Organic Acids of Plants and Mushrooms: Are they Antioxidants?

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INTRODUCTION

Many of the beneficial effects in human health resulting from the consumption of vegetables are strongly associated with their content in antioxidants, which can protect from a number of diseases, from atherosclerosis to cancer.

Within phytochemicals that contribute to this activity, some groups, such as flavonoids, are, today, almost unequivocally regarded as strong antioxidants.

However, when it comes to organic acids, besides the well known case of ascorbic acid (vitamin C), the attribution to these compounds of antioxidative behaviour is sometimes doubted and looked down.

In this review, we intend to present some works in which organic acids content of many crops and relation with their antioxidant activity was pursued. Studies involving similar experimental conditions were preferred, thus allowing an easier comparison of results.

CHEMISTRY OF ORGANIC ACIDS

Organic acids are water-soluble, colourless liquids or solids with relatively low melting temperatures. With the exception of α-keto acids, that readily undergo decarboxylation, these compounds are generally stable. The chemical structures of the main organic acids in nature can be found in Fig. 1. Ascorbic, shikimic and quinic acids are quite widespread through a number of species and ascorbic acid, a compound in lactone form, is universal in plants (Naidu 2003). Shikimic and quinic acids, two cyclohexane carboxylic acids, are very important as they are precursors of aromatic compounds in plants.

The simplest monocarboxylic acid is formic, followed by acetic. Acetic acid may be considered the most important one, once it is a universal precursor of lipids, fatty acids and other organic compounds, when as acetyl coenzyme A, together with malonyl coenzyme A, the active forms. In what concerns to dicarboxylic acids, the simplest one is oxalic, followed by succinic, whose unsaturated derivatives are fumaric and maleic acids, two geometrical isomers, with fumaric corresponding to the cis one. Maleic acid is equivalent to monohydroxysuccinic acid, while tartaric acid is the dihydroxylated form of succinic

Keywords: antioxidant activity, Brassica genus, Cydonia oblonga, edible mushrooms, organic acids, Rumex induratus

Abbreviations: ABTS, 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); EDTA, ethylenediaminetetraacetic acid; HPLC-UV, high performance liquid chromatography-ultraviolet detection; DPH, 2,2-Diphenyl-1-picrylhydrazyl; DNA, deoxyribonucleic acid; FRAP, ferric reducing ability of plasma; ROS, reactive oxygen species; RNS, reactive nitrogen species; X/XO, xanthine/xanthine oxidase

REFERENCES
Dehydroascorbic acid
Ascorbyl radical
OH
OH
OH
vents oxidation of that sub- substrate”, with “oxidizable sub- Those of an oxidizable substrate, significantly delays or pre-

DNA (Halliwell
living tissues, including proteins, lipids, carbohydrates and

mizes rancidity, retards the formation of toxic oxidation

products, maintains nutritional quality and increases shelf-

life (Jadhav 1978).

ANTIOXIDANTS

Nowadays there is increasing evidence that antioxidants in foods and beverages play an important role in the maintenance of health and prevention of disease. These antioxidants are believed to play a very important role in the body defence system against the various aggressions we encounter daily, such as the reactive oxidant species that are generated during several physiological and pathological processes (Cantuti-Castelvetri et al. 2000). From a food technology point of view, addition of antioxidants minimizes rancidity, retards the formation of toxic oxidation products, maintains nutritional quality and increases shelf-life (Jadhav et al. 1995).

A broad definition of an antioxidant may be “any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate”, with “oxidizable sub-

strate” being almost every molecule found in foods and in living tissues, including proteins, lipids, carbohydrates and DNA (Halliwell et al. 1995).

A compound exerting antioxidant actions may do so either by inhibiting the generation of reactive species, or by directly scavenging free radicals. Additionally, an antioxidant might act indirectly by raising the levels of endoge-

nous antioxidant defences in vivo (e.g. by upregulating the expression of the genes encoding superoxide dismutase, catalase or glutathione peroxidase) (Halliwell et al. 1995).

When reactive species, like those of oxygen or nitrogen (ROS or RNS), are produced in excess, some pathological conditions, related to the impairment of the oxidative/anti-

oxidative balance in favour of the former, may occur as a consequence of the oxidative stress that takes place. Examples of ROS include the free radicals superoxide anion (O2•−), peroxyl (ROO•), alkoxyl (RO•), hydroxyl (HO•) and the non radical species singlet oxygen (O2•), hydrogen peroxide (H2O2), hypochlorous acid (HOCl) and lipid hydro-

peroxides. The RNS of primary concern are nitric oxide (NO) and peroxynitrite (ONOO•), resultant from the com-
bination of O2•− and ‘NO. Other potentially important RNS include nitrogen dioxide radical (NO2•), nitrosion (NO•) and nitronium (NO2+) ions (Seabra et al. 2006).

The antioxidant activity of ascorbic acid is deeply related to its chemistry: when involved in radical scavenging it gives a single electron to the free radical species, thus origin-

ating the less reactive ascorbyl free radical. This ascorb-

yl radical can be reduced back to ascorbic acid or oxidized to dehydroascorbic acid (Fig. 2), which, in turn, can be re-
duced to ascorbic acid by reducing agents such as gluta-

thione (Seabra et al. 2006).

Although ascorbic acid is the most studied organic acid, other compounds of this group can also be regarded as anti-

oxidants. As stated earlier, oxalic acid is the simplest dicar-

boxylic acid. The most relevant chemical impact of this compound is related to its strong chelating ability for multi-

valent cations. Other carboxylic acids, such as tartaric, malic, citric, succinic and hydroxyglutaric, behave as anti-

oxidants because they also have the ability to chelate metals. They are, therefore, classified as “preventive” or synergistic (Seabra et al. 2006).

BRASSICA SPECIES

Brassica vegetables, including all cabbage-like ones, are widely consumed throughout the world and are very important in human nutrition. They are reported to reduce the risks of some cancers, especially due to its content of glucosinolates and their derived products (Park and Pezzuto 2002; Chun et al. 2004), although phenolic compounds are also considered to contribute to this capacity (Hollman et al. 1996; Galati and O’Brien 2004). Some Brassica oleracea varieties, namely cauliflower (Proteggente et al. 2002; Llor-
rach et al. 2003), broccoli (Kurilich et al. 2002; Proteggente et al. 2002; Ninfali and Bacchiocca 2003; Lin and Chang 2005) and several cabbages (Vinson et al. 1998; Chu et al. 2002; Proteggente et al. 2002) have already been studied for their antioxidant capacity in different experimental models, although no conclusion about the organic acids contribution was postulated.

Brassica oleracea L. var. costata DC (tronchuda cabbage)

1. Internal leaves

Tronchuda cabbage internal leaves presented a chemical profile composed by seven identified organic acids: aconitic, oxalic, ascorbic, malic, succinic, shikimic and fumaric acids (Fig. 3A). Quantitatively, the aqueous lyophilised extract exhibited a high content of organic acids (ca. 23 g/kg), with
citric acid representing ca. 43% of total identified compounds, followed by the pair malic plus quinic acids (ca. 28% of total acids). Shikimic acid was the one present in minor amounts, accounting for ca. 0.2% of total acids (Ferrer 2006a).

Regarding its antioxidant activity, this matrix was tested against 2,2-diphenyl-l-picrylhydrazyl radical (DPPH), O2•−, and HO• and also for its xanthine oxidase (XO) inhibitory activity.

In DPPH radical assay, internal leaves revealed an IC25 of 1192 μg/mL and a value for IC25 against O2•− equal to 101 μg/mL. The extract was also tested against HO•, where it revealed an IC25 of 27 μg/mL, and weak inhibition of XO with an IC10 of 273 μg/mL (Ferreres et al. 2006a) (Table 1). The IC25 values found against superoxide and hydroxyl radicals were quite low, which could be the result of the presence of almost 50% of citric acid in samples composition. With just one sample, the correlation of organic acids with the antioxidant activity of the extract is a challenging exercise, which would make necessary to remove these compounds from the matrix and retesting the material, in order to search for a reduction of activity. However, the removal of organic acids and subsequent testing of these molecules would be equally troublesome, as their activity is highly affected by synergism and antagonism phenomena.

The existence of additional works, aiming the study of organic acids and antioxidant activity of different parts of this species, and even of other related ones, allows inferring about the influence of both the qualitative and quantitative profiles of organic acids in the antioxidant potential of matrices.

2. External leaves

External leaves of B. oleracea var. costata revealed the presence of the same organic acids already identified in internal leaves (Fig. 3B). However, when it comes to quantitative profile, some differences can be pointed. In internal leaves ascorbic acid represented 26% of total compounds (Ferreres et al. 2006a). In external leaves, however, ascorbic acid was the major compound, corresponding to 35% of the organic acids found in the matrix, followed by the pair malic/quinic acids (33.7%) and citric acid (31.8%) (Ferreres et al. 2006a). Given the well established role of ascorbic acid as an antioxidant, this could explain, at least partially, the stronger activity of external leaves. In fact, external leaves revealed to be more active than internal ones in all assays performed: DPPH (440 μg/mL vs 1192 μg/mL), superoxide radical (43 μg/mL vs 101 μg/mL) and hydroxyl radical (10 μg/mL vs 27 μg/mL) (Table 1).

Some caution must be taken when extrapolating the influence of organic acids in the antioxidant activity of these samples, as the authors also identified a different profile of phenolic compounds in both internal and external leaves, with the first presenting mainly phenolic acids and the latter, flavonoids, whose antioxidant activity is also well recognized.

3. Seeds

Tronchuda cabbage seeds presented an organic acids profile constituted by aconitic, citric, ascorbic, malic, quinic, shikimic and fumaric acids, totaling ca. 16 g/kg (Ferreres et al. 2007). This content is similar to that previously found in the leaves (Ferreres et al. 2006a; Vrchovska et al. 2006). However, seeds exhibit a distinct quantitative profile, with ascorbic acid being the main compound, representing about 52% of total identified organic acids, followed by citric acid (28% of compounds).

These quantitative differences seem to exert a strong influence in the antioxidant activity displayed, as seeds were considerably stronger in all assays besides superoxide, for which the IC25 found was similar to external leaves (Table 1). Comparatively with the leaves of the same species, both internal and external, seeds revealed to be the material with the strongest antioxidative properties in vitro and, simultaneously, the one with higher amounts of ascorbic acid (Ferreres et al. 2007).

These results make sense as seeds constitute a reserve for many storage compounds, namely lipids, which need to be protected from oxidative stress, contributing to the viability of seeds and their rapid germination when oxygen demand is high (Andarwulan and Wattimena 1999; Randhir and Shetty 2003; Satller et al. 2004).

4. Sprouts

The screening of organic acids showed the presence of oxalic, fumaric, citric, malic, pyruvic, shikimic and ascorbic acids (Sousa et al. 2007). With the exception of oxalic and pyruvic acids, described for the first time in sprouts of B. oleracea var. costata, the remaining compounds had already been identified in leaves and seeds (Ferreres et al. 2006a, 2007).

In this work, besides the identification of the organic acids and its quantification, the authors tried to establish the qualitative and quantitative changes in sprouts’ chemical composition through a twelve day germination period. The total organic acids content increased ca. 46% from 45.7 g/kg, after 2 days of germination, to 66.9 g/kg on day 12 (Fig. 4) and changes in individual compounds were also monitored (Fig. 5). This increase may be explained by the increased metabolic activity of the seeds, which rapidly resume the glycolytic and the tricarboxylic acid cycle and the β-oxidation of fatty acids after germination (Li et al. 2005; Pracharoonwattana et al. 2005).

Seeds had an amount of organic acids lower than that present in the sprouts. Ascorbic acid, one of the major compounds found in the seeds (ca. 13.3 g/kg) was present only in vestigial amounts in the sprouts, being greatly depleted since the beginning of germination, may be due to its antioxidant effect (Sousa et al. 2007).

Citrinic and malic acids were the major organic acids
Table 1 Organic acids composition and antioxidant activity of some Brassica species. * malic + quinic acids; † citric + ketoglutaric acids

<table>
<thead>
<tr>
<th>Organic Acids</th>
<th>Aox. activity&lt;sup&gt;2&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>DPPH&lt;sup&gt;†&lt;/sup&gt;</td>
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<td>B. oleracea var. costata</td>
<td></td>
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<tr>
<td>Internal leaves&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.191</td>
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<tr>
<td>(0.004)</td>
<td>(0.143)</td>
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<tr>
<td>External leaves&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.022</td>
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<tr>
<td>(0.008)</td>
<td>(0.518)</td>
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<tr>
<td>Seeds&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.170</td>
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<tr>
<td>(0.003)</td>
<td>(0.438)</td>
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<tr>
<td>Sprouts&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.081</td>
</tr>
<tr>
<td>(0.008)</td>
<td>-</td>
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<tr>
<td>B. rapa var. rapa&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Flower buds&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.608</td>
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<tr>
<td>(0.359)</td>
<td>-</td>
</tr>
<tr>
<td>Leaves and stems&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.219</td>
</tr>
<tr>
<td>(0.209)</td>
<td>-</td>
</tr>
<tr>
<td>Roots&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.642</td>
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<td>(0.016)</td>
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Brassica inflorescences<sup>2</sup>

|               | DPPH<sup>†</sup> | IC<sub>50</sub> |
| B. oleracea var. costata |               |              |
| (0.008) | - | (1.657) | (0.079) | (0.005) | (16.039) | - | - | 0.137 | 49.024 | DPPH<sup>†</sup> | IC<sub>50</sub> = 754 |
| B. oleracea var. acephala | 0.097 | - | 48.373 | 0.018 | 108.159 | - | - | 0.065 | 47.055 | - | IC<sub>50</sub> = 470 |
| (0.003) | - | (1.864) | (0.000) | (0.079) | (0.009) | (0.005) | (0.001) | (0.001) | (0.001) | (0.001) | (0.001) |
| B. rapa var. rapa | 0.0423 | - | 13.177 | 1.261 | 22.350 | - | - | 0.143 | 51.108 | - | IC<sub>50</sub> = 560 |
| (0.000) | - | (0.075) | (0.005) | (0.021) | (0.002) | (0.001) | (0.001) | (0.001) | (0.001) | (0.001) | (0.001) |

<sup>1</sup> mean (SD) of three determinations, in g/Kg (dry basis). <sup>2</sup> µg/mL. <sup>3</sup> from Ferreres et al. 2006a. <sup>4</sup> from Ferreres et al. 2007. <sup>5</sup> from Sousa et al. 2007. <sup>6</sup> from Fernandes et al. 2007. <sup>7</sup> from Sousa et al. 2008.

Fig. 4 Evolution in total organic acids content of tronchuda cabbage sprouts with germination time. * p<0.05, compared with the previous germination time. From Sousa C, Lopes G, Pereira DM, Teixeira M, Valenço P, Seabra RM, Pereira JA, Baptista P, Ferreres F, Andrade PB (2007) Screening of antioxidant compounds during sprouting of Brassica oleracea L. var. costata DC. Combinatorial Chemistry & High Throughput Screening 10, 377-386, ©2007, with kind permission of Bentham Science Publishers.

found in sprouts at all germination times. Citric acid, accounting for more than 15% of the total organic acids content, decreased ca. 37% from day 2 to day 12 (Fig. 5). However, despite the significant decrease between days 2 and 4 and increase between days 6 and 8, its variation during the germination period did not show a clear tendency. Malic acid increased from 27.9 g/kg (61% of the total organic acids) on day 2 to 51.6 g/kg (77% of the total organic acids) on day 12, which represents an increase of 85% along the studied germination period. This was the organic acid that registered the highest raise, which can indicate that besides oxidation, the glyoxylate cycle in which fatty acids are converted to sugars having malate as an intermediate product, was active (Eastmond and Graham 2001).

Differently from other tronchuda cabbage materials presented until now, in the referred work (Sousa et al. 2007) no antioxidant activity was evaluated. However, this parameter should be assessed in a near future as a deep knowledge about the variations on organic acids, at both qualitative and quantitative levels, was achieved with this work. Consequently, a connection with antioxidant activity could be established, given the great amount of information available, namely the behaviour of each organic acid through time.

Brassica rapa var. rapa L. (turnip)

In the work of Fernandes et al. (2007), three turnip edible parts (leaves and stems, flower buds and roots) were analysed, revealing similar organic acids composition. Generally, aconitic, citric, ketoglutaric, malic, shikimic and fumaric acids were detected in all parts.

The quantification of the identified compounds revealed a high organic acids content (ranging from 36 to 51 g/kg, dry basis), with higher amounts of these compounds in flower buds and in leaves and stems than in the roots, in a general way. Three distinct quantitative organic acids profiles were obtained. Flower buds and leaves and stems presented an equivalent amount of organic acids (47.1 and 51.1 g/kg, respectively), with citric acid accounting for 41.8% in the first and 28.1% in the later. Roots were the sample presenting lower organic acids content, with citric acid constituting only 14.4% of the total amount. As it will be dis-
Turnip edible parts displayed a scavenging activity against DPPH that was concentration-dependent. IC$_{25}$ was determined in order to compare the results, once it was not possible to reach 50% scavenging activity with all samples and considering that it corresponds approximately to the middle activity of each curve. The flower buds revealed to be the most active part (mean IC$_{25}$ of 470 μg/mL), followed by the leaves and stems (mean IC$_{25}$ at 560 μg/mL). Turnip roots showed a considerably higher amount (163.1 mg/kg) against 49.0 g/kg and 38.0 g/kg in B. oleracea L. var. acephala and B. oleracea L. var. acephala and B. rapa L. var. rapa inflorescences, respectively. It should be emphasized that ascorbic acid, present in leaves and seeds of B. oleracea var. costata (Sousa et al. 2005; Ferreres et al. 2006a, 2007) was not detected in its inflorescences. As it was discussed later, this quantitative profile may be responsible, at least in part, for the results obtained in antioxidant activity assays (Fig. 6, Table 1).

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The antioxidant potential exhibited by the different turnip edible parts is obviously determined by their composition and, in all samples, the values of IC$_{25}$ in DPPH assay were correlated with citric acid content. The role of phenolics in the antioxidant activity displayed cannot be ignored: hydroxycinnamic acids and their derivatives (Plumb et al. 1997; Fukumoto and Mazza 2000) and flavonol glycosides (Tang et al. 2001; Braca et al. 2003) are known to exert antioxidant activity and they were also present in turnip.

**B. oleracea L. var. acephala (kale)**

In the work of Sousa and colleagues (2008), two Brassica oleracea varieties (B. oleracea L. var. costata DC and B. oleracea L. var. acephala) and Brassica rapa L. var. rapa inflorescences were studied for their chemical composition and antioxidant capacity. These three Brassica shared a profile composed by six organic acids (aconitic, citric, pyruvic, malic, shikimic and fumaric acids), but B. oleracea L. var. acephala presented a considerably higher amount (163.1 g/kg against 49.0 g/kg and 38.0 g/kg in B. oleracea var. costata and B. rapa var. rapa, respectively). It should be emphasized that ascorbic acid, present in leaves and seeds of B. oleracea var. costata (Sousa et al. 2005; Ferreres et al. 2006a, 2007) was not detected in its inflorescences. As it...
will be discussed later, this fact may explain some of the results found in antioxidant activity.

Each inflorescence was investigated for its capacity to act as a scavenger of DPPH radical and reactive oxygen species (superoxide radical, hydroxyl radical and hypochlorous acid), exhibiting antioxidant capacity in a concentration dependent manner against all radicals.

Against DPPH, *B. oleracea* var. *acephala* revealed to have a stronger capacity (IC{sub}25 = 565 μg/mL) than *B. oleracea* var. *costata* (IC{sub}25 = 774 μg/mL), which exhibited a similar behavior (Fig. 7, Table 1). The fact that, from a quantitative point of view, *B. oleracea* var. *acephala* inflorescences showed the highest organic acids content (ca. 163 g/kg), corresponding to about three and four times the amount found for *costata* variety and *B. rapa* var. *rapa*, respectively, can explain, at least partially, its higher antioxidant activity.

In superoxide radical assay, the most active matrix was *B. oleracea* var. *acephala* – IC{sub}25 = 10 μg/mL, which had been determined for *costata* (IC{sub}25 = 244 μg/mL) or using a NADH/PMS one (*B. oleracea* var. *acephala* – IC{sub}25 = 281 μg/mL) (Table 1).

Against hydroxyl radical, as it had been determined for superoxide and DPPH radical, the most active matrix was the one presenting higher amounts of organic acids, *B. oleracea* var. *acephala* (IC{sub}10 = 10 μg/mL). When it comes to hypochlorous acid assay, the most active vegetable was *B. oleracea* var. *costata* (IC{sub}10 = 639 μg/mL), which was the sample with higher relative amount of citric acid (56.9%) (Table 1).

At this point, it should be highlighted that, in *B. oleracea* var. *costata*, ascorbic acid was present in leaves and seeds but absent in inflorescences. This may explain, in part, the weaker antioxidant activity displayed by inflorescences when compared with the other referred plant parts.

**RUMEX INDURATUS**

Several species of the *Rumex* (Polygonaceae) genus, namely its leaves and roots, have been used in traditional medicine for inflammation, blood purification and constipation (Medical Economics Co. 1998; Newall et al. 1996). However, oxalic acid intoxication, mainly in children, is a problem due to the high oxalic acid content of the species (Der Marderosian and Beutler 2002; Newall et al. 1996).

Guerra and colleagues (2008) proceeded to a targeted metabolite analysis, organic acids included, and evaluation of the antioxidant potential of *Rumex induratus* leaves. The HPLC-UV analysis of the aqueous lyophilized extracts revealed the presence of malic, oxalic, citric, ascorbic and shikimic acids, which were described for the first time, with the exception of oxalic acid (Fig. 8).

In addition, in this work the chemical composition of field samples was compared with that of greenhouse ones, both origins being tested to determine the influence of maturation stage in the chemical composition. In a general way, field samples depicted a slight decrease in the total amount of organic acids during plant growth.

The sample chosen for antioxidant activity assays displayed the presence of malic, oxalic, citric, ascorbic and shikimic acids, which were described for the first time, with the exception of oxalic acid (Fig. 8).
The lyophilized aqueous extract of *R. induratus* leaves exhibited a strong concentration-dependent antioxidant potential against DPPH (IC$_{50}$ = 106.5 μg/mL).

Nitric oxide and hypochlorous acid can be responsible for the formation of more reactive species, such as hydroxyl radical (Halliwell et al. 2005). The aqueous extract of *R. induratus* leaves showed a potent scavenging activity against nitric oxide and hypochlorous acid in a concentration dependent manner, with an IC$_{50}$ of 92.7 μg/mL. The extract exhibited a lower activity against hypochlorous acid. Nevertheless, a concentration-dependent antioxidant potential was observed (IC$_{50}$ = 171.3 μg/mL). These findings, along with the fact that *R. induratus* leaves can act as superoxide radical scavenger and xanthine oxidase inhibitor (Ferrer et al. 2008a) are extremely valuable: the simultaneous scavenging capacity of superoxide radical and nitric oxide can prevent the formation of peroxy nitrite and ultimately, hydroxyl radical (Halliwell et al. 2005).

Despite the considerable number of samples used for chemical characterization, when it comes to antioxidant activity assays, only one sample was tested. As so, it is difficult to associate the activity displayed only with organic acids composition, given the fact that phenolics were also present in samples, thus contributing to antioxidant capacity. However, the contribution of organic acids to this activity cannot be despised.

**CYDONIA OBLONGA MILLER (QUINCE)**

Quince fruit is a pome with numerous seeds. The fruits are big and exhibit a characteristic fragrance. The pulp is acidic and astringent and, so, it is not suitable for consumption when raw. The most important utilization of this fruit is in the production of jams and jellies, which are very appreciated in many countries.

Silva and colleagues (2004) studied the organic acids composition of both fruit (pulp, peel and seed) and jam and antiradical activity was assessed against DPPH.

Pulp, peel and jams extracts presented a similar profile composed by seven identified organic acids: ascorbic, oxalic, citric, quinic, malic, shikimic and fumaric acids, which had already been described by Silva et al. (2002). In seed extract, only oxalic acid could not be found.

In pulp, peel and jams extracts the sum of malic plus quinic acids always represented at least 95% of the total organic acid content, with all other acids present in small amounts.

### Table 2 Organic acids composition and antioxidant activity of some mushrooms and vegetable foods.

| Organic Acids | Ascorbic | Citric | Fumaric | Ketoglutaric | Malic | Oxalic | Shikimic | Succinic | Quinic | Total | Assay | IC |
|---------------|----------|-------|---------|-------------|-------|--------|---------|---------|-------|-------|-------|------|----|
| **Aconitic** | nq       | -     | -       | -           | -     | 0.018  | 0.163   | 0.001   | -     | -     | -     | 0.182| IC$_{50}$ = 107 |
| **Ascorbic** | (0.000)  | (0.002)| (0.000) | (0.000)     | (0.000)| (0.000)| (0.000) | (0.000) | (0.000)| (0.000)| (0.000)| (0.000)| IC$_{50}$ = 93 |
| **Citric**   | (0.006)  | (0.001)| (0.000) | (0.000)     | (0.000)| (0.000)| (0.000) | (0.000) | (0.000)| (0.000)| (0.000)| (0.000)| IC$_{50}$ = 171 |
| **Fumaric**  | (0.007)  | (0.015)| (0.000) | (0.000)     | (0.000)| (0.000)| (0.000) | (0.000) | (0.000)| (0.000)| (0.000)| (0.000) |
| **Ketoglutaric** | -       | -     | -       | -           | -     | 5.984  | 0.030   | 0.100   | -     | -     | -     | 4.235| IC$_{50}$ = 16 |
| **Malic**    | (0.000)  | (0.006)| (0.000) | (0.000)     | (0.000)| (0.000)| (0.000) | (0.000) | (0.000)| (0.000)| (0.000)| (0.000) |
| **Oxalic**   | 0.000    | 0.000 | 0.000   | 0.000       | 0.000 | 0.000  | 0.000   | 0.000   | 0.000 | 0.000 | 0.000 | 0.000 |
| **Shikimic** | 0.000    | 0.000 | 0.000   | 0.000       | 0.000 | 0.000  | 0.000   | 0.000   | 0.000 | 0.000 | 0.000 | 0.000 |
| **Succinic** | 0.000    | 0.000 | 0.000   | 0.000       | 0.000 | 0.000  | 0.000   | 0.000   | 0.000 | 0.000 | 0.000 | 0.000 |
| **Quinic**   | 0.000    | 0.000 | 0.000   | 0.000       | 0.000 | 0.000  | 0.000   | 0.000   | 0.000 | 0.000 | 0.000 | 0.000 |
| **Total**    | 0.018    | 0.163 | 0.001   | 0.000       | 0.182 | 4.135  | 0.047   | 0.052   | 0.055 | 0.004 | 0.182 | 0.191 |
| **DPPH**     | (0.000)  | (0.002)| (0.000) | (0.000)     | (0.000)| (0.000)| (0.000) | (0.000) | (0.000)| (0.000)| (0.000)| (0.000) |
| **NO**       | (0.000)  | (0.000)| (0.000) | (0.000)     | (0.000)| (0.000)| (0.000) | (0.000) | (0.000)| (0.000)| (0.000)| (0.000) |
| **HOCI**     | (0.000)  | (0.000)| (0.000) | (0.000)     | (0.000)| (0.000)| (0.000) | (0.000) | (0.000)| (0.000)| (0.000)| (0.000) |

- * mean (SD) of three determinations, in g/Kg (dry basis). μg/mL. 
- * from Guerra et al. 2008. 
- from Ribeiro et al. 2007. -- concentration in mg/mL.
Mushrooms have been used as food and food-flavouring material in soups and sauces for centuries, due to their unique and characteristic flavour. Their high amounts of proteins, carbohydrates and fibres and low fat contents is often referred in literature in relation to their nutritional value. Furthermore, they contain significant levels of vitamins, namely thiamine, riboflavin, ascorbic acid and vitamin D2, as well as minerals (Mattila et al. 2000). Regarding their medicinal value, mushrooms revealed to be effective as antitumor, antibacterial, antiviral, haematological and in immunomodulating treatments (Wasser and Weis 1999; Yang et al. 2002).

Trás-os-Montes region (northeast of Portugal) is recognized as one of the richest regions of Europe in wild edible mushroom species, of considerable gastronomic relevance. Russula cyanoxantha, Amanita rubescens, Suillus granulatus and Boletus edulis are among the more common and eaten species.

In the work of Ribeiro et al. (2008), the four above mentioned mushrooms species were studied for their chemical composition, including organic acids. The organic acids profile showed that all of the species contained oxalic, citric, malic and fumaric acids. Some also exhibited ketoglutaric, quinic, succinic and shikimic acids. In a general way, the highest total organic acids content was found in A. rubescens, followed by R. cyanoxantha, S. granulatus and B. edulis.

In A. rubescens, citric, ketoglutaric, succinic, oxalic, malic, quinic, shikimic and fumaric acids were identified. The authors stated that organic acids weren’t accumulated at a special part, since for each one of the three A. rubescens samples tested, the material presenting the highest organic acids content was different. In the cap, they were found significantly higher citric acid contents than in the stipe. On the other hand, quinic acid concentrations were significantly higher in the stipe (Fig. 9, Table 2).

R. cyanoxantha showed a profile composed by citric, oxalic, quinic, malic, and fumaric acids. The results indicated that these compounds accumulate mainly in the cap. In what concerns to the quantitative profile, the different mushroom parts were found to be similar, presenting quinic...
and malic acids as the major compounds, with oxalic acid being the minor one. However, some differences were observed: the cap showed a tendency for higher concentrations of citric and quinic acids, but its malic acid content was significantly smaller than the one exhibited by the stipe (Fig. 9, Table 2).

The analysis of S. granulatus samples yielded the identification of citric, malic, quinic, succinic, shikimic, oxalic and fumaric acids with clear evidence that the cap presented the highest contents of these compounds. The analysis of the mushrooms’ quantitative profiles obtained made it possible to realize that succinic and shikimic acids were those appearing in lower quantities and that the major compounds differed according to the different mushroom parts (Fig. 9, Table 2): fumaric and citric acids in stipe and quinic acid in cap. Malic, oxalic and succinic acids presented important differences in their relative amounts in the different mushrooms materials.

B. edulis presented an organic acids profile composed by oxalic, citric, malic, succinic and fumaric acids. The total organic acids amount was higher in the cap, while stipe and entire mushrooms contained similar amounts of these compounds. These results indicate that, like in R. cyanoxantha and S. granulatus, organic acids of B. edulis species are mainly fixed in the cap. In a general way, the quantitative profile (Fig. 9, Table 2) showed malic acid as the major compound, while succinic and fumaric acids only appeared in low amounts. Moreover, cap and stipe had a tendency to concentrate malic and citric acids, respectively.

The antioxidant potential of the different mushroom materials was evaluated by their DPPH scavenging effect, which was, in a general way, concentration-dependant. The IC$_{25}$ values were used to compare their antioxidant properties.

In R. cyanoxantha species, results found for antioxidative activity did not follow the total organic acids contents order. Nevertheless, citric acid concentration was considerably higher in the cap, with this material presenting higher antioxidative activity than the stipe, which contained the lowest amount of this acid. These data suggest that citric acid may be important for the antioxidant capacity of the species. The alkaloids could also have a relevant role, since their quantitative profile (Fig. 9, Table 2) showed malic acid as the major compound, while succinic and fumaric acids only appeared in low amounts. Moreover, cap and stipe had a tendency to concentrate malic and citric acids, respectively.

**VITAMIN C: A CASE STUDY**

Undoubtedly, ascorbic acid (which, together with dehydroascorbic acid, constitutes what we call vitamin C) (Naidu 2003) is the most important organic acid when addressing antioxidant activity and its impact on human health.

This compound can act as a scavenger/neutralizer of a wide range of both oxygen and nitrogen reactive species, such as hydroxyl radical, alkoxyl radicals, peroxy radicals, superoxide anion/hydroperoxyl radical, hypochlorous acid, ozone, singlet oxygen, nitrogen dioxide radical, peroxynitrite/peroxynitrous acid (Carr and Frei 1999).

Besides this direct action against oxidizing species, ascorbic acid amplifies the activity of other antioxidants, as it can regenerate α-tocopherol, urate and β-carotene radical cation from their radical species (Niki et al. 1995; Niki 1987; Naidu 2003). We can, therefore, speak of synergism of several antioxidants.

The antioxidant properties of ascorbic acid are recognized as the most probable explanation for the role that it seems to play on atherosclerosis (Frei 1997; Martin and Frei 1997) and cancer prevention (Campbell et al. 1999; Naidu 2003).

Recently some concern has appeared in the literature given the fact that in in vitro experiments, ascorbic acid, when in low concentrations (0.2 M), was able to catalytically reduce active metal ions and therefore contribute to oxidative damage through the production of hydroxyl and alkoxyl radicals (Bast et al. 1991; Buettner and Jurkiewicz 1996; Protegente et al. 2000). Nevertheless, this data does not necessarily imply that ascorbic acid can be a pro-oxidant in vivo and many controversies are still unresolved in this area (Halliwell 1996; Carr and Frei 1997; Chen et al. 2000). Many studies indicate that, in fact, both actions can be linked with ascorbic acid, depending on the medium conditions.

In the work of Martinez-Sánchez et al. (2008), a comparative study of flavonoid compounds, vitamin C and antioxidant properties (against DPPH and ABTS’$^+$) of baby leaf Brassicaceae species was performed. The species involved were watercress (Nasturtium officinale), mizuna (Brassica rapa subsp. nipposinica), wild rocket (Diplotaxis tenuifolia) and salad rocket (Erba vesicaria).

Besides important information regarding phenolic com-
position of these matrices, a correlation between phenolics and Vitamin C presence in leaves and antioxidant activity could be achieved, although Pearson’s correlation coefficient was lower for Vitamin C than for flavonoids. The vitamin C content, measured as ascorbic acid and dehydroascorbic acid, ranged from 64 to 104 mg per 100 g of fresh weight. The highest content of Vitamin C was observed in watercress, followed by wild rocket and salad rocket and the lowest content of vitamin C found in mizuna leaves. Antioxidant activity followed the same order. Ascorbic acid was the predominant form of Vitamin C in all of the studied species. Differences in ascorbic acid content can be related to both leaf age and the irradiance arriving at the leaf surface, among other factors (Foyer 1993).

Other study (Llorach et al. 2008) characterised polyphenols, Vitamin C and antioxidant properties of five varieties of lettuce (Lactuca sativa L.), three green varieties (iceberg, romaine, continental) and two red varieties (red oak leaf, lollo rosso) and one escarole one (Cichorium endivia var. crispa) “frissè”. Vitamin C levels ranged between 2.8 (romaine) and 19.5 (continental) mg/100 g (fresh weight). The highest amount of Vitamin C was found in green lettuce, followed by red lettuce varieties. Antioxidant activity was also evaluated against DPPH and ABTS™. In both screening tests, red varieties showed higher scavenging activity, a fact that was not in line with their content in Vitamin C, which was lower when compared with green varieties. However, it should be taken into account that the authors found anthocyanins in red varieties, which could contribute to the antioxidant activity in a higher degree than ascorbic acid, thus diminishing its influence in scavenging activity of samples.

CONCLUSION

After analysing several works dedicated to the influence of organic acids on antioxidant activity of vegetable matrices, one cannot state, without a doubt, their contribution, or not, to the activity showed. This happens due to many synergistic and antagonistic behaviour of compounds found in the plant matrices. These phenomena increase with matrix complexity, rendering almost impossible to clearly associate the presence of an acid(s) with the displayed antioxidant activity. In cases in which total organic acids content is correlated with antioxidant potential of the samples, ascorbic acid is, most of the times, a compound present in significant amounts. In fact, ascorbic acid is a well known antioxidant which is sold worldwide as a diet supplement given its beneficial effects. However, some caution may be taken as an increasing number of studies show how this compound can act as a pro-oxidant in physiological conditions. In the end, the main factor contributing to antioxidant effects of organic acids is the complex chemical medium in which these compounds are included, which can dictate their neutral, deleterious or beneficial properties. Works on this theme, however, are rather scarce and therefore, more efforts should be put in this matter as a way to understand the complex biochemistry behind organic acids’ biological activity.

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