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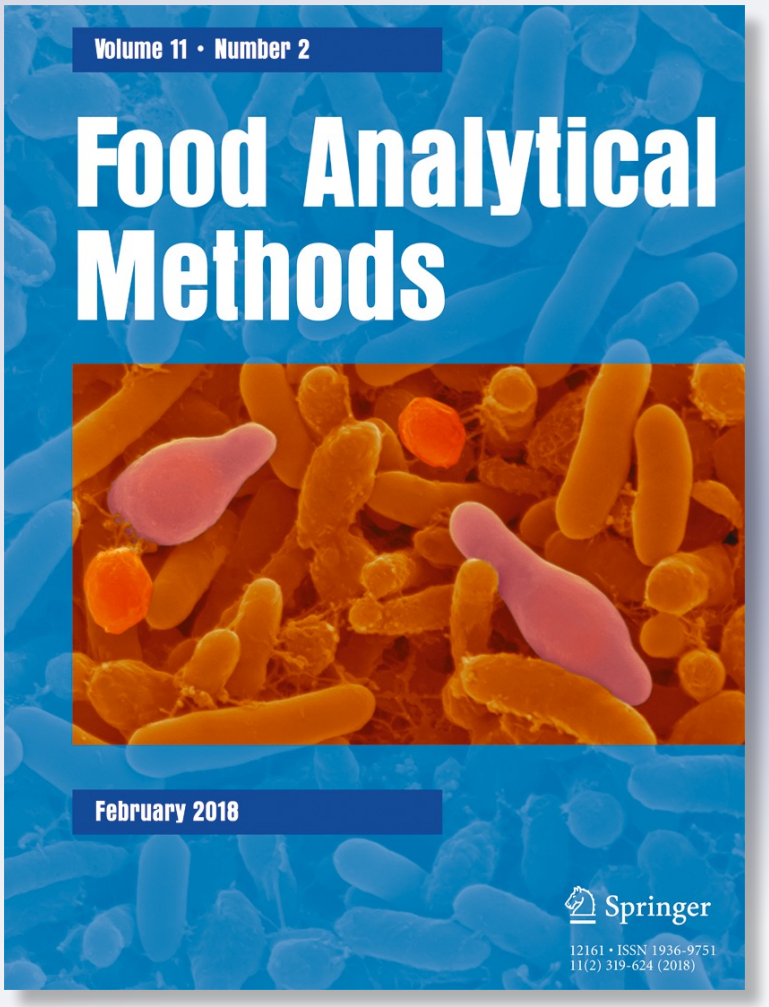
Food Analytical Methods

ISSN 1936-9751
Volume 11
Number 2

Food Anal. Methods (2018) 11:432-439
DOI 10.1007/s12161-017-1014-z



Springer

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Simultaneous Kinetic and Thermodynamic-Based Assay to Determine the Hydrogen Peroxide (H_2O_2) Scavenging Activity of Berry Extracts by Using Reaction Calorimetry

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Received: 7 June 2017 / Accepted: 31 July 2017 / Published online: 18 August 2017

Abstract This work determines the radical scavenging activity of antioxidants and berry extracts based on the heat generated during their reaction with hydrogen peroxide, under isothermal condition (25 °C). After addition of H_2O_2 to a water solution containing antioxidants, an exothermic heat flow appeared. After an initial damping time, the signal decayed exponentially, following a first-order kinetic. Through an iterative fitting routine, both thermodynamic (ΔH) and kinetic (k) information were achieved. Such approach was applied toward relevant food antioxidants, revealing that the fastest reactivity (k) was for tannic acid > gallic acid > caffeic acid > ascorbic acid. Interestingly, k was inversely correlated with ΔH ($r = -0.96$) and with the DPPH test ($r = -0.98$). Apparently, strong radical scavengers show faster kinetics and lower ΔH-values, as expected, respectively, from a high reactivity toward peroxyl radical and efficient delocalization capacity. Such approach was finally applied to berry extracts (mixed grape seed and skin; chokeberries; grape seed; goji berries). The resulting ΔH-values were correlated with three indices, namely, total phenol, amperometry, and DPPH test. However, k-values largely deviated from these indices. Such discrepancy was explained considering that none of these indices is a "true" measure of the kinetic of the reaction, but only express an apparent concentration. Conversely, reaction calorimetry provides directly and simultaneously both thermodynamic and kinetic properties of the radical scavenging reactivity of antioxidants or natural extracts.

Keywords Reaction calorimetry · Antioxidants · Berry extracts · Hydrogen peroxide · Scavenging activity

Introduction

During oxidative stress, cells metabolism may lead to a number of reactive oxidative species (ROS), which result in a significant damage of cell structures. A relevant contribution to oxidative damage is given by hydrogen peroxide (H_2O_2). H_2O_2 may form highly reactive hydroxyl radical (HO*), which can damage proteins, lipids, carbohydrates, and DNA (Gill and Tuteja 2010). To cope with such kind of oxidative stress, biological systems synthesize certain bioactives, namely antioxidants, which have the capacity to reduce the rate of formation of hydroxyl radicals (HO*). The capacity of certain antioxidants to scavenge H_2O_2 is of practical importance not only to biological systems, but also in many other fields, such as foods, pharmaceuticals, feeds and cosmetics processing, or in all those situations where an additive, an ingredient or a substance is used to retard an oxidation process (Sroka and Cisowski 2003; Wang and Jiao 2000; Zhou and Elias 2011).

Accordingly, the measurement of the radical scavenging activity is of great importance to identify the best ingredients, preservatives, or natural extracts. Nowadays, one of the most

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straightforward assays for the measurement of the radical scavenging activity is based on the direct measurement of H_2O_2 in the UV region at 230 nm (Ruch et al. 1989). As scavenger compounds decrease the concentration of H_2O_2, the absorbance value at 230 nm also decreases. However, such approach cannot be applied in most plants and food extracts as both may contain chemical species that also absorb at this wavelength without contributing to the radical scavenging activity. This, in turn, may compromise both precision and accuracy of the method. It should be also mentioned that such assay does not use any catalyst for generating HO* radicals. Accordingly, the number of free radicals generated during the assay is relatively small. This ultimately affects the sensitivity of the assay, because the measurement is based on the available H_2O_2 (which is in excess amount) and not on the generated HO* (which is in trace amount). Not surprisingly, many works report similar scavenging activities for very different antioxidants (Ak and Gülçin 2008; Bozin et al. 2008; Ruch et al. 1989; Yen and Chen 1995).

To overcome such problems, many other indirect assays have been proposed. In general, they are all based on the oxidation of a probe by H_2O_2. The reaction is typically monitored by fluorescence, spectrometry, or chemiluminescence (Özyürek et al. 2010). In detail, fluorescence assays are based on the use of fluorophores, the intensity of which changes as a function of the amount of H_2O_2 present in the solution (Gomes et al. 2005). The assays based on spectrometry monitor the oxidation of phenol red by H_2O_2 mediated by horseradish peroxidase (HRPO). This results in the formation of a compound with increased absorbance at 610 nm (Pick and Keisari 1981). Finally, the chemiluminescence measurement of luminol and peroxyoxalates was also successfully used to test the scavenging activity (Bartosz 2006).

In spite of their practical importance, these assays suffer from a number of drawbacks, such as time-consuming sample pre-treatments, measurements limited by sample transparency, and results dependent on the experimental conditions (i.e., pH and solvents). These aspects, in turn, limit the transferability of the results. A possible way to improve the performance of such radical scavenging assays may be through to use heat-flow reaction calorimetry.

Differently to optical-based detectors, reaction calorimetry is based on the direct measurement of the heat flow generated during the occurrence of a reaction (Nilsson and Hess 2008). Moreover, as described by Beezer, one of the main advantages of such technique is that it allows the direct and simultaneous measurement of both thermodynamic and kinetic information of a reaction, under the maintenance of isothermal conditions (Willson et al. 1995).

Accordingly, the purpose of this work was to apply reaction calorimetry to evaluate the radical scavenging activity of different antioxidants by monitoring the heat generated during their reaction with H_2O_2. Assuming that the reaction is overall exothermic (Kamrul et al. 2016), a simple fitting procedure may likely be used to obtain both the enthalpy and the rate constant of the reaction, leading to fundamental thermodynamic and kinetic information of the antioxidants.

Materials and Methods

Samples

The commercial bio-product of dried whole chokeberries (*Aronia*), goji berries (dried whole fruit of *Lycium barbarum L.*) and commercial grape seed flour (*Traubenkern mehl*) were purchased from local bio-shop (Bolzano, Italy). In addition, a mixture of dried grape seeds and skins was obtained from local biological producer "Othmar Sanin" (Bolzano, Italy).

Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu reagent (2.0 N), hydrogen peroxide (H_2O_2) sodium bicarbonate, gallic acid, caffeic acid, tannic acid, ascorbic acid, boric acid, phosphoric acid, acetic acid, and sodium hydroxide (NaOH) were of analytical grade (Sigma Aldrich, Germany). Methanol, ethanol, and acetonitrile (99.8%) were HPLC grade (Sigma Aldrich). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased form ACROS (Denmark).

Preparation of Standards

A 127-μM stock solution of DPPH and trolox (0.1, 0.2, 0.3, 0.4 and 0.5 mM) was prepared by dissolving the desired amount in ethanol/water (1:1) mixture. A 50-mM stock solution of gallic acid, caffeic acid, tannic acid, and ascorbic acid was prepared by dissolving the desired amount in distilled water (18 MΩ) or ethanol. The Britton-Robinson buffer was prepared from an equal mixture of 0.04 M boric acid, 0.04 M phosphoric acid, and 0.04 M acetic acid. The pH value was adjusted by using 0.2 M NaOH.

Extraction

Dried samples were grinded into uniform powder (Retsch MM400, Retsch GmbH, Germany). Two grams of dry powder were mixed with 5 mL of solvent (80% methanol, 20% water) in a glass tube. The tube was vortexed for 60 s (IKA Genius 3 Vortex) and then sonicated (Bandelin Sonorex, 40 K Hz) for 15 min at room temperature. Afterwards, the sample was centrifuged at 5000 rpm (Thermo Scientific SL 16R) for 25 min. An aliquot of the supernatant was transferred into a 10-mL plastic syringe and filtered through a 0.45-μm filter before the analysis.

Reaction Calorimetry

Reaction calorimetry was performed with a CPA 202 system (ChemiSens AB, Lund, Sweden). Details of the analysis are reported elsewhere (Kamrul et al. 2016). Briefly, the reactor cell was filled with 100 mL of a non-deaerated buffer solution (Britton-Robinson, pH = 6.5) and tightly closed with a lid. Finally, the reactor cell was immersed in the thermostat unit and kept under isothermal conditions (25 °C). The true heat flow signal was recorded under stirring conditions (200 rpm). When the base line was stable (signal ≤0.1 mW), an aliquot of the antioxidant or berry extract (1 mL) was injected in the cell. After a rest of ~30 min, a further 1 mL of H_2O_2 (30%) was added into the reactor cell. The integral of the resulting heat flow signal (in Watt) leaded to the heat of the reaction (in Joule).

Kinetic Fitting of the Thermogram

The analysis of calorimetric data was based on previous works (Gaisford et al. 1999; Willson et al. 1995), where both kinetic and thermodynamic parameters can be recovered once a kinetic equation describing the reaction under study was defined. In this work, the experimental thermograms were fitted assuming a first-order kinetic model:

$$A \xrightarrow{k} B$$

The thermal power associated with the reaction is equal to:

$$\frac{dq}{dt} = -k \cdot \Delta H \cdot [A]_0 e^{-k \cdot t} \quad (1)$$

Where k is the rate constant, ΔH is the enthalpy of the reaction, and $[A]_0$ is the initial amount of antioxidants. The overall heat of the reaction can be expressed as:

$$Q_{\text{tot}} = DH[A]_0 \quad (2)$$

When initial guessed values for k and ΔH are given, and when $[A]_0$ is known, any curve-fitting package can easily estimate the kinetic and thermodynamic parameters of the reaction mechanism by minimizing the difference between the simulated and observed thermogram toward an iteration process.

Total Phenolic Content by Folin-Ciocalteu Method

A 20 μL of sample extract was mixed with 5 mL Folin-Ciocalteu solution. Then 20 mL of sodium bicarbonate (20% solution) was added to the mixture and finally adjusted to the mark with distilled water to make 100 mL solution. The solutions were left for 30 min at 22 °C. Then, the absorbance was measured at 750 nm. Gallic acid (0–2000 μM) was used for calibration. The calibration curve was linear ($R^2 = 0.996$). The results were expressed as mean of the concentration of gallic acid equivalent (GAE) per gram of dried sample ± standard deviation (SD). All measurements were performed in triplicate.

Antioxidant Power Measurement

Flow measurements were performed using Thermo Fischer Ultimate 3000 LC Auto sampler. Extracts were diluted 500 times with a mixture of 0.1 M LiClO4 in acetonitrile and ethanol (ratio 1:1). The following measurement settings were used: flow rate of 1 mL min^{-1} and injection volume of 20 μL. Electrochemical measurements were performed using a thin-layer cell (ALS, Japan), with a layer thickness of 50 μm, equipped with a non-aqueous reference electrode (Ag/Ag+) and a working electrode made of glassy carbon (3 mm diameter). The working electrode was set at the potentials of + 0.4 V or + 0.8 V vs Ag/AgCl. The lower potential (+ 400 mV) was used to measure strong antioxidants. The + 800 mV was instead used for the estimation of total phenol content. The data was recorded with Autolab analyzer (Metrohm, Netherlands). Data were recorded using Nova 1.10 software. The results were expressed as ascorbic acid equivalent (AAE) in mM per gram of dried sample.

DPPH Assay

Antioxidant activity of samples was determined by a free radical scavenging assay, by using DPPH as the source of the free radicals (Brand-Williams et al. 1995). DPPH can react directly with most of the antioxidants and be captured by them. The reduction of DPPH is measured as a function of time (120 min) by the decrease in absorbance at 515 nm. Briefly, a 1-mL $DPPH^{\bullet}$ solution was transferred into a cuvette. The absorbance was measured immediately and after 120 min, Trolox solutions (0.1, 0.2, 0.3, 0.4, and 0.5 mM) were used for building the calibration curve. Similarly, the Trolox equivalent (TE) antioxidant capacity of the samples was obtained by measuring the absorbance of the $DPPH^{\bullet}$ solution after its mixing with the extracts (50 μL) diluted (100 times). The absorbance spectra were recorded with a Cary-100 UV-VIS spectrophotometer (Agilent Technologies, USA). All the analyses were performed in triplicate. The results were expressed as:

$$DPPH \quad scavenging \quad capactity(\%) = \frac{A\,control - A\,sample}{A\,control} \times 100$$
$$A = absorbance\ at\ 515\ nm$$

Statistical Analysis

Statistical analysis was carried out using XLSTAT software (Addinsoft). Analysis of variance (ANOVA) and Tukey's test were used for multiple comparisons of means. The differences were considered statistically significant at $P \leq 0.05$.

Results and Discussions

Reaction Calorimetry

Figure 1 shows the heat flow response measured during the sequential addition of antioxidants (1 mL) and H_2O_2 (1 mL) on a reactor vessel equipped with true-type heat flow sensor (Nilsson and Hess 2008). Initially, the reactor vessel was filled with distilled water (100 mL) while stirring at 200 rpm. Under steady state conditions (see Fig. 1a), the recorded heat flow signal was below 0.1 mW. Such value comprised mainly the heat of stirring. Any thermal unbalances between the reactor vessel and the surrounding were minimized by drowning the reactor in a thermostat tank with recirculating water. After reaching steady state, 1 mL of ascorbic acid (final concentration in vessel, 0.50 mM) was added (Fig. 1b). The resulting signal increased up to 7.0 ± 0.3 mW (n = 5) in about 1 min, with a heat—i.e., the area under the heat flow curve—of 2.0 ± 0.2 J. As soon as the heat flow signal returned steady, 1 mL of H_2O_2 (final concentration in the vessel, 98 mM) was added. Figure 1c shows the resulting signal, leading to a heat flow of 27 ± 2 mW (n = 5) and an overall heat of 21 ± 2 J. For comparison, the heat generated by mixing H_2O_2 in water resulted in heat values ten times smaller (2.0 ± 0.2 J). Accordingly, the extent of the heat flow signal after the addition of H_2O_2 should be viewed as a direct measurement of the dynamic reactivity of antioxidants toward an induced oxidative stress exerted by hydrogen peroxide.

Time Constant of the Calorimeter

It is often important to determine the time constant of the calorimeter. In general, this accounts for the delay related to the heat transfer from the sample to the surrounding, where the sensors are located. Such delay typically results in a mismatch between the recorded and the real signal. Figure 2 shows the impulse response of the calorimeter when an electrical input (7 mW) is applied inside the reaction vessel. When the electrical input is removed, the resulting thermal trace can be described by plotting the logarithm of the heat flow signal vs time. The slope provides the reciprocal of the time constant, which in this case is equal to 37 s. However, it should be noted that in case of long processes, the use of a time constant to correct the observed thermal traces can be misleading. Especially when the signal is produced by a multi-step process, a further time delay reflects the real kinetic of the process and not a delay of the instrument. In the specific case observed in Fig. 1, the maximum of the calorimetric signal of ascorbic acid appears at ~300 s after the injection of the reagent. Thus, $t >> \tau$. In such circumstances, the use of a time correction should be avoided. Accordingly, all the next experiments simply neglected the first points of the thermogram.

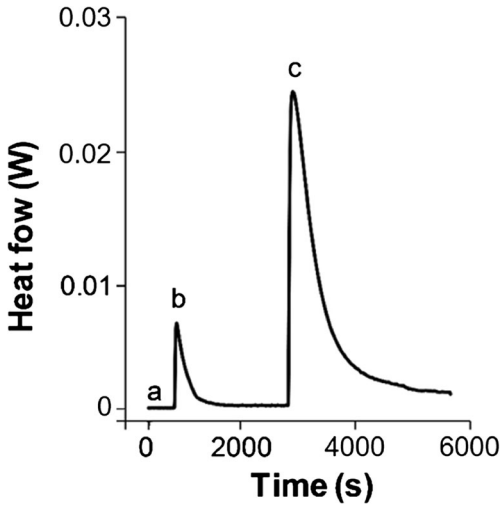

Fig. 1 Calorimetic signal recorded from the reaction between ascorbic acid and H_2O_2. (**a**) is the background signal with the reactor cell filled with water (degassed); (**b**) addition of 1 mL of ascorbic acid (50 mM); (**c**) addition of 1 mL of H_2O_2 (30% *w*/*w*)

Figure of Merit of the Reaction with Ascorbic Acid

The analytical performance of the proposed assay was characterized by adding different concentrations of ascorbic acid (from 0.05 to 2 mM) to H_2O_2 (98 mM). The resulting overall heat produced was linearly dependent (R^2 = 0.99) on the concentration, showing a sensitivity of 29 ± 1 J mM^{-1}. The limit of detection (LOD) was determined as 3 s / b, where b is the slope of the calibration line obtained with at least six increasing concentrations of ascorbic acid and s is the standard deviation of the calibration line. The resulting LOD was 0.08 mM. The repeatability, expressed as relative standard deviation (RSD), was obtained from 5 independent additions of ascorbic acid (0.50 mM, final concentration in the vessel), resulting in a precision always better than 3%.

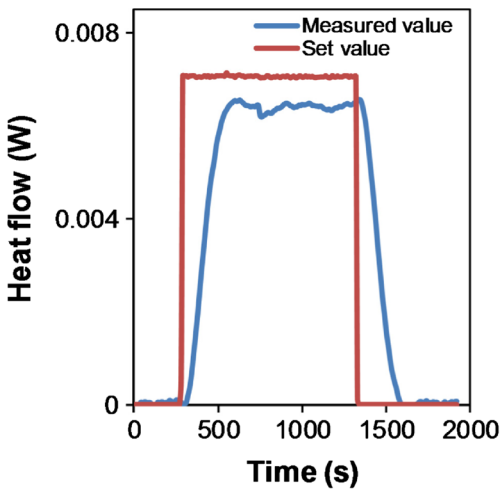

Fig. 2 Heat flow signal for the validation of system using electrical input (0.007 W)

Reaction Calorimetry of Individual Antioxidants

The dynamic reactivity of individual antioxidants toward H_2O_2 was next investigated utilizing the developed reaction calorimetry assay. Figure 3 shows the resulting thermograms of some standard solutions of antioxidants, namely, ascorbic acid ($C_6H_8O_6$), caffeic acid ($C_9H_8O_4$), gallic acid ($C_7H_6O_5$) and tannic acid ($C_{76}H_{52}O_{46}$). It is known that all these species can scavenge the hydroxyl peroxide by a combination of electron and hydrogen transfer (Liang and Kitts 2014). Accordingly, all these antioxidants generate a thermogram with similar pattern, although different overall heat. This likely reflects their different reactivity. To explain such reactivity, the thermograms were fitted with simulated equations obeying to the same fundamental rate law. From previous studies, the oxidation of ascorbic acid sustained by H_2O_2 was described by a two-consecutive first-order reactions (Kamrul et al. 2016), where H_2O_2 triggers the consumption of antioxidants via the formation of an intermediate radical species (A*) followed by the formation of a variety of oxidation products. However, in the presence of an excess of H_2O_2 relative to the antioxidant, the reaction could be furtherly simplified into a single irreversible process, with k and ΔH characterizing the thermogram.

In line with this simplification, the thermograms of all the antioxidants were described by a single irreversible first-order kinetic model, leading to R^2 ranging from 0.997 to 0.998 (Fig. 4). Table 1 reports the pseudo-first-order rate constants (k, expressed as 10^{-3} s^{-1}). Ascorbic acid showed the smallest k (1.55) followed by caffeic (3.68) < tannic (4.09) < gallic (4.20) acids. Overall, the extent of such rate constants reflects the rate at which each antioxidant quenches peroxyl radicals and, thus, its reactivity toward free radicals.

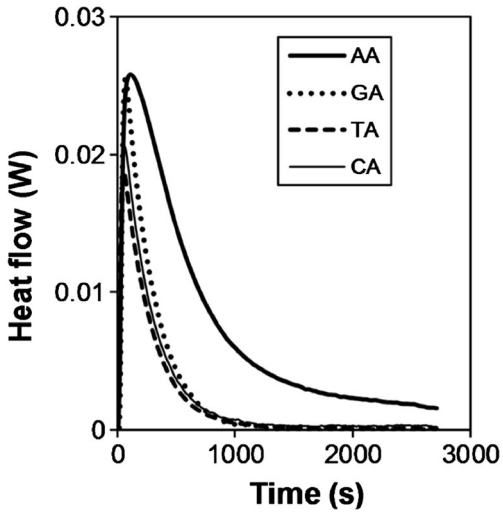

Fig. 3 Isothermal calorimetric record of caffeic acid with corresponding fitting (dotted line) using eq. (1). Shaded area under the curve is integral area

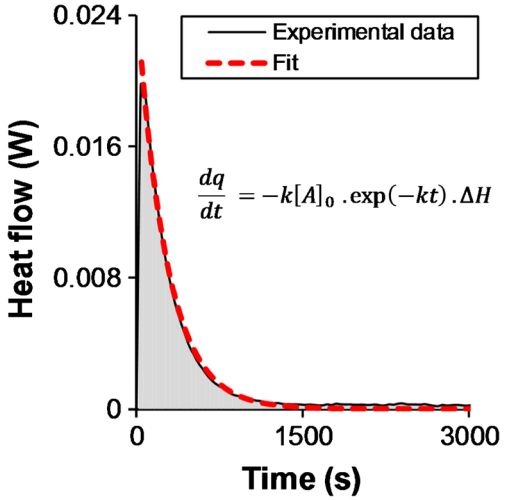

Fig. 4 Heat flow signal for different antioxidant compound. AA = Ascorbic acid; GA = Gallic acid and TA = Tannic acid

Also, Table 1 reports the resulting thermodynamic information. The enthalpy of each reaction followed the trend: ascorbic acid (409 ± 40 kJ/mol) > gallic acid (167 ± 24 kJ/mol) > caffeic acid (132 ± 20 kJ/mol) tannic acid (119 ± 20 kJ/mol). A small ΔH-value corresponds presumably to antioxidants (AH) having the most efficient π-conjugated system. This ultimately leads to more stable radical products (A*). Accordingly, tannic acid shows the best delocalization capacity, followed by caffeic, gallic, and ascorbic acids. Overall, the rate constants are inversely correlated with ΔH-values (r = −0.96). Apparently, antioxidants with a faster kinetic show also lower ΔH-values, in line with a more efficient delocalization capacity.

Comparison Between the Reaction Calorimetry and DPPH Test

Next experiments aimed to investigate the correlation between the kinetic and thermodynamic parameters obtained by reaction calorimetry in comparison with those obtained by other antioxidant capacity indices. The DPPH test in the version of

Table 1 Kinetic and thermodynamic properties of the radical scavenging activity of selected antioxidants (each 500 μM, final concentration in the cell) and their antioxidant capacity value as determined by DPPH test (expressed as 50% inhibition concentration, IC50)

Sample	Reaction calorimetry assay			DPPH assay
	Q_{tot} (J)	ΔH (kJ/mol)	k ($\times 10^{-3}$ s^{-1})	IC50 (μM)
Ascorbic acid	20.5 ± 3.9	409 ± 40	1.55 ± 0.16	28.8
Caffeic acid	6.6 ± 1.4	132 ± 20	3.68 ± 0.23	6.1
Tannic acid	5.9 ± 1.2	119 ± 20	4.09 ± 0.20	7.9
Gallic acid	8.3 ± 1.4	167 ± 24	4.20 ± 0.20	4.4

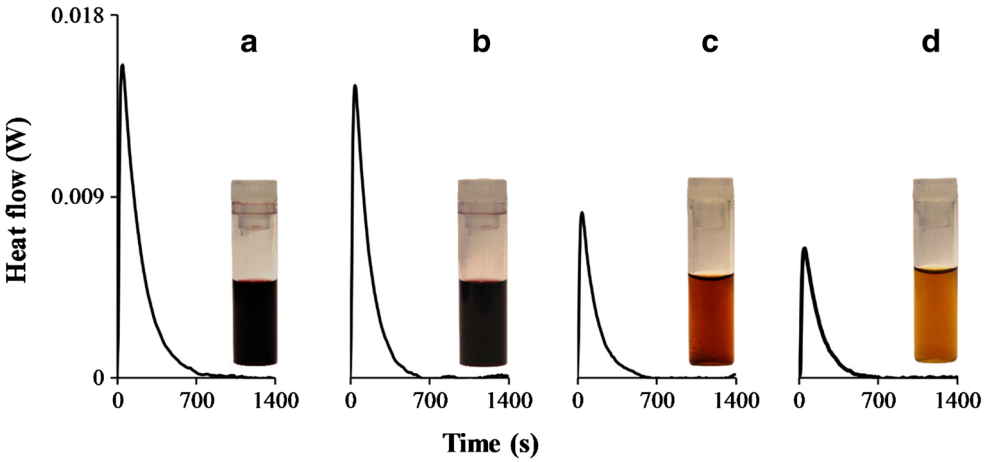

Fig. 5 Calorimetric heat flow record for different berry extracts. **a** Mixture of grape seed and skin, **b** commercial dried chokeberries, **c** commercial grape seed flour, and **d** commercial dried goji berries. Inserted vial show the physical appearance of corresponding berry extracts

Brand-Williams (Brand-Williams et al. 1995) is a quick and widespread spectrometric assay, which expresses the antioxidant capacity in terms of inhibitory concentration (IC50). This is the concentration of the sample that quenches 50% of the initial DPPH• radical in a specific, although arbitrary, time interval. In practice, the lowest concentration of the IC50 corresponds with the highest antioxidant activity.

The highest IC50 was recorded for ascorbic acid (28.8 μM), followed by tannic acid (7.9 μM), caffeic acid (6.1 μM), and gallic acid (4.4 μM). The fact that gallic acid has a smaller IC50 value than caffeic acid can be explained considering that the DPPH scavenging activity of phenolics is positively correlated with the number of their hydroxyl groups (Sroka and Cisowski 2003). Although the DPPH test does not provide a true measurement of the reaction kinetic, within the specific case of standard solution of antioxidants, the correlation between the DPPH test and the *k*-values is excellent (r equals −0.98). Apparently, the highest DPPH values correspond to the lowest rate constant.

These findings provide evidence that to establish whether a compound behaves as a good antioxidant, such ability should be simply represented by the rate at which the putative compound quenches peroxyl radicals (Foti 2015). Eventually, this information should be also combined with some thermodynamic information about the capacity of an antioxidant to give more stable products. Unlike DPPH, reaction calorimetry leads to both kinetic and thermodynamic information. Through the enthalpy value of the reaction with peroxyl radicals, the assay reveals the capacity of antioxidants (AH) to delocalize electrons and generate more stable radicals (A*). At the same time, through the estimates of the rate of reaction, the assay provides a direct measure of how fast a bioactive reacts with peroxyl radicals. Ultimately, this leads to a direct measurement of the antioxidant efficiency.

Antioxidant Capacity of Berry Extracts

The proposed reaction calorimetric assay was finally applied to the analysis of the antioxidant activity of berry extracts. Berry extracts are known to contain a mixture of phenolic compounds with good antioxidant properties (Forino et al. 2016; Gorinstein et al. 2013; McDougall et al. 2016; Yahui et al. 2017). Figure 5 shows the heat flow signal recorded during the reaction between H_2O_2 and different berry extracts. Each extract contained the following amount of antioxidants

Table 2 Kinetic, thermodynamic, and antioxidant properties of different berry extracts

Sample	Reaction calorimetry			TP (μM g^{-1})	DPPH (mM g^{-1})	Tot AOX (mM g^{-1})	AOX power (mM g^{-1})
	k ($\times 10^{-3}$ s^{-1})	ΔH (J g^{-1})	[*AA*] (μM g^{-1})				
Goji berries	7.78 ± 0.62	2.61 ± 0.30d	65	236.2 ± 0.2c	13.2 ± 0.3d	9.4 ± 0.1b	2.2 ± 0.2c
Grape seed flour	7.76 ± 0.43	3.03 ± 0.17c	76	236.4 ± 0.3c	23.4 ± 0.6c	9.6 ± 0.1b	3.4 ± 0.2b
Chokeberries	8.02 ± 0.56	5.57 ± 0.27b	139	448.4 ± 2.3b	31.3 ± 0.2b	14.3 ± 0.2a	8.5 ± 0.6a
Grape seed-skin	6.20 ± 0.51	7.14 ± 0.41a	179	493.9 ± 0.3a	33.1 ± 0.3a	14.6 ± 0.1a	3.2 ± 0.3b

k and ΔH of reaction calorimetry refer, respectively, to the first-order rate constant and the reaction enthalpy. [*AA*] is the equivalent concentration of ascorbic acid that is determined by the calibration plot with Q_{tot}. Total phenol (TP) is determined by the Folin-Ciocalteu method and expressed as μM of gallic acid equivalent per g of berry extract; DPPH test is expressed as mM of Trolox equivalent per g of berry extract. Total antioxidant (Tot AOX) and antioxidant power (AOX power) are determined by amperometry with a glassy carbon electrode poised at, respectively, + 800 and + 400 mV vs Ag/AgCl and expressed as ascorbic acid equivalent per gram of berry sample; different letters in the same column indicate a significant difference ($P < 0.05$)

(expressed as μM of ascorbic acid): (a) grape seed and skin (179), (b) chokeberries (139), (c) grape seed flour (76), and (d) goji berries (65). The estimation of the apparent rate constant and enthalpy of each sample was determined with the previously described fitting procedure. Both kinetic and thermodynamic parameters of each berry extract were correlated with that obtained with other assays (Folin-Ciocalteu, DPPH assay, and amperometric detection method) (Table 2). In all cases, the enthalpy values were positively correlated with the indices of other assays. Instead, the rate constants were only poorly correlated. Interestingly, such lack of correlation may highlight the fact that none of these three indices express a "true" kinetic information, but only an apparent concentration. Accordingly, the results of the Folin-Ciocalteu, DPPH assay and amperometric detection method may fail to reveal the "true" radical scavenging ability. Instead, reaction calorimetry provides a direct measurement of the reaction rate occurring between the antioxidants and hydrogen peroxide, overcoming the aforementioned limitation. Overall, reaction calorimetry should be regarded as a "true" measurement of the radical scavenging activity of antioxidants and natural extracts in light of the fact that the signal is directly proportional to the rate of the reaction under study.

Conclusion

In conclusion, the radical scavenging activity determined by the reaction calorimetry assay provides a simple and direct way to express the enthalpy (ΔH) and rate constant (k) of the reaction with hydrogen peroxide. The proposed assay is advantageous in comparison to classical UV-method (Ruch et al. 1989) as the measurement of the heat flow is unaffected by the interference from secondary metabolites present in many plants, which all absorb in UV region (Özyürek et al. 2010). Also, unlike the DPPH test, the proposed assay allows to express both thermodynamic and kinetic information.

Acknowledgements We are grateful to the Province of Bolzano for financial support (Landesregierung mittels Beschluss Nr. 1472, 07.10.2013).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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