Antioxidant activity and total phenolic compounds of New Zealand spinach (*Tetragonia tetragonioides*): Changes during boiling

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Abstract

Antiradical activity against DPPH* and total phenolic content of ten samples of New Zealand fresh spinach leaves (*Tetragonia tetragonioides*) cultivated in Portugal were evaluated. The EC₅₀ values concerning the radical scavenging activity of the samples ranged between 97.5 µg/ml and 319 µg/ml. The total phenolic content of raw New Zealand spinach varied between 27.9 and 58.2 mg GAE/ g of extract. Differences of maturity at harvest, growing conditions and soil state can explain the variability observed. Four batches of 140 g of spinach fresh leaves were boiled in 2 L of water in different times: 1; 2.5; 5; 10 minutes. Significant reduction of antiradical activity was observed up to five minutes boiling time, no changes were observed between 5 and 10 minutes cooking time. No significant changes were observed on total phenolic content after cooking.

Introduction

Fruits and vegetables contain large amounts of natural antioxidant molecules, which can protect against several chronic diseases¹. These protective effects are generally attributed to the presence of various functional components, such as, vitamin C, vitamin E, provitamins, minerals, fibre and phenolic compounds. Many of these compounds have bioactive mechanisms for effectively scavenging reactive oxygen species (ROS) and reducing cell proliferation in cancer cell². Oxidative stress appears to be the critical factor in the pathogenesis
of many diseases because ROS have the ability to damage macromolecules like DNA, protein and lipids
To minimize the harmful effects of oxidative stress in the human body, it is necessary to supply adequate amounts of ROS-scavengers, and fruits and vegetables are considered to be the major contributors of ROS-scavenging antioxidants; however the health effects of polyphenols depend on the amount consumed and also on their bioavailability.

The composition of fruits and vegetables depends both on species and on subtype, as well as on the environmental, farming, production, and storage conditions and some vegetables, often termed salad vegetables, are commonly eaten raw, many are cooked before they are eaten. In most case, whether a vegetable is eaten raw depends on personal choice. Most forms of cooking reduce the total nutrient content of vegetables, although the degree to which this happens varies between nutrients and with cooking methods. However, cooking also increases the bioavailability of some nutrients.

Spinach (Spinacea oleracea L.) is one of the vegetables considered to have a high nutritional value. Because of its abundance of phenolic compounds, spinach ranks high among vegetables in terms of antioxidant capacity, suggesting that spinach consumption may protect against oxidative stress mitigated by free-radical. However, spinach is often consumed fresh or stored frozen after cooking in boiling water. New Zealand spinach is a drought-resistant annual trailing vine with small triangular shaped, thick, dark green and succulent leaves. This species is grown when temperatures are too high for Spinacea Oleracea, and it is widely cultivated and consumed in temperate regions. Antioxidant activity and total phenolics content fresh and cooked spinach, were already evaluated, but there are no similar studies made for New Zealand Spinach.

The aims of this study were to evaluate antiradical activity against DPPH and total phenolic of New Zealand raw spinach (Tetragonia tetrogonioides) cultivated in Portugal, and study the effect of boiling time on this type of spinach.

Materials and methods

Samples

Samples of New Zealand spinach (1 kg each) were collected in ten different regions of Portugal between February 27th and March 9th of 2009. Samples were numbered from 1 to 10. After harvesting, samples were immediately transferred to the laboratory, stems and leaves were separated and leaves frozen at -20 °C before analysis.
Sample preparation

Extraction procedures: for antioxidant capacity assay and total phenolics determination, methanolic extracts were prepared. Leaves were lyophilized and yields were calculated (minimum was 6.1% for sample 2 and maximum was 16.9% for sample 4). Then, three powdered sub samples (~4 g; 50 mesh) were extracted with 100mL of methanol for 20 min and filtered through 30 ml borosilicate Robu filter 3.3, pore 4. This procedure was repeated three times. The resulting extracts were pooled and the solvent evaporated in a rotary evaporator (Büchi® Rotavapor RE 111 equipped with a Büchi® 461 waterbath and Büchi® Vac V-500 vacuum pump). Then, the extract was lyophilized in a freeze dried apparatus. The lyophilized extracts were kept in a desiccator, in the dark.

Cooking procedures: batches of 140g of fresh leaves of sample 10 were boiled in 2 L of boiling water in different times: 1; 2.5; 5; 10 minutes. These samples were labelled as 10A, 10B, 10C and 10D. Raw leaves were used as control (sample 10). After cooking, samples were rapidly cooled and frozen until extraction procedures. Cooking water was changed for each experiment.

Radical scavenging activity using DPPH

The radical scavenging activity of the extracts was determined spectrophotometrically in a Biotek® ELX808 plate reader (Biotek Corporation, USA), by monitoring the disappearance of DPPH* at 515 nm, according to a described procedure. The optimisation of the assay was studied for the samples in two steps: extractions were performed with pure and 50% (v/v) methanol and reaction conditions were tested with pure and 70% (m/v) ethanol extract and DPPH solutions. Kinetic behaviour of the different conditions was evaluated for determination of the incubation time. After selecting the optimal conditions, the assay was carried out as described below: for each extract, a dilution series composed of five different concentrations (15, 53 – 1000 µg/ml) of the methanolic extracts was prepared in a 96-well plate. The reaction mixtures in the sample wells consisted of 100 µL methanolic extract and 100 µL DPPH*, both dissolved in ethanol 70%, prepared daily. Absorbances at 515 nm were measured during 3 h in 1 min intervals, until the reaction reached a plateau. These experiments were performed in triplicate.

The DPPH scavenging effect was expressed as EC50, which is the concentration of extract required to scavenge 50% of the radical present in the reaction medium, and was calculated according to the formula:
DPPH scavenging effect (%) = \(100 - \left( \frac{A_{\text{extract}} - A_{\text{blank1}}}{A_{\text{control}} - A_{\text{blank2}}} \times 100 \right)\)

with \(A_{\text{extract}}\) as the absorbance of the extract against the DPPH solution, \(A_{\text{control}}\) the absorbance of the DPPH solution, \(A_{\text{blank1}}\) the absorbance of the extract alone and \(A_{\text{blank2}}\) the absorbance of ethanol 70% in the well. The percentage of remaining DPPH against the extract was then plotted to obtain EC50.

Total phenolic content (TPC)
The amount of total phenolics in the extracts was determined using the Folin-Ciocalteu colorimetric method, according to a described procedure. Briefly, 1 mL of Folin-Ciocalteu reagent was added to 300 \(\mu\)L of the lyophilized extract dissolved in ethanol:water (7:3) solution, followed by the addition of 5 mL of 20% sodium carbonate solution. The mixture was made up to 10 mL with water and thoroughly shaken and the absorbance was read after 20 min, at 735 nm (V 530, UV-VIS Spectrophotometer, Jasco Corporation, Japan). The contents are expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract. The standard calibration equation for gallic acid (concentration range 60–175 \(\mu\)g.mL\(^{-1}\)) was \(y = 0.0033x - 0.0032\) (R\(^2\) = 0.9992). The measurements were performed in triplicate.

Statistical treatment
The differences of the mean values were tested using Duncan’s test (\(\alpha = 0.05\)), with SPSS software, version 14.0.

Results and discussion
Antiradical activity and total phenolic content in raw New Zealand spinach
The EC50 values concerning the radical scavenging activity of the samples are shown in Table 1. The extract which presented the lowest EC50 value, hence the highest antiradical activity, was sample 10, with a value of 97.5 \(\mu\)g/ml. The extract with the highest EC50 was sample 2, with a value of 319 \(\mu\)g/ml. Both values had significant differences when compared with the other samples.

Concerning sample 2, the lower antiradical activity could be explained by the fact that the leaves were in a younger maturity stage when collected, since even under similar culture conditions the development of this vegetable depends on climatic conditions of the region. Pandjaitan referred that spinach leaves collected at midmaturity growth stages presented higher levels of total
phenolics, total flavonoids and antioxidant activity than immature or mature leaves7.

The total phenolic content of raw New Zealand spinach varied between 27.9 and 58.2 mg GAE/g of extract, as shown in Table 1. Variation in total phenolic content (TPC) within raw vegetables can be explained by many factors such as species, variety, natural chemical composition, maturity at harvest, growing conditions, soil state and conditions of post-harvest storage 10.

Table 1. Antiradical activity and total phenolic content of raw New Zealand spinach

<table>
<thead>
<tr>
<th>Sample</th>
<th>EC50 (µg/ml)</th>
<th>TPC (mg GAE/g of extract) (n=4)</th>
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<tbody>
<tr>
<td>1</td>
<td>224±32c,d</td>
<td>33.2±1.2e</td>
</tr>
<tr>
<td>2</td>
<td>319±36a</td>
<td>27.9±1.6b</td>
</tr>
<tr>
<td>3</td>
<td>176±7b</td>
<td>40.8±1.3d</td>
</tr>
<tr>
<td>4</td>
<td>155±32b</td>
<td>41.8±1.4d</td>
</tr>
<tr>
<td>5</td>
<td>242±27c</td>
<td>39.9±2.1d</td>
</tr>
<tr>
<td>6</td>
<td>190±38bc</td>
<td>42.5±2.8d</td>
</tr>
<tr>
<td>7</td>
<td>169±23b</td>
<td>35.0±4.4c</td>
</tr>
<tr>
<td>8</td>
<td>204±18bc,d</td>
<td>40.9±1.3d</td>
</tr>
<tr>
<td>9</td>
<td>229±48c</td>
<td>32.4±2.2c</td>
</tr>
<tr>
<td>10</td>
<td>97.5±10.9a</td>
<td>58.2±2.4a</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts are statistically different (p<0.05)

In this work, a strong correlation between EC50 and TPC was found (-0.471; p<0.05). Some studies report a strong correlation between EC50, indicating that phenolic compounds have the main role in antioxidant activity. However, there are some authors finding weak values for this correlation, attributable to the characteristics of the DPPH radical.
Influence of cooking time on antiradical activity and TPC of New Zealand spinach

Sample 10 was selected to evaluate the influence of cooking time on antiradical activity and TPC of New Zealand spinach since it presented the best value for antiradical activity (EC₅₀ equal to 97.52 μg/ml). The changes of antiradical activity after thermal processing are presented in Table 2. The samples subjected to thermal processing for 1 minute, 2.5 minutes, 5 and 10 minutes were significantly different at p < 0.05, compared to raw sample, with EC₅₀ values between 140.6 and 257.5 μg/ml. We also observed significant differences between groups with different times of thermal processing, except for 10C and 10D that presented very similar EC₅₀ values (Table 2).

Table 2. Antiradical activity and total phenolic content in New Zealand spinach boiled during different times.

<table>
<thead>
<tr>
<th>Boiling time (min)</th>
<th>Sample</th>
<th>EC₅₀ (μg/ml) (n=4)</th>
<th>TPC (mg GAE/g of extract) (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>97.5±10.9⁹</td>
<td>58.2±2.4⁹</td>
</tr>
<tr>
<td>1</td>
<td>10A</td>
<td>141±8</td>
<td>55.4±3.1⁹</td>
</tr>
<tr>
<td>2.5</td>
<td>10B</td>
<td>189±15</td>
<td>57.7±2.5⁹</td>
</tr>
<tr>
<td>5</td>
<td>10C</td>
<td>257±15</td>
<td>59.1±7.3⁹</td>
</tr>
<tr>
<td>10</td>
<td>10D</td>
<td>257±20</td>
<td>59.2±6.1⁹</td>
</tr>
</tbody>
</table>

⁹, b, c, d Means within columns with different superscripts are statistically different (p<0.05)

When analyzing the results of EC₅₀ values over 10 minutes, the antiradical efficiency of methanolic extracts of spinach leaves in New Zealand decreased linearly up to 5 minutes of cooking (sample 10C), as shown in Table 3. The samples 10C and 10D showed no significant differences between them, suggesting that the anti-radical activity and TPC values tend to stabilize after a certain time of cooking.

Studies performed on different vegetables after cooking showed that the total polyphenol content and antioxidant capacity could be either higher or lower in comparison to the fresh food ⁵, ¹¹, ¹². Food processing and domestic cooking could result in higher polyphenol concentration when compared to raw samples.
12. In contrast, the deleterious effects of heat treatment have already been shown for polyphenols as well as for their antioxidant capacity4,8,13.

Conclusions
The antiradical activity of New Zealand spinach was strongly correlated with TPC. The differences found suggested a greater influence of environmental and genetic factors in the synthesis and accumulation of phenolic compounds in this species. Boiling spinach up to five minutes significantly reduced antiradical activity, with no changes observed after this cooking time. After this initial analysis, it’s important to trace a phenolic profile of New Zealand spinach, as well as a study of their antioxidant activity with the use of different methodologies, in order to characterize them and their possible health benefits.

References


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