibited the same phenolic compounds (**Table 2**), corresponding to ∼15.5 mg/kg. No significant differences in the phenolics profile were obtained
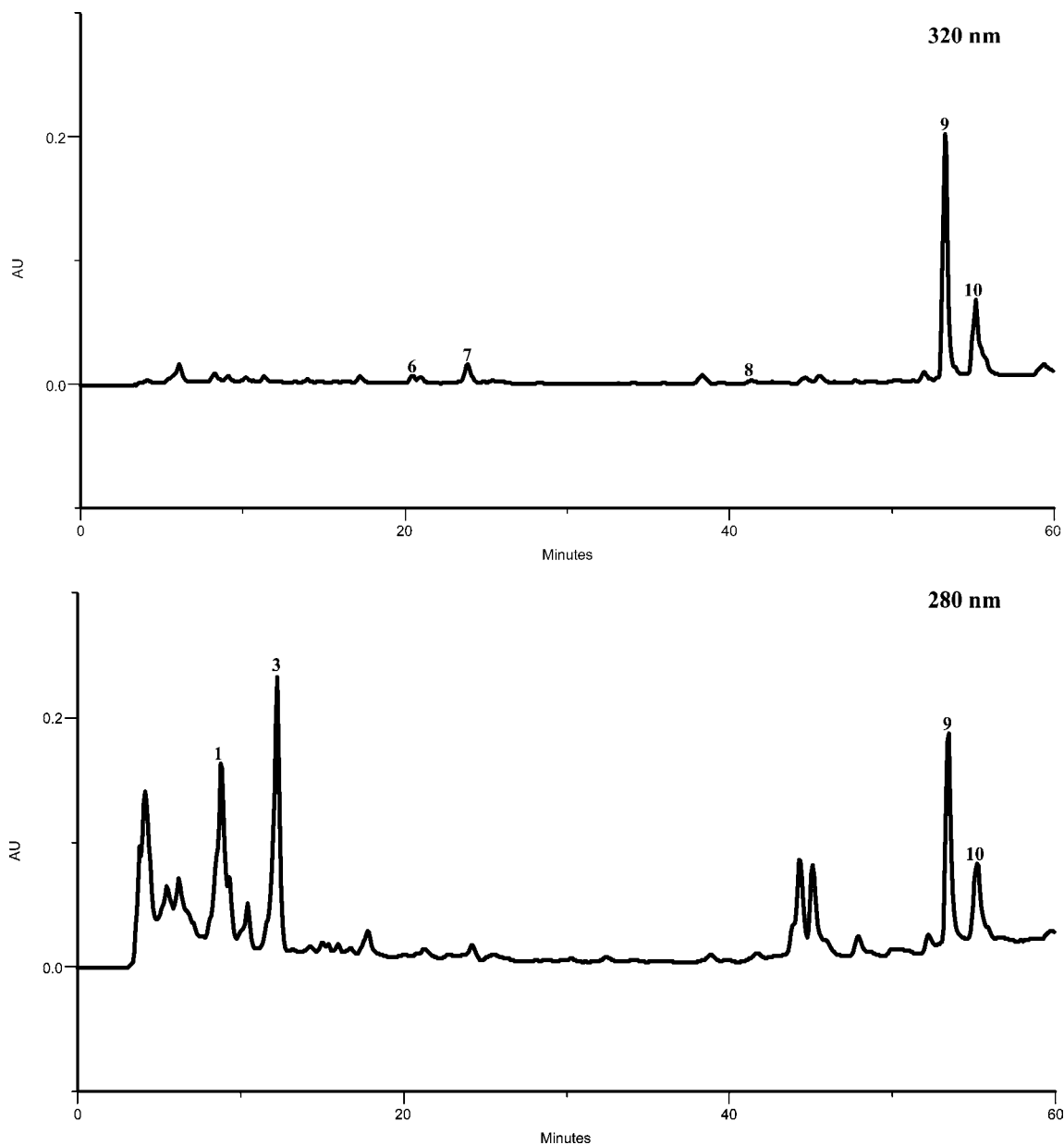
Figure 2. HPLC-DAD chromatogram of phenolic compounds in chanterelle (sample in olive oil). Peaks: (1) hydroxytyrosol; (3) tyrosol; (6) caffeic acid; (7) *p*-coumaric acid; (8) rutin; (9) luteolin; (10) apigenin.

with these samples, in which 3-*O*-caffeoylquinic acid was still the compound present in highest amounts, representing 61% of total identified phenolics (**Figure 3**).

Caffeoylquinic acids were not detected in the samples preserved in olive oil (samples 10 and 11), which may be explained by their degradation during boiling of the samples in the mixture of vinegar and water with salt. However, besides the presence of caffeic acid, *p*-coumaric acid, and rutin, these samples additionally exhibited another four phenolic compounds (**Figure 2**; **Table 2**): hydroxytyrosol, tyrosol, luteolin, and apigenin. The presence of these compounds in chanterelle may be attributed to the olive oil, because they were not found in frozen samples and they are reported to exist in olive oil (*36*, *37*). Samples preserved in olive oil were those that, by far, exhibited the highest amounts of phenolic compounds (∼900.4 mg/kg) (**Table 2**). The phenolics profile of these samples is distinct from those of the frozen ones, presenting hydroxytyrosol and tyrosol as the major compounds (each corresponding to ∼44%) (**Figure 3**). Despite the presence of these two com-

pounds and luteolin and apigenin, no significant differences were found for caffeic acid, *p*-coumaric acid, and rutin, when compared with frozen samples.

Samples preserved in wine vinegar (samples 7−9) exhibited the same phenolics detected in frozen samples, with the exception of 3-*O*-caffeoylquinic acid, probably lost during boiling. Additionally, 4- and 5-*O*-caffeoylquinic, caffeic, and *p*-coumaric acids existed in trace amounts (**Figure 3**; **Table 2**). These samples also presented hydroxytyrosol and tyrosol (**Table 2**), which may be provided by the vinegar, as they are described to occur in this matrix (*38*, *39*). The phenolic profile of these samples is different from the previous ones, exhibiting hydroxytyrosol as the major compound (∼86% of total identified phenolics) (**Figure 3**), which is mainly responsible for the high contents of phenolic compounds (∼339.4 mg/kg).

Organic Acids. Organic acids are known to have a lower susceptibility to change during processing and storage than other components such as pigments and flavor compounds (*40*). The HPLC-UV analysis showed that all of the samples presented a

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Dehydrated

Frozen
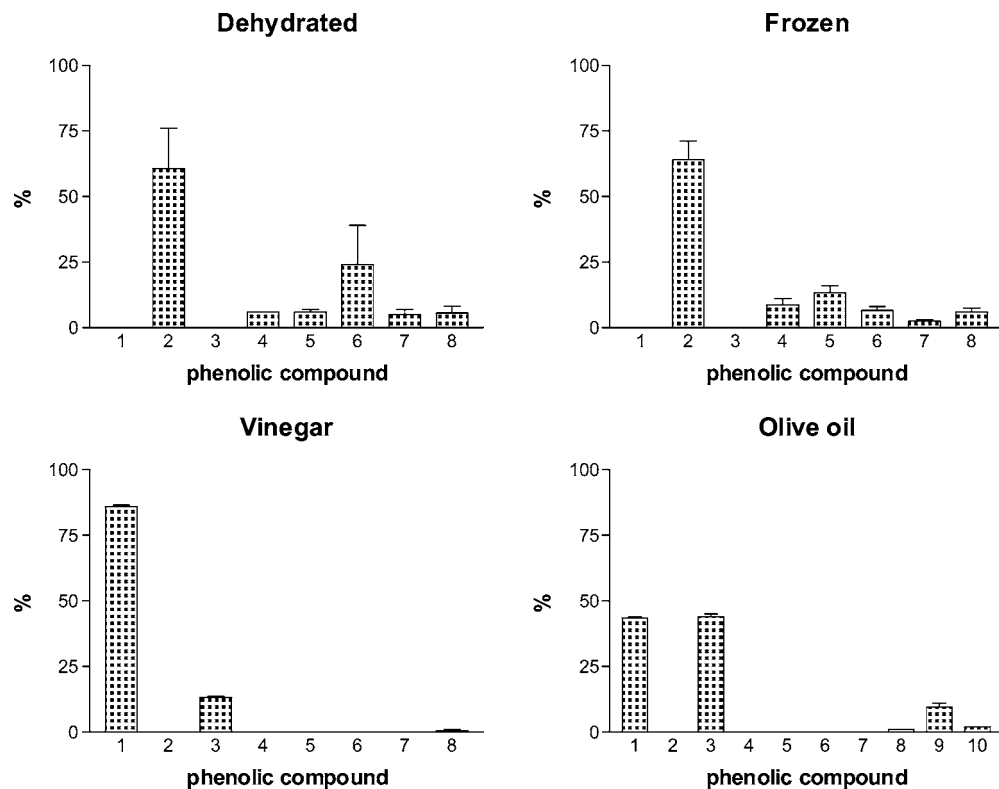
Vinegar

Olive oil

Figure 3. Phenolics profile of chanterelle preserved under four procedures. Values represent mean, and standard error bars are on the top of each column. (1) Hydroxytyrosol; (2) 3-*O*-caffeoylquinic acid; (3) tyrosol; (4) 4-*O*-caffeoylquinic acid; (5) 5-*O*-caffeoylquinic acid; (6) caffeic acid; (7) *p*-coumaric acid; (8) rutin; (9) luteolin; (10) apigenin.
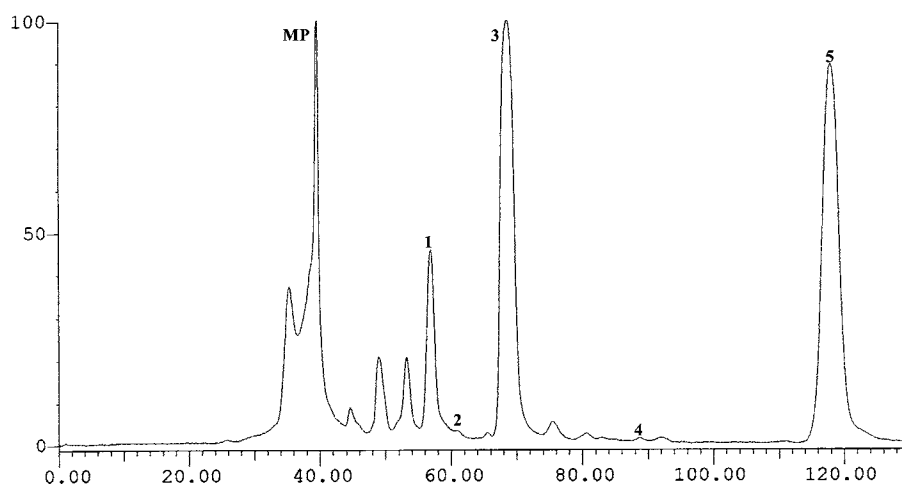
Figure 4. HPLC-UV chromatogram of organic acids in chanterelle (frozen sample). Detection was at 214 nm. Peaks: (MP) mobile phase; (1) citric acid; (2) ascorbic acid; (3) malic acid; (4) shikimic acid; (5) fumaric acid.

profile composed of at least five organic acids: citric, ascorbic, malic, shikimic, and fumaric acids (**Figure 4**; **Table 3**). Samples preserved in vinegar and in olive oil also exhibited tartaric acid, which coeluted with ascorbic acid.

The quantification of the identified compounds in chanterelle frozen samples (samples 4−6) ranged from 5 to 8 g/kg (**Table 3**), with malic acid, followed by citric acid, as the main compounds, corresponding to 85 and 15% of total compounds, respectively. Shikimic acid was present in very small amounts, below 0.1% of the total quantified compounds (**Figure 5**; **Table 3**).

The qualitative composition of dehydrated samples (samples 1−3) was similar to that of the frozen ones. Differences were noted at a quantitative level: in dehydrated samples the sum of the identified acids was lower (∼3 g/kg) (**Table 3**), with malic

acid as the major compound, although it represented only 78% of total identified acids. In addition, the amount of ascorbic acid was noticeably higher (∼6% of total acids) (**Figure 5**; **Table 3**).

The quantification of the identified organic acids in the samples preserved in vinegar (samples 7−9) also ranged from 5 to 8 g/kg (**Table 3**). Under the analytical conditions used, ascorbic acid coeluted with tartaric acid, which was also found in the samples and came from the vinegar, as it is a very important compound in that matrix (*41*). Therefore, those two organic acids exhibited the highest content (∼92% of total compounds), significantly higher than the amount of ascorbic acid in frozen samples (**Figure 5**; **Table 3**). Malic acid, which was the main compound in the frozen samples, was present in significantly lower levels (∼5%).
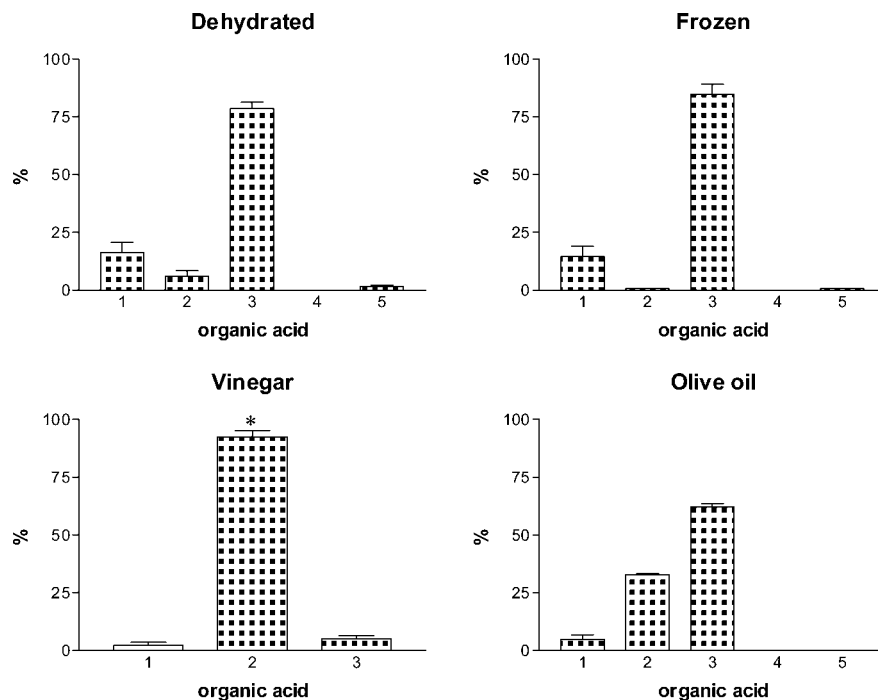
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Figure 5. Organic acids profile of chanterelle preserved under four procedures. Values represent mean, and standard error bars are on the top of each column. (1) Citric acid; (2) ascorbic acid; (3) malic acid; (4) shikimic acid; (5) fumaric acid; (∗) ascorbic plus tartaric acid.

The same observations were found for the samples preserved in olive oil (samples 10 and 11): when compared with the frozen samples, a significant increase in the contents of ascorbic acid and a significant decrease in malic acid amounts (ca. 33 and 62% of total acids, respectively) were noted (**Figure 5**; **Table 3**). Because these samples were also treated with vinegar, the rise of ascorbic acid (although not so high has that found for the samples preserved in vinegar) could be due to the existence of tartaric acid in the samples, as previously mentioned.

As far as we know, this is the first report concerning the phenolic compounds and organic acids composition of chanterelle mushroom. The work herein indicates that the preservation in olive oil and in vinegar affects the chemical composition of this species, which may be important when nutritional and health aspects are considered. However, the existence of chemical variability within the species cannot be excluded considering that, as each individual results from the cross-breeding of different hyphas, each mushroom presents a distinct genotype. Additionally, more samples should be analyzed, to clearly establish the influence of the conservation procedure on the phenolics and organic acids profiles of chanterelle.

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