The antioxidative power AP—A new quantitative time dependent (2D) parameter for the determination of the antioxidative capacity and reactivity of different plants

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Abstract

In the last decade, naturally occurring antioxidants continue to play an important role in the food-supplement industry. The content of antioxidants in a plant depends on the species, temperature, humidity, period of growth, harvest month, part of the plant used and many other variables. Herein, we present a new method able to determine the all over antioxidative power (AP) of plant extracts or lyophilised plant parts based on the reducing activity against a stable test radical. The method is performed by ESR spectroscopy and is based on the well-known 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method with the major difference that both the antioxidative capacity and the antioxidative activity are used to characterise an antioxidant. The resulting antioxidative power is expressed in antioxidative units (AU), where 1 AU corresponds to the activity of a 1 ppm solution of Vitamin C as a benchmark. This method allows a rapid, inexpensive and general applicable technique for the measurement of the antioxidative power of very different kinds of substances. The inclusion of the kinetic behaviour of the reducing process of the antioxidant for the determination of the AP allows the identification of the main antioxidant present in a sample. Herein, we present the application example of seeds, sprouts and adult parts of dandelion, amaranth, quinoa, fenugreek, broccoli, red clover and mugwort, where the AP method permits to characterise the plants with the highest antioxidative capacity and reaction velocity. The method permits to select active plant extracts for the food and nutrition industry.

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1. Introduction

Vegetables are important to the human diet and many studies have shown that a close relation exists between the intake of vegetables and prevention of serious widespread diseases as cancer and cardiovascular diseases [4–5]. One important factor for the anticancer activity is thought to be the abundant natural antioxidants, such as Vitamin C and Vitamin E and β-carotene, in vegetables. Vitamin C is a major antioxidant to quench free radicals in blood. Both tocopherol and β-carotene exhibit antioxidative activity under lipophilic and hydrophilic conditions. Vitamin E is located in cell membranes and capable of reducing the free radicals in the cell membrane. β-Carotene is the major antioxidant in cell membranes and lipoproteins, although it shows weaker antioxidant activity.

Vegetables contain not only the above major antioxidants but also a great quantity of substances with antioxidant activity, such as flavonoids, flavones and polyphenols.

Flavonoids are able to block the formation of cancerogenous nitrosamines and nitrosamides and they are very efficacious antioxidants [6]. They are present mainly in leaves, flowers and fruits of the plants. Also, polyphenols are strong antioxidants and their biosynthesis is strongly influenced and regulated by light irradiation [7].

In a single botanic species the amount of antioxidants depends on several parameters such as the part of the plant (seeds, germs, leaves, roots and fruits), the germination time, the environmental conditions of sprouting and growth (pH, temperature, irradiation and availability of macro- and micro-nutrients), and the procedure of preserving (different drying methods and conge-
The outdoor plants were cultivated at a cropping farm. All sprouts and plants were freeze-dried at \(-25\) °C, at the end of the lyophilisation at 22 °C.

Herbs (five subspecies of Artemisia vulgaris) were hand-gathered adult plants from different cultivations and air-dried for 4 weeks (June–July). All samples were ground to powder with a standard laboratory mill at 5 °C. The ground samples were filled under nitrogen atmosphere in tubes and stored at \(-21\) °C.

2.2. General procedure

The 2D parameter called AP is characterised by the capacity to remove a certain number of free radicals in a certain time interval. The AP is described by the following relation:

\[
AP = \frac{RA \times N_{\text{DPPH}}}{w_c \times t_r}
\]  

where \(RA\) is the constant reduction amplitude (1/e²), \(N_{\text{DPPH}}\) the quantity of reduced free radicals characterised by free electrons (spins) of DPPH, \(w_c\) the characteristic weight of the antioxidant product and \(t_r\) is the reduction time. The measuring unit is defined by \(N\), number of spins; \(w_c\), weight in milligrams; \(t_r\), reduction time in minutes, so that:

\[
AP (\text{spins/(mg min)}) = (\text{mol DPPH/(mg min)})
\]

At least three different weights of the samples were dissolved in ethanol 50%, agitated for 3 min and after centrifugation the supernatant was used for analysis. DPPH was dissolved in 96% ethanol and was added to the samples to reach a final concentration of 0.1 mM.

Thirty seconds after mixing the test radical with the antioxidant solution the first ESR spectrum is registered. The measurements were performed with the ESR spectrometer Miniscope 200 (Magnettech, Germany) and the following technical parameters were used: 100 G sweep width, 100 Gain, 1 G modulation amplitude, 7 mW microwave power, 3365-G central field. At a minimum of three different time intervals, the intensity of the remaining DPPH concentration in each sample is measured. The reduction curves of DPPH of each sample concentration are normalised to the initial DPPH intensity of 0.1 mM. The reduction of DPPH in each antioxidant concentration is described by a monoexponential decay.

From the plots the reaction time \(t_r\) of each concentration is calculated by the following equation:

\[
f_m = -\ln(1 - 1/e) (\text{min})
\]

where \(f_m\) is the reaction constant of the monoexponential decay.

Then, the DPPH intensity is plotted against the antioxidant concentration and the curve is fitted monoexponentially. The characteristic weight \(w_c\) of the tested substance is calculated using the following equation:

\[
w_c = \frac{-\ln(1 - 1/e)}{k_w} (\text{mg/ml})
\]

where \(k_w\) is the rate constant of the monoexponential decay.
Fig. 1a and b and Table 1 show the different AP values found in sprouts and seeds of broccoli, dandelion, fenugreek, quinoa, red clover and amaranth. The AP is expressed in antioxidative units (AU), where 1 AU corresponds to the AP of 1 ppm Vitamin C.

### 3. Results and discussion

The AP of seeds and sprouts of broccoli, dandelion, fenugreek, quinoa, red clover and amaranth is shown in Fig. 1a and b. The AP values range from 27 in amaranth seeds to 9750 in broccoli sprouts. All data are expressed as median values.

The AP of the seeds is lower than the AP of the sprouts, indicating a fast biochemical synthesis of antioxidants during germination processes. For fenugreek, the AP of seeds is 10 times higher than in the sprouts (1462 rsp. 116 AU), indicating that high levels of antioxidants are stored in the protein-rich seeds. The scattered data in the figures refer to the right y-axis and report the reaction time $t_r$ of each compound. The shorter the reaction time is, the faster is the reaction between the antioxidants and the test radical DPPH.

Table 2 reports the AP and $t_r$ of freeze-dried sprouts and seeds.

### Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>AP of seeds</th>
<th>AP of sprouts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli</td>
<td>5789</td>
<td>9750 ± 1847</td>
</tr>
<tr>
<td>Dandelion</td>
<td>8204</td>
<td>3100</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>1462</td>
<td>542 ± 29.2</td>
</tr>
<tr>
<td>Quinoa</td>
<td>458</td>
<td>468 ± 101</td>
</tr>
<tr>
<td>Red clover</td>
<td>1162 ± 332</td>
<td></td>
</tr>
<tr>
<td>Amananth</td>
<td>27</td>
<td>409 ± 205</td>
</tr>
</tbody>
</table>

**Notes:**
- Six samples, sprouts between 3 and 8 days.
- Four samples, sprouts between 7 and 8 days.
- Six samples, sprouts between 2 and 8 days.
- Five samples, sprouts between 2 and 4 days.
- Four samples, sprouts between 3 and 4 days.
- Five samples, sprouts between 3 and 5 days.

The AP is expressed in antioxidative units (AU), where 1 AU corresponds to the AP of 1 ppm Vitamin C.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Main antioxidant</th>
<th>Reference</th>
<th>$t_r$, means ± S.D. (min)</th>
<th>$t_r$, pure antioxidant (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli seeds</td>
<td>Vitamin C</td>
<td>[4]</td>
<td>0.243</td>
<td>Vitamin C=0.24</td>
</tr>
<tr>
<td>Broccoli sprouts</td>
<td>Vitamin C, carotene</td>
<td>[12]</td>
<td>0.26 ± 0.02</td>
<td>Vitamin C=0.24</td>
</tr>
<tr>
<td>Fenugreek seeds</td>
<td>Polyphenols</td>
<td>[3]</td>
<td>0.46</td>
<td>nd</td>
</tr>
<tr>
<td>Quinoa seeds</td>
<td>Vitamin C</td>
<td>[15]</td>
<td>0.223</td>
<td>Vitamin C=0.24</td>
</tr>
<tr>
<td>Quinoa sprouts</td>
<td>Riboflavin, Vitamin E</td>
<td>[12]</td>
<td>0.65 ± 0.2</td>
<td>Vitamin E=0.38</td>
</tr>
<tr>
<td>Amananth seeds</td>
<td>Polyphenols</td>
<td>[10]</td>
<td>0.035</td>
<td>nd</td>
</tr>
<tr>
<td>Amananth sprouts</td>
<td>Vitamin C, Vitamin E, tocotrienol</td>
<td>[12,17]</td>
<td>0.50 ± 0.14</td>
<td>Vitamin C=0.24, Vitamin E=0.38</td>
</tr>
<tr>
<td>Red clover sprouts</td>
<td>Clovamide</td>
<td>[18]</td>
<td>1.88 ± 0.04</td>
<td>clovamide = 1.78</td>
</tr>
</tbody>
</table>

**Notes:**
- Six samples, sprouts between 3 and 8 days.
- Four samples, sprouts between 7 and 8 days.
- Six samples, sprouts between 2 and 8 days.
- Five samples, sprouts between 2 and 4 days.
- Four samples, sprouts between 3 and 4 days.
- Five samples, sprouts between 3 and 5 days.

In Table 2, the reaction times of the analysed samples are reported. The main antioxidant for each species, as reported in literature, is listed. For Vitamin C, Vitamin E and clovamide the AP was determined and the reaction time is listed in the table. There is a good accordance between the reaction time measured in the plant samples and the reaction time measured for the main antioxidant present in the sample as a pure substance. For instance, broccoli seeds and sprouts are rich in Vitamin C and the measured $t_r$ was calculated between 0.243 and 0.257 min. The reaction time of pure Vitamin C was 0.24 min. The same accordance is found for broccoli sprouts, quinoa seeds and quinoa sprouts. Red clover is enriched in clovamide (caffeoyl-DOPA), a special antioxidant compound present also in cacao, which has a reaction time of 1.78 min and the reaction time of red clover sprouts was found to be 1.87 min. The reaction time of polyphenols and mixtures of antioxidants is variable. In fenugreek seeds, $t_r$ was 0.46 min, whereas in fenugreek sprouts $t_r$ was 4.04 min. Phenolic compounds are the major source of natural antioxidants.
The antioxidant activity of polyphenols can greatly vary depending on several variables such as the chemical structure of the molecule and its concentration and the oxidation degree. It has been recently pointed out that processing can have many effects not all of which result in a loss of content and activity of natural antioxidants.

In order to select the parts of the plants with the highest antioxidant activity, the AP determination of sprouts, leaves, flowers and roots from one single species were performed. In the case of dandelion (*Taraxacum officinalis*), the AP of the roots was 10–12-fold higher than in the green parts of the plant (Fig. 2). Sprouts, leaves and flowers differed only marginally in their AP values, but the reaction time \( t_r \) changed dramatically from the sprouts to the adult plant samples: in 7 days old sprouts the reaction time was 0.36 min and decreased to 0.345 min in 8 days old sprouts. In adult tissues, leaves, flowers and roots, the reaction time was 0.257 \( \pm \) 0.002 min, indicating the presence of other antioxidants in the adult plant.

Also, for amaranth (Fig. 3a) the highest AP was found in the green parts of the adult plant, whereas seed and sprouts had a 20-fold lower AP. Also, the reaction time \( t_r \) changed significantly during the germination process. In the adult plant the reaction time was 0.38 min, corresponding to the reaction time of Vitamin E. The reaction times in the seeds and sprouts were considerably longer. Also, for quinoa (Fig. 3b) the AP of the adult plant was much higher than the AP of seeds and sprouts. In contrast to amaranth, the antioxidants present in the seeds had the highest reactivity. During the germination and growing process, the reaction time became longer, indicating a change in the composition of antioxidants in the sprouts.

The variation of the AP in fenugreek (Fig. 4) during germination was inverted with respect to amaranth and quinoa. The seeds had the highest AP values and the shortest reaction time compared to the sprouts. The reaction time of the sprouts was nearly 10 times lower than the reaction time in the seeds.

In Fig. 5, the AP of five subspecies of mugwort is represented. All plants were adult hand-gathered and air-dried plants from different cultivations. Since they belong to the same species (*A. vulgaris*), it is reasonable that all plants contain the same antioxidants, although in different amounts. The reaction time of all types were nearly the same (0.233 \( \pm \) 0.014 min), confirming the presence of the same main antioxidant. This reaction time of 0.24 min is identical to the reaction time of pure Vitamin C and mugwort is a rich font of Vitamin C. The AP values of all plants were high, ranging from 12,000 to 72,000 AU.
Fig. 5. AP and $t_r$ of five subspecies of mugwort (Artemisia vulgaris).

4. Conclusion

A new method for the determination of antioxidant power of numerous botanical samples was presented inclusive a proposal of a reference standard, the antioxidative unit AU for labelling antioxidative products.

The AP method bases on the reduction of the semi stable test radical DPPH whose reaction with the antioxidant sample is detected by ESR spectroscopy. The total DPPH reduction and its kinetic are used for the calculation of a new 2D factor characterising the capacity and reactivity of a product containing antioxidants. This new 2D parameter called AP represents a new quality of characterising the free radical scavenging activities of different antioxidative products. Two properties of the antioxidative product are integrated: (a) the antioxidative capacity (stationary part) and (b) the dynamic of its reactivity (dynamic part). The differentiation in two parts enables a better and more comprehensive description of the antioxidative properties of a product.

The inclusion of the kinetic behaviour of the reducing process of the antioxidant for the determination of the AP allows the identification of the main antioxidant present in a sample. During germination and growth of a plant, numerous factors influence the content of antioxidants. The AP represents a rapid method to determine the all over antioxidant activity in plants during different growth phases and under different growth conditions.

References