

Food and Ascorbic Scavengers of Hydrogen Peroxide: a Reaction Calorimetry Investigation

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ABSTRACT

Common spectrometric methods to determine hydrogen peroxide scavenging activity require time consuming extraction protocols and become poorly reliable because of the sample turbidity or the presence of UV absorbing compounds. The present work suggests the use of reaction calorimetry (RC) to determine the antioxidant capacity of fruit juices, fruit puree, tea, coffee and alcoholic beverages, like wines. This experimental approach, that does not imply the above drawbacks and does not require any extraction protocol, allows the direct monitoring of the reaction between food beverages and H₂O₂. The overall exothermic effect reflects the extent of the scavenging activity of the samples versus hydrogen peroxide. The reliability of the approach is assessed through the study of the reaction between hydrogen peroxide and ascorbic acid at different concentration and pH at room temperature.

Key words: reaction calorimetry; antioxidant capacity; hydrogen peroxide; food; peroxide scavenging capacity.

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INTRODUCTION

Antioxidants in food products contribute to a number of important peculiar processes, like slowing oxidation rate [1], preserving nutritional quality [2] and promoting the health-status [3]. Because of the multi-component and multi-phase nature of most food products, a short-cut evaluation of food antioxidants is not easily achievable. In most cases the assessment of the antioxidant content of a given food comes from experimental data drawn with independent methods, each accounting for different aspects related to the antioxidant functionality [3]. The current practice involves two main kinds of experimental approaches. One includes methods that measure the capacity of transferring hydrogen atoms (HAT), such as DPPH (2,2-Diphenyl-1 Picryl Hydrazyl), ORAC (Oxygen Radical Absorbance Capacity) and TEAC (Trolox Equivalent Antioxidant Capacity) assays. The second deals with the measurement of the electron transfer (ET) capacity, such as Folin-Ciocalteu and FRAP (Ferric Ion Reducing Antioxidant Power) [4]. Recently, we proposed a third method that allows the simultaneous determination of the HAT and ET capacity through an electrochemical approach [5].

All these methods require preliminary time-consuming treatments of the samples and show serious drawbacks when applied to opaque or viscous systems.

The present work aims to overcome these difficulties applying a calorimetric approach. Either differential scanning calorimetry (DSC) or reaction calorimetry (RC) indeed allows determination of the reaction heat, regardless to the physical form of the sample and without the need of sample pre-treatments.

Some difficulties are still to overcome. For instance, during DSC investigations with open pans, the water contained in the sample evaporates with a large endothermic effect that conceals any other thermal signal. Another limit of the DSC approach comes the small size of the cells that makes difficult the addition of *in situ* peripherals for dosing, sampling or stirring. Therefore, fast reactions following the mixing of reagents are normally difficult to monitor with this technique. RC instruments too show some drawbacks. They generally host large-volume samples (typically more than one liter) and therefore require extensive calibration checks throughout the reaction time span to compensate baseline shifts. Their response depends on the liquid level, sample viscosity and stirring speed [6].

An innovative chemical process analyzer CPA202 allows overcoming such drawbacks. In the present investigation the instrument allowed detection of the heat flux (HF) released during the reaction between antioxidants and hydrogen peroxide.

The choice of the hydrogen peroxide comes from the knowledge of its involvement in the oxidative stress of plants and human cells and the initiation of many degradation mechanisms in food products [7].

The paper presents examples of the peroxide scavenging activity of various beverages and food preparations [8]. Our hypothesis is that the higher the scavenging activity, the larger the heat of reaction.

MATERIALS AND METHODS

Materials

Ascorbic acid (AA) was of analytical grade (Sigma Aldrich). Stock solutions (5, 10, 25, 50, 100, 200, 500 and 1000 mM) were prepared by dissolving the desired amount in distilled water (18 M Ω). For the IC investigations 1 mL of each solution was mixed with 1 mL of H₂O₂ (30% \approx 8.8 M) and added to 100 mL of an aqueous buffer. Therefore the starting concentrations of AA were 100 times smaller. The Britton Robinson buffers in the 2-7 pH range of were prepared from a

1:1:1 mixture of 0.04 M acetic acid, 0.04 M boric acid and 0.04 M phosphoric acid. The pH value was adjusted with a 0.2 M NaOH solution. Reagents were purchased from Sigma Aldrich.

Fruit juice samples (apricot, apple, and grapefruit), wine (Chianti 2010, Lagrein 2011 and 2012), baby fruit puree (apple, banana and plum), tea (green and white), and coffee (with and without caffeine) were purchased from local market. Tea and coffee beverages were prepared by soaking the powder sample (contained in a paper bag, net content, 1 g) in 50 mL of boiling water for 5 min. All the other samples were used as received. 1 mL or 1 g of food sample was diluted (dispersed) either in 100 mL of distilled water or in 100 mL of Britton-Robinson buffer at pH 6.5. The AA solutions were studied in buffered conditions, at various pH.

Reaction calorimetry apparatus (Figure 1).

Originally developed by ChemiSense® [6], the reactor calorimeter CPA 201 (ChemiSens AB, Lund, Sweden) suppresses uncontrolled temperature differences between the content of the reactor and its surrounding jacket. This means that all the heat from the reactor to the thermostat bath flows through the heat transducer, which is located between the bottom of the reactor and a Peltier element used as a heat pump CPA202 performs rather well with either solid or liquid samples, without need of calibration, and without requiring any sample pre-treatment [6].

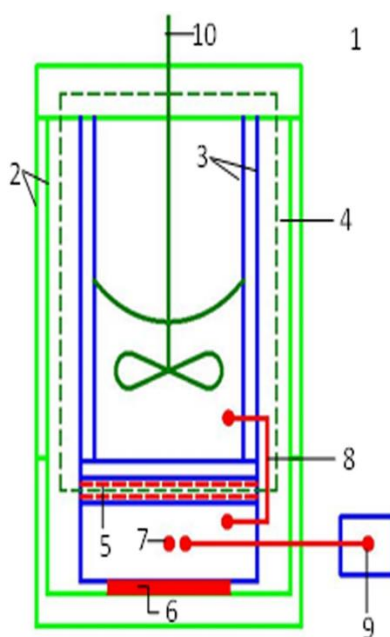


Figure 1. Scheme of the Reaction Calorimeter. 1. Reference Thermostat, 2. Insulated jacket, 3. Reaction vessel, 4. Gap between reaction vessel and insulated jacket, 5. Heat flow transducer, 6. Peltier element, 7. Temperature sensor (Heat sink), 8. Temperature sensor (Reactor-heat sink), 9. Temperature sensor (Reference-Heat sink), and 10. Stirrer.

The cell (250 mL) is a double-walled pyrex glass, tightly closed by a lid and a Hastelloy base. A water-bath thermostat surrounding the cell insures a constant temperature. The reactor is equipped with a stirrer, a torque transducer and a true-heat flow sensor. The heat-flow signal is the on-line output of the instrument transduced with the help of ChemiCall v2 ProFind® software. The instrument does not need calibration throughout the experiment. The highly stable baseline returns to ± 0.0001 W level at the end of the reaction. The generation of an electrical power (from 0.007 to 0.04 W, provided by an internal electrical power heater) allows, periodically, the check of the performance of the instrument. The accuracy

always is higher than 99%. The time constant is around 20 s (according to the producer), although a little larger value (about 35 s) affects the data of the present work.

Optimized procedure

Immersion of the CPA reactor into a water-bath thermostat set at 25°C allows achievement of isothermal conditions. Prior of the analysis, the two syringes hosting the sample and the hydrogen peroxide, respectively, attain the desired temperature after immersion in the same thermostat. Before each measurement, the reactor cell, filled with 0.1 L of a buffer solution (Britton-Robinson, pH = 6.5), de-aerated and tightly closed with a lid, is immersed in the thermostat. The stirrer rate is set at 200 rpm. Once attained the thermal equilibrium (reflected by a stable baseline: background signal ≤ 0.1 mW), 1 mL of sample is injected in the reactor cell. After stabilizing rest (~30 min), 1 mL of H₂O₂ (30%) is added into the reactor cell and the resulting thermal power signal (W) monitored. The integral of the HF- vs- time record yields the heat of the reaction (J).

RESULTS

Solutions of Ascorbic Acid

The RC records collected from AA buffered (pH = 6.5) solutions are reported in Figure 2. The overall thermal effect is exothermic (positive sign throughout the text and figures).

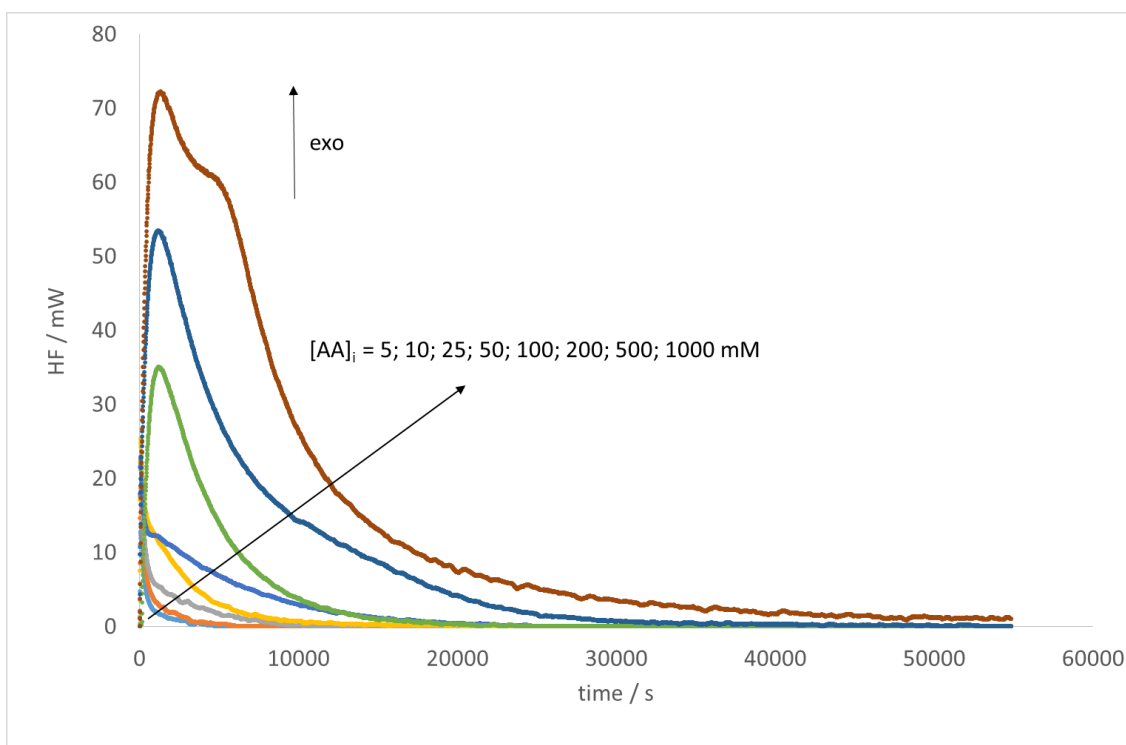


Figure 2. Isothermal RC records from buffered solutions of AA. The HF values refer to 1 mL of the original solution (lettering indicates the relevant concentration) that underwent a 100 fold dilution in the calorimetric cell.

Figure 2 suggests that the overall thermal effect (areas beneath the signals) may be proportional to the starting content of AA, no matter the amount of H₂O₂ available. Integration of the signals confirms this impression (Figure 3).

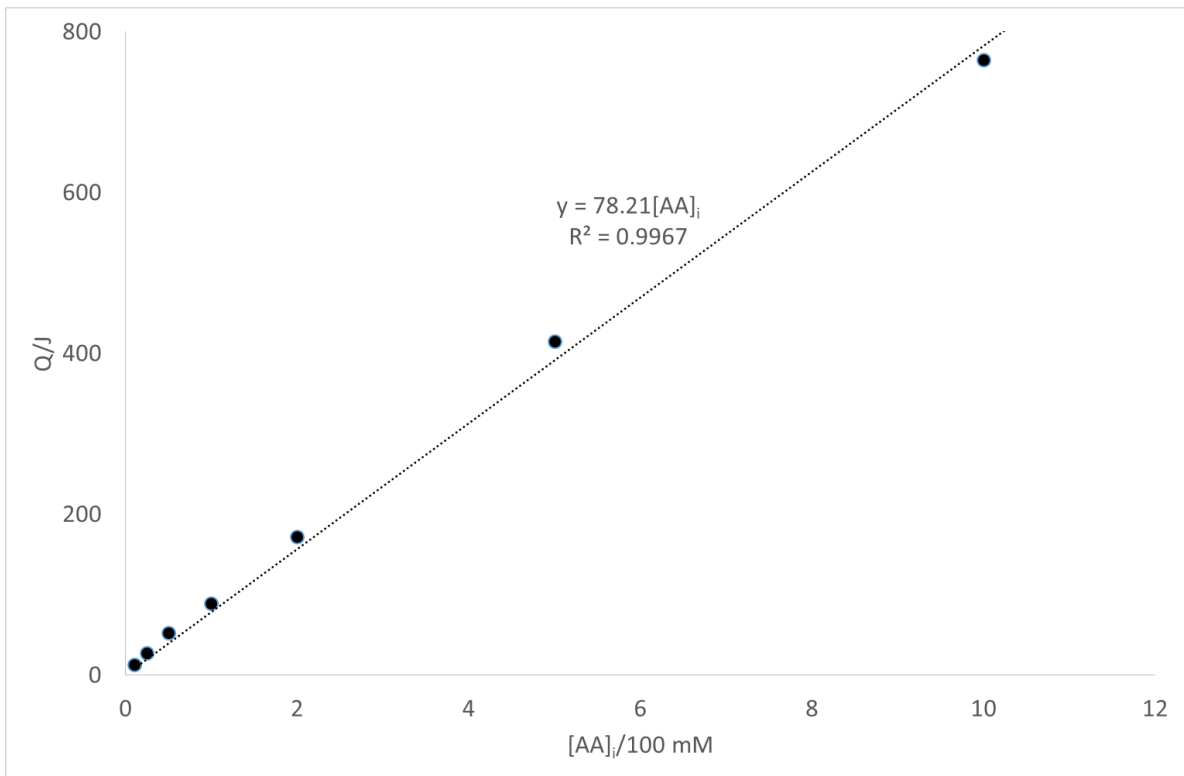


Figure 3. Integrated IC signals show a straight-line increasing trend with a slope that corresponds to 782.1 kJ per mole of ascorbic acid.

When the starting concentration is fixed, $[AA]_i = 50 \text{ mM}$, and the pH of the solution is varied (2, 4, 5, 6.5, 7), the overall thermal effect increases with increasing pH (Figure 4).

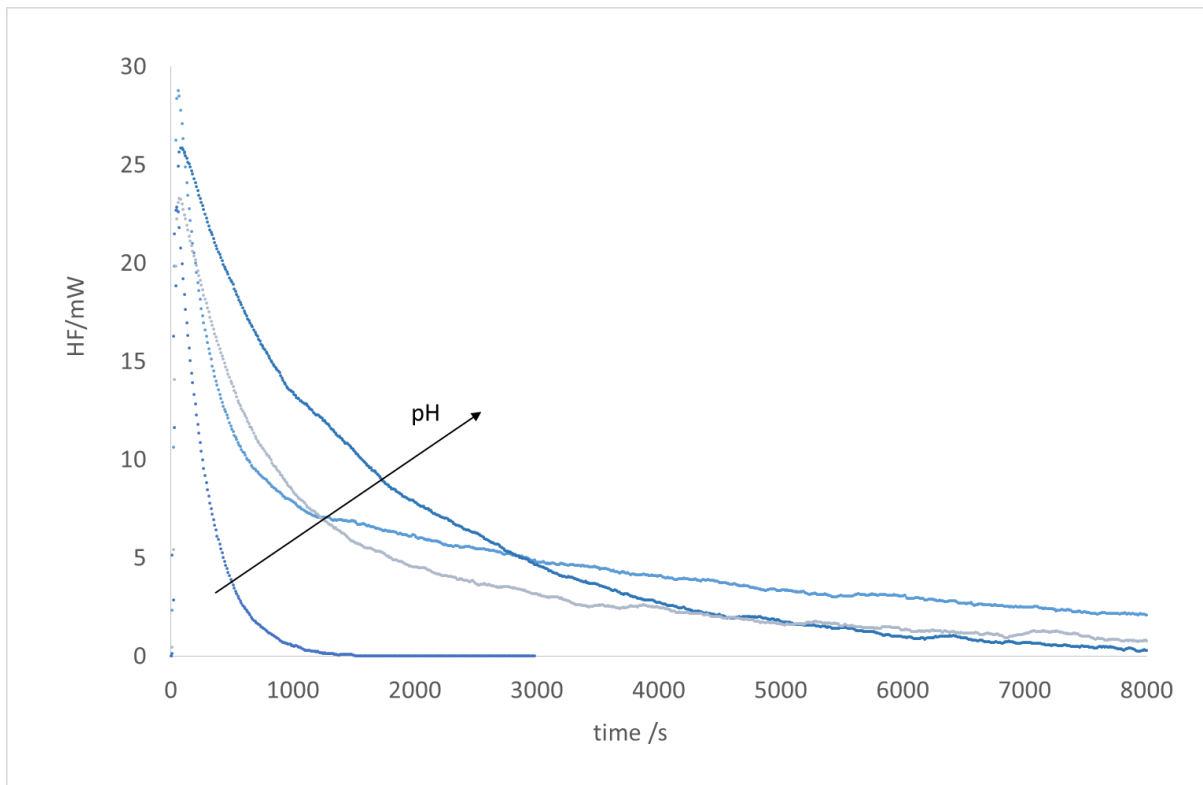


Figure 4. Isothermal RC records from 50 mM solutions of AA (0.5 mM after dilution in Britton-Robinson buffer at different pH (2, 4, 5, 7).

Scavenging Properties of Some Food Products.

Many food products that contain antioxidant compounds behave like AA when treated with H_2O_2 . Some products, like various kinds of wine, tea, coffee, fruit juices and purée, allow an IC investigation and a direct comparison with the AA. The relevant IC records are indeed similar to those collected for AA, namely, a sharp single peak that ends in less than one hour (Figure 5).

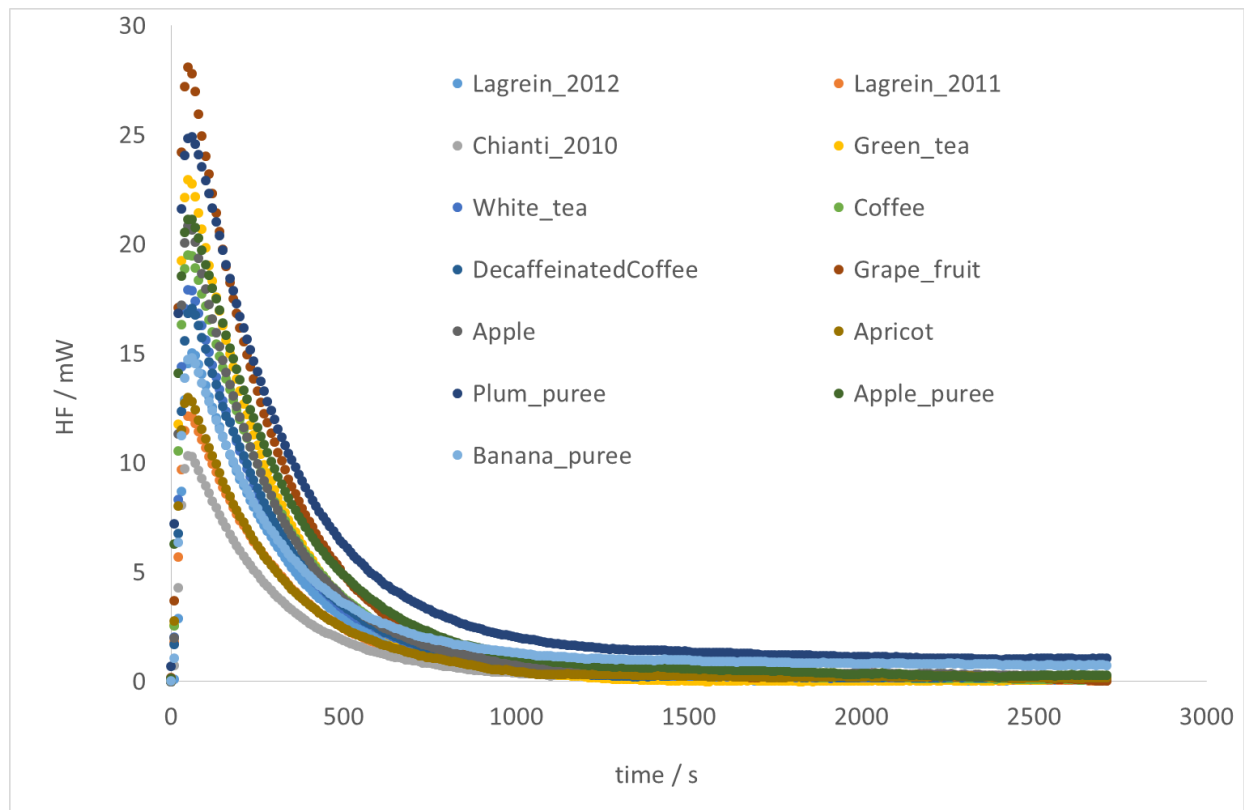


Figure 5. Isothermal calorimetric records from various food samples.

All baby foods, normally fortified with ascorbic acid, show the highest thermal powers. The overall thermal effects calculated by integration of the IC records increases with the order reported in the following histogram (Figure 6).

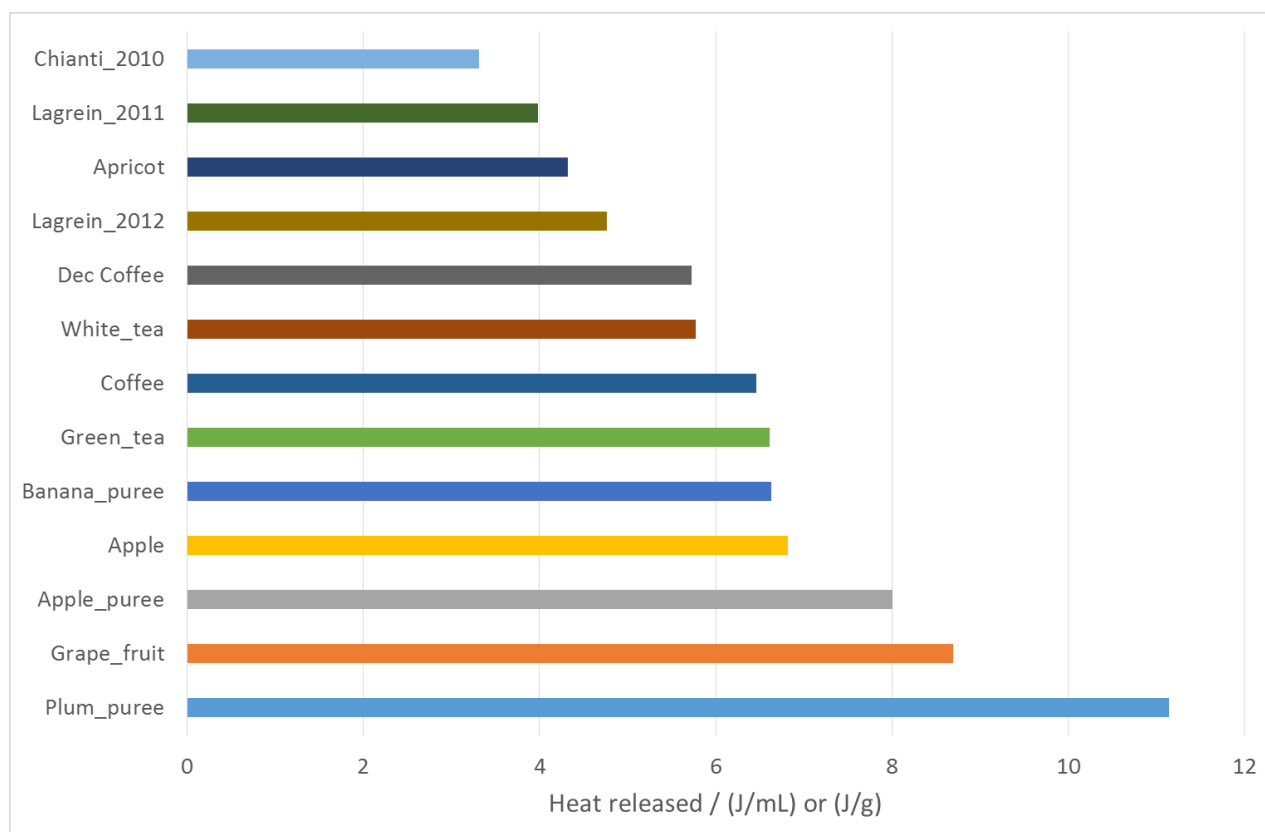


Figure 6. Histogram of the overall thermal effects evaluated by integration of the IC records collected from various food diluting (dispersing) 1 mL or 1 g in 100 mL of distilled water, (heat units: J/ mL and J/g, respectively).

DISCUSSION

Figure 3 reflects a purely phenomenological evidence that can be somewhat misleading, as the same data, once scaled with respect to $[AA]_i$ (Figure 7), show that the overall thermal effect decreases with increasing $[AA]_i$. This result suggests that the overall heat exchanged per mole of AA is the result of a balance between endo- and exothermic effects and that the underlying mechanism of the oxidation may change with the AA/H₂O₂ molar ratio.

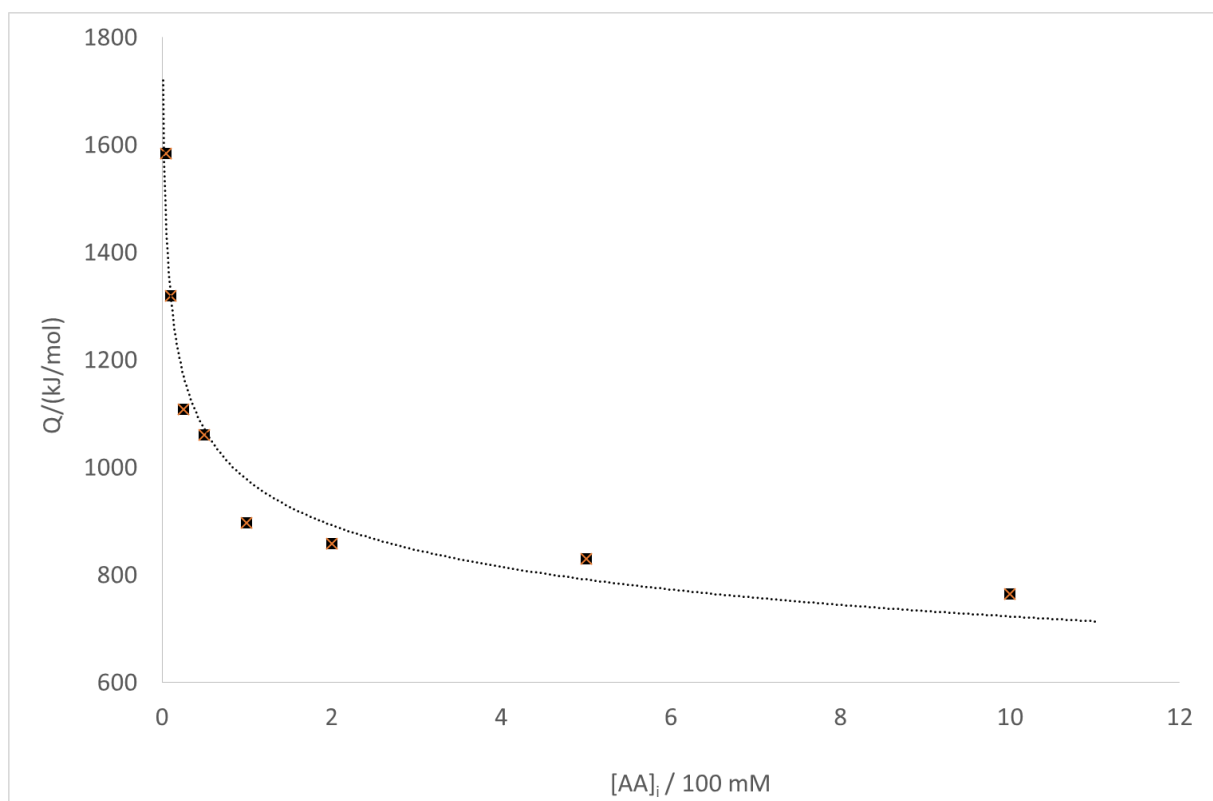
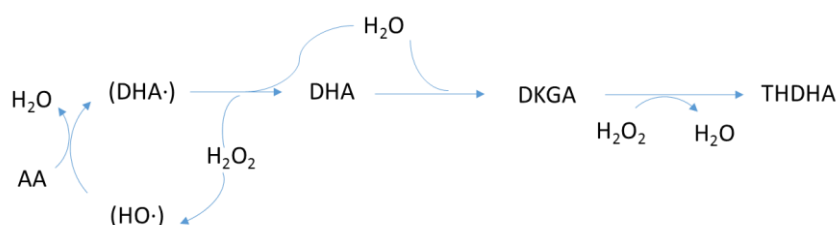


Figure 7. The integrated HF data (Figure 2. $[AA]_i$ is the starting concentration of AA (it underwent a 100 fold dilution in the calorimetric cell).

Looking at the literature [9, 10], a likely kinetic model for the whole process would include free radicals. More specifically, J.C. Deutsch [9] reported that AA and dehydro-ascorbic acid, DHA, undergo oxidation along the same reaction pathway and that the early oxidation of AA occurs via the formation of an intermediate radical species, namely, the semi-dehydro-ascorbic radical, $(DHA\cdot)$. This radical would undergo a further oxidation step to form DHA that, after hydrolysis to di-ketogulonic acid, DKGA, would also react with H_2O_2 to give some final oxidation products, like THDHA (4,5,5,6 – tetra hydroxyl – 2,3 – di – keto - hexanoic acid) and threonic acid.

This interpretation is in line with the expectation that many, if not all, oxidation processes sustained by hydrogen peroxide take place thanks to the early formation of free radicals, like $(HO\cdot)$, which trigger further steps of the process.

The suggestions of these authors may correspond to the following scheme:



Scheme 1. Oxidation of Ascorbic Acid (AA) with Hydrogen Peroxide. Loop of reactions implying formation of hydroxyl $(HO\cdot)$ and semi-Dehydro-Ascorbic $(DHA\cdot)$ radicals that generates Dehydro-Ascorbic Acid (DHA) that finally yields 4,5,5,6 – tetra hydroxyl – 2,3 – di – keto - hexanoic acid (THDHA) through the oxidation of Di-Ketogulonic Acid, DKGA.

An isothermal calorimetric investigation would detect the overall heat exchanged that, in the present case, is the sum of a number of exo- and endo-thermic effects difficult to single out. Assuming that the major effects come from the steps that involve H_2O_2 , a reasonable simplification could be:



Scheme 2. Simplified model to account for the overall thermal effect observed. Lettering is the same as for Scheme 1.

namely, a couple of consecutive steps with apparent kinetic constant k_1 and k_2 , where DKGA, the product of the hydrolysis of DHA, is involved in the second oxidation. In the presence of an excess H_2O_2 , (namely for large $[\text{H}_2\text{O}_2]/[\text{AA}]$ ratios) a pseudo-first order kinetics may likely describe either step. This kinetic model implies the following equations for the molar fractions of the compounds involved:

$$\alpha_{AA} = 1 - e^{-k_1 t} \quad \text{with} \quad \dot{\alpha}_{AA} = k_1 e^{-k_1 t} \quad (1)$$

$$\alpha_{DKGAA} = \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad \text{with} \quad \dot{\alpha}_{DKGAA} = \frac{k_1}{k_2 - k_1} (k_2 e^{-k_2 t} - k_1 e^{-k_1 t}) \quad (2)$$

$$\alpha_{THDHA} = 1 - (\alpha_{AA} + 1 - \alpha_{DKGAA}) \quad \text{with} \quad \dot{\alpha}_{THDHA} = \frac{k_1 k_2}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad (3)$$

where α and t stand for the molar fraction and time, respectively, and the upper dot stands for time derivative.

In line with the simple principle that the calorimetric signal directly reflects the underlying kinetics [11, 12], the relevant heat power is:

$$\begin{aligned}
 \dot{Q} &= \dot{Q}_1 + \dot{Q}_2 = \dot{\alpha}_{AA} \Delta H_1 + \dot{\alpha}_{THDHA} \Delta H_2 = [k_1 e^{-k_1 t}] \Delta H_1 + \left[\frac{k_1 k_2}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \right] \Delta H_2 = \\
 &= \left[k_1 \Delta H_1 + \frac{k_1 k_2}{k_2 - k_1} \Delta H_2 \right] e^{-k_1 t} - \left[\frac{k_1 k_2}{k_2 - k_1} \Delta H_2 \right] e^{-k_2 t} = a e^{-c t} - b e^{-d t}
 \end{aligned} \quad (4)$$

A couple of exponentials should therefore satisfactorily fit the calorimetric trace, provided that the earliest part of the record (about 50 s) is neglected, as it reflects the lag time of the calorimeter [12]. Integration of such expression between the limits $(0, \infty)$ leads to

$$Q = (\Delta H_1 + \Delta H_2) \quad (5)$$

The quantities in equation (5) are in Joule units; when multiplied by $V \times [\text{AA}]_i$ (where $V = 1$ mL is the sample volume), they should correspond to the experimental evidence (see Figure 3).

The four fitting parameters, a , b , c and d , allow evaluation of the kinetic constants and the enthalpies:

$$(a-b)/c = \Delta H_1; b(d-c)/dc = \Delta H_2; c = k_1 \text{ and } d = k_2.$$

Table 1 reports the values of the relevant enthalpies and kinetic constants and Figure (8) shows the fits obtained.

[AA] _i /mM	[AA] _i /[H ₂ O ₂] _i	$k_1/10^{-3}s^{-1}$	$k_2/10^{-4}s^{-1}$	$\Delta H_1/kJ\ mol^{-1}$	sd	$\Delta H_2/kJ\ mol^{-1}$	sd
5	1712	4.35	5.98	790.91	57.1	-982.58	70.9
10	856	4.37	5.61	539.82	34.8	-868.52	56.0
25	342	4.81	3.04	247.23	13.7	-949.91	52.8
50	171	7.99	3.36	82.90	4.11	-976.19	48.4
100	86	10.20	1.58	31.05	1.38	-914.10	40.5
200	43	2.04	2.76	-31.37	1.24	-880.37	34.8
500	17	3.03	1.49	1.29	0.04	-803.42	27.4
1000	9	1.17	1.36	14.43	0.44	-707.53	21.5

Table 1. Kinetic constants and reaction enthalpies from the fits of the experimental data according to equation (4). The calculated value of ΔH_1 and ΔH_2 comes from equation (4) taking into account that the volume of the solution is 0.1 L. AA concentrations are those in the calorimetric cell. Standard deviation (sd) of enthalpy drops in the neighbor side columns.

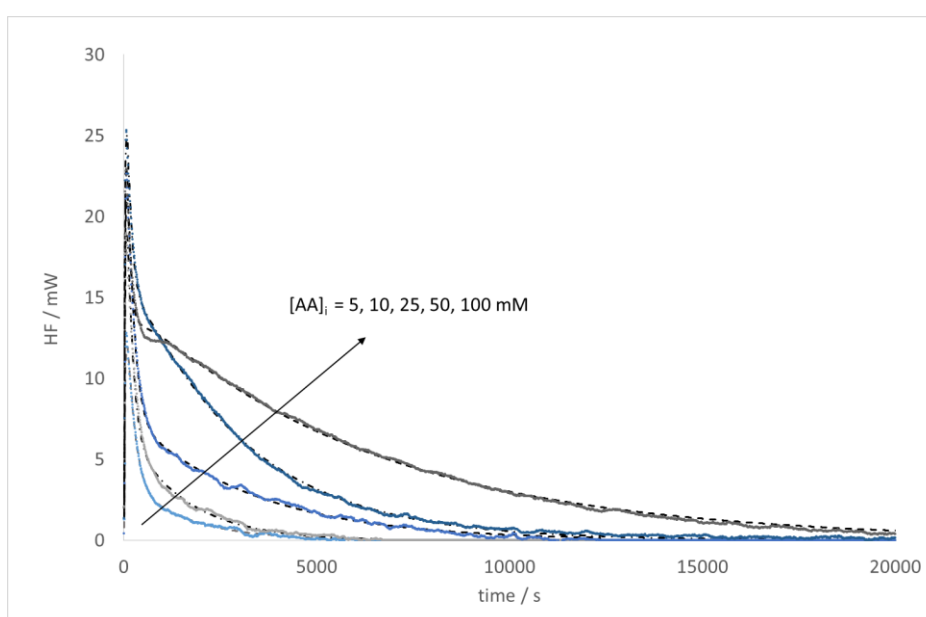


Figure 8. Isothermal calorimetric records from buffered ($pH = 6.5$) aqueous AA (0.05, 0.1, 0.25, 0.50 and 1.00 mM in the calorimetric cell) in the presence of excess H_2O_2 . Dashed lines are drawn according to equation (4). For larger $[AA]_i$ the fit is still satisfactory ($R^2 > 0.98$), but can be improved adding a further step to the kinetic model assumed (see below).

In spite of the satisfactory appearance of the fits, the calculated values of the kinetic constants and the enthalpy changes with $[AA]_i$ (see Table 1) and do not lead to an unequivocal interpretation. Would the model reflect the true mechanism of the process, the values of the kinetic constants and reaction enthalpy should not change with $[AA]_i$. While the values of k_1 and k_2 coming from the best fitting treatment may allow a likely averaging (at least for $[AA]_i < 100$ mM), namely, $6.4 \cdot 10^{-3} s^{-1}$ and $3.9 \cdot 10^{-4} s^{-1}$, respectively, this is not the case of ΔH_1 and ΔH_2 . The consistency of the model seems rather poor. However, one has to take into account the oversimplification assumed: in particular, the first step of the model actually includes several concomitant processes related to the reactions of the free radicals, some of which, like the cleavage of H_2O_2 , imply endothermic effects that partially counterbalance the exothermic ones.

The ΔH_1 values in Table 1 actually tend to vanish with increasing $[AA]_i$. This trend suggests that endo- and exothermic effects related to the first step tend to achieve a zero balance for $[AA]_i > 100$ mM, namely, for $([H_2O_2]_i / [AA]_i < 86.5$ ($[H_2O_2]_i = 8.8$ M). The overall exothermic effect determined with IC therefore tends to coincide with the reaction enthalpy of the second step.

This could be the reason why other authors [10], based on calorimetric evidences collected in the presence of a poorer oxidant (either O_2 or H_2O_2) concentration, were inclined to describe the oxidation of AA as a pseudo first order process, namely with a single main thermal effect.

As for the ΔH_2 , a reasonable average value may be -929 kJ mol^{-1} for $[AA]_i < 500 \text{ mM}$. At larger AA the IC signal shows an extra shoulder (mainly at $[AA]_i = 1000 \text{ mM}$) which suggests the occurrence of a further thermal effect to account for. Figure 9 shows the split of the overall recorded heat flux that reflects the two consecutive steps of the assumed kinetic model. Figure 10 reports the corresponding trend of the molar fractions of the species involved. The intermediate species DKGA goes through a maximum, its formation from AA being rather fast with respect to its oxidation to THDHA. This means that, in the presence of excess H_2O_2 , the observed thermal effect would mainly deal with the oxidation of DKGA.

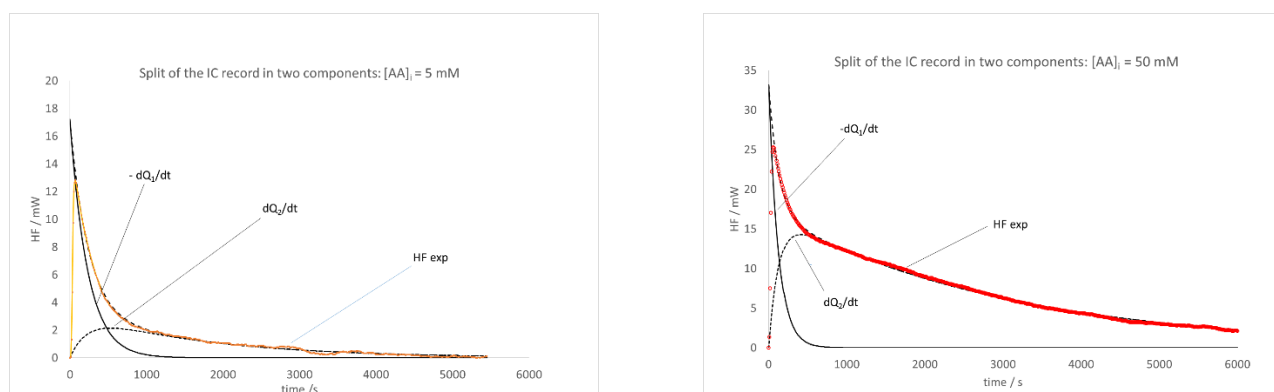


Figure 9. Split of the recorded HF data (left, 0.0 5 mM; right, 0.50 mM in the calorimetric cell) in two contributions related to the consecutive steps of the assumed kinetic model. The first contribution is endothermic, while the second is exothermic.

The signal collected for $[AA]_i \geq 50 \text{ mM}$ shows a shoulder that looks like a double peak for $[AA]_i = 1000 \text{ mM}$. The fitting model used for lower AA concentration cannot reproduce this kind of trend that suggests the occurrence of a third thermal effect. Such an addition implies a kinetic model with three consecutive steps, the third of which has a rate that can be predicted accurately [13]. For the present scope, namely, just the fit of an experimental record, it is enough to say that the corresponding trend is close to that of a broad Gaussian peak.

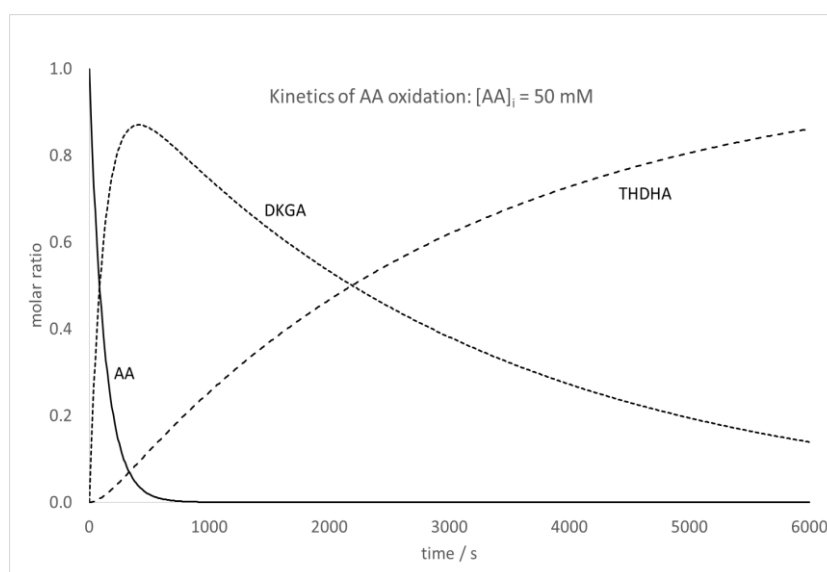


Figure 10. Trend of the molar fraction of the compounds involved in the scavenging process according to a two-consecutive-step model (equation 4).

If one adds such a function to the expression of equation (4) and assumes a naught neat enthalpy for the first step (see above), the obtained fits are very satisfactory (Figure 11), although the adjustment accounts only for about 2% of the overall thermal effect.

The reason for such extra step may be related to oxygen-accepting antioxidants, like threonic and oxalic acids, that arise during ascorbate oxidation [14, 15].

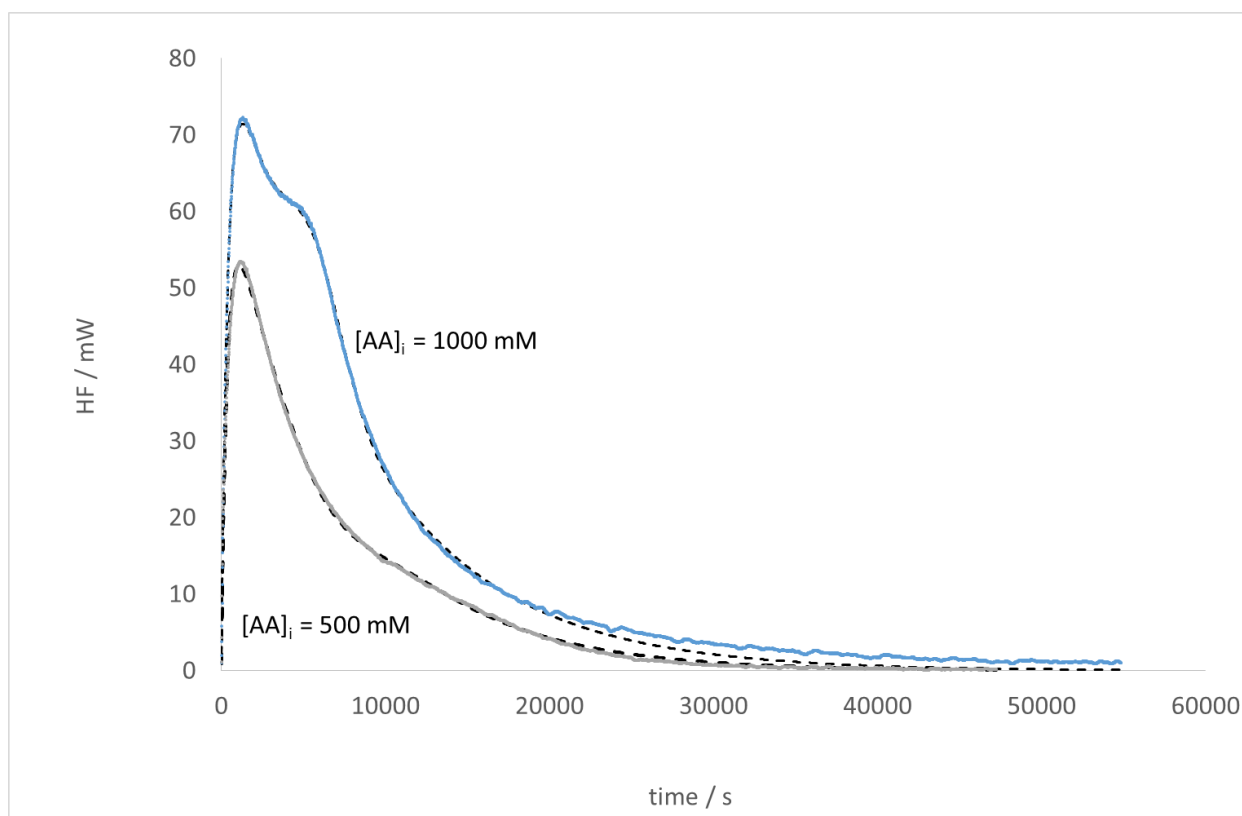


Figure 11. Fit of the IC records collected from $[AA]_i = 500$ and 1000 mM (100 fold diluted in the calorimetric cell) buffered solutions according to a modified kinetic model (see text).

When $[AA]_i$ is fixed (e.g. 50 mM) and the pH is changed, the IC record shows a sharp and small-area peak at pH = 2, while the signal becomes broader and larger at higher pH (Figure 12). Taking into account the equilibrium of dissociation of AA ($pK_a = 4.17$) and that $([AA^-] + [AA]) = [AA]_0$, one can easily obtain

$$[AA^-] = [AA]_0 \frac{K_a}{K_a + 10^{-pH}} \quad (7)$$

$[AA^-]$ is therefore supposed to increase with increasing pH, which suggests that the truly reactive species may be the ascorbate anion, the signal from low-pH solutions being small since the ascorbate is a small fraction of $[AA]_0$. One simply has to multiply α and (dQ/dt) by $[AA^-]$ in equations (2 - 4) to apply the model of two consecutive steps described above and fit the IC records (Figure 12), while Figure 13 shows the trend of the overall oxidation heat, Q .

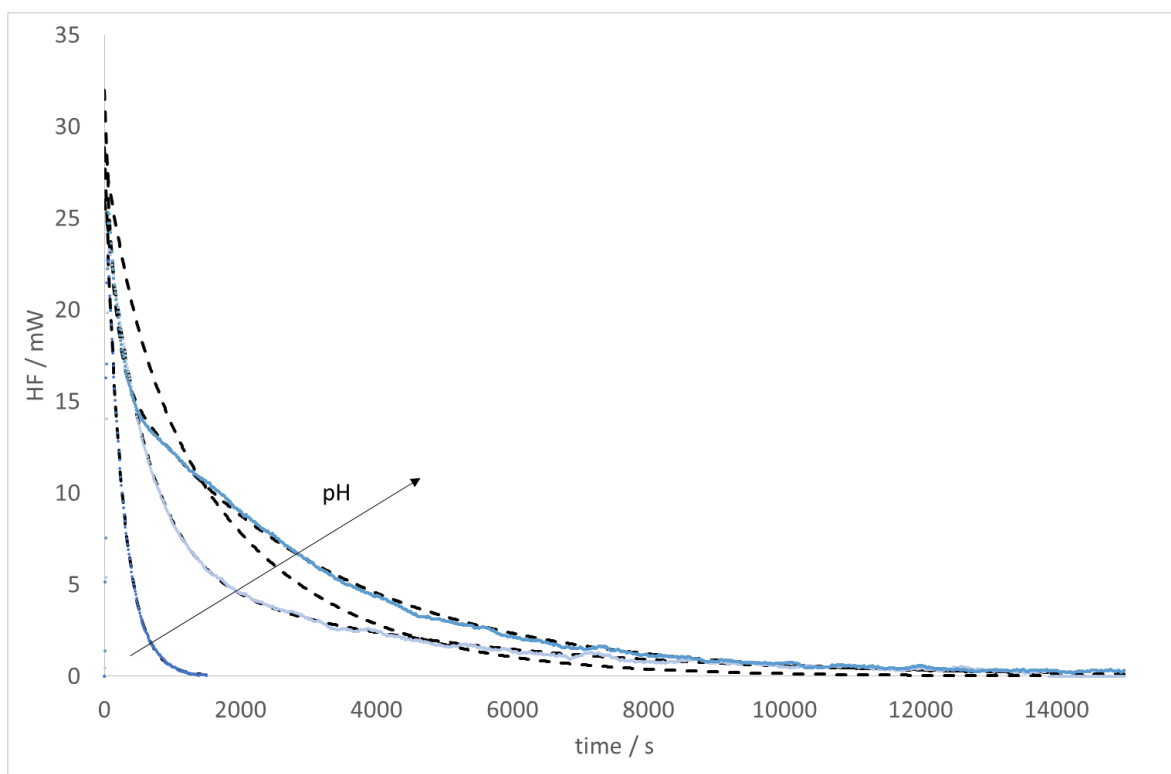


Figure 12. Fits (dashed lines) of the IC traces of aqueous $[AA]_i = 50 \text{ mM}$ solutions (100 fold diluted in the calorimetric cell) at different pH (2, 4, 5, 7).

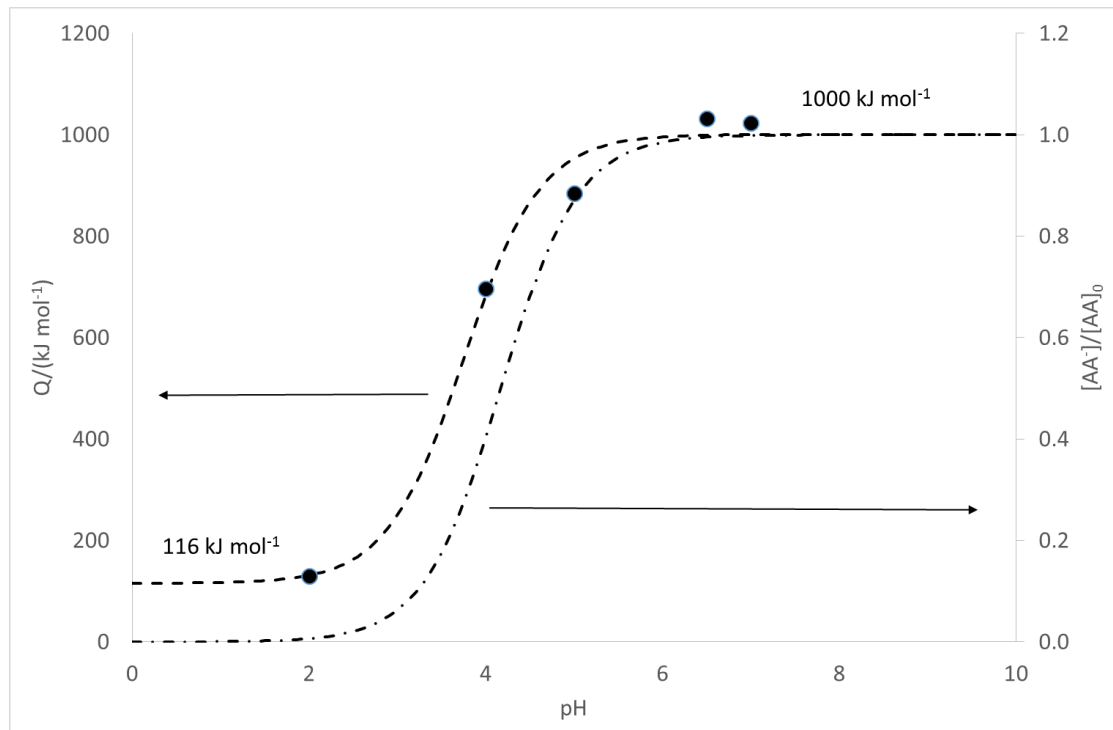


Figure 13. Overall heat of oxidation of AA in the presence of excess H_2O_2 , at various pH.

The sigmoid trend of Q indeed parallels that of the $[AA^-]/[AA]_0$ molar ratio and implies a low-pH and a high-pH steady levels, at about 100 and 1000 kJ per mole of AA, respectively. At pH = 7, the anion AA^- practically accounts for 100% of the ascorbic species. The corresponding oxidation heat accordingly is the largest one for $[AA]_i = 50 \text{ mM}$. At pH = 2,

the anion $[AA^-]$ is 1% of $[AA]$; and the corresponding Q value reflects the lowest scavenging effect for $[AA]_i = 50$ mM. This finding seems in line with the evidence of low pH increase oxidative deterioration of fish oil in the presence of ascorbate [16].

The fit of the experimental IC records with a two consecutive process model allows evaluation of the relevant parameters. k_1 remains around $3.5 \pm 1 \times 10^{-3} \text{ s}^{-1}$ with a mean endothermic effect of -155 kJ mol^{-1} , at every pH considered, while k_2 and the related enthalpy drop depend on pH. Figure 14 shows the overall picture that reflects these evidences.

The broader signal observed at larger pH is therefore related to a smaller rate of the second step, that however implies a larger thermal effect.

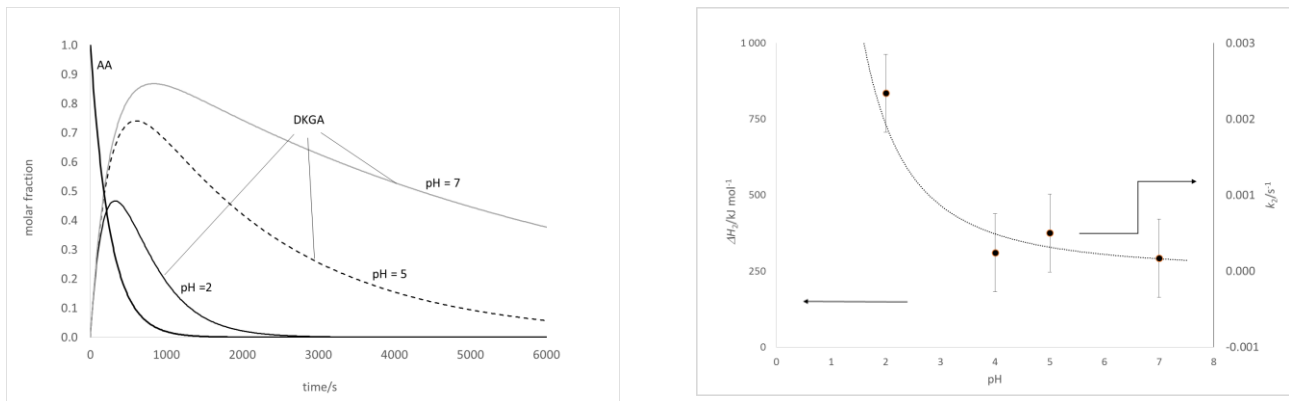


Figure 14. Trends of the molar ratios of AA and DKGA at various pH. Kinetic constant and enthalpy drop of the second step of the two consecutive step model.

As for the food products considered in this work, no simple kinetic model seems appropriate to describe their scavenging activity, inasmuch as each food contains different antioxidant components [17 - 20] and, above all, metal ions that can catalyze the oxidation [10, 21]. Most of them show a scavenging effect that follows a first order law, which is a purely empirical evidence with no physical meaning. Table 2 reports the kinetic constants of the scavenging process and the concentration of ascorbic acid at pH 6.5 that would release the same heat per mL.

It is worth mentioning that the pH conditions experienced by any food through the gastro-intestinal tract change passing from the mouth to the great intestine. Our results from buffered AA solutions suggest that the respective scavenging potential would be smaller in the acidic tracts (because of the slow reaction rate), and much larger in the basic tract of the gut where most of the adsorption takes place.

This seems indeed the case also for the antioxidants present in some food samples. When the same amounts of food are diluted (dispersed) in 100 mL of buffer at pH = 6.5, a significant shift toward higher scavenging activity occurs for wines and grapefruit juice, while other remain almost unchanged or even a little reduced (Table 3).

Food	$k_1/10^{-3} \text{ s}^{-1}$	Q/(J/mL, or J/g)	[AA] / mM equivalent
Chianti_2010	3.79	2.80	4.23
Lagrein_2011	3.59	3.52	5.10
Apricot	3.67	3.59	5.53
Lagrein_2012	3.74	4.16	6.09
Decaff Coffee	3.60	4.83	7.32
White_tea	3.74	4.95	7.38
Coffee	3.62	5.62	8.26
Apple	3.77	5.66	8.45
Green_tea	4.09	5.91	8.48
Banana_puree	3.80	6.07	8.72
Apple_puree	3.26	6.81	10.23
Grape_fruit	3.88	7.46	11.12
Plum_puree	3.53	10.06	14.24

Table 2. Apparent kinetic constant (first order) and heat released per mL or per g of food. Column 4 reports the concentration (mM) of ascorbic acid that would release the same heat per mL at pH 6.5 (after dilution in the buffer).

CONCLUSIONS

Although many innovations have been so far proposed to assess the scavenging properties of natural antioxidants present in many food [22, 23], the use of reaction calorimetry performed with a suitable instruments offers a further possibility, based on the determination of the heat released (exothermic effect) by systems that contain ascorbic acid or other natural antioxidants. A tentative kinetic model, including two or three consecutive first-order steps, allows a satisfactory fit of the experimental IC records. However, the values of the relevant kinetic constants and reaction enthalpies actually are the result of the combination of intermediate steps involving free radicals that imply a balance of endo- and exothermic effects that can depend on the H_2O_2 /antioxidant molar ratio. The pH conditions have a substantial effect on the scavenging power of AA solutions: this increases with increasing pH and attains its maximum for pH = 6.5, i.e., when ascorbate anions represent almost 100% of the ascorbic species.

Assuming that the overall heat released reflects the scavenging capability of the system considered, one may define the antioxidant potentiality of several food products with reference to that of a buffered (pH = 6.5) AA solution that releases the same amount of heat. The scavenging capability of the food products considered seems enhanced (with few exceptions) when buffered at pH 6.5, namely, above the original value. Since the pH conditions experienced by any food change along the gastro-intestinal tract, the respective scavenging potential would be larger in the basic tract of the gut.

Food	Q /(JmL ⁻¹ or g ⁻¹) diluted in water	Q /(JmL ⁻¹ or g ⁻¹) at pH 6.5
Plum_puree	11.14	10.35
Grapefruit juice	8.69	10.51
Apple_puree	8.00	9.68
Apple juice	6.82	9.92
Banana_puree	6.63	6.83
Green_tea	6.61	7.92
Coffee	6.46	5.53
White_tea	5.78	6.81
Dec Coffee	5.72	5.40
Lagrein_2012	4.76	9.05
Apricot juice	4.32	5.66
Lagrein_2011	3.98	8.92
Chianti_2010	3.31	6.43

Table 3. Heat released by food samples diluted in distilled water or in buffer at pH 6.5.

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