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Relationship between redox potential and chain-breaking activity of model systems and foods

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Abstract

The relationships between the redox potential and the chain-breaking activity of a glucose-glycine model system as well as of natural or heat-induced antioxidant-containing foods, when they undergo changes of their oxidative status, were studied. Results showed that redox potential may represent an interesting indicator of the antioxidant efficiency of food products when coupled with the determination of the chain-breaking activity. In fact, while chain-breaking activity measurements provide information on the capacity of antioxidant compounds to quench radicals, redox potential determinations give indications of the overall reducing properties of all the food antioxidants, including those which react slowly with radical species. The latter, in fact, could represent an antioxidant reservoir whose action is deferred during storage.

Keywords: Chain-breaking activity; Redox potential; Food antioxidants

1. Introduction

Following the disclosure of the relationships between oxidative stress and human diseases, in recent years increasing attention has been focussed upon those foods, food components, dietary supplements and drugs that potentially exert antioxidant capacity (Various Authors, 2001). Besides their well documented technological role in extending the shelf-life of foods by slowing down undesirable oxidation reactions, antioxidants are now proposed as important dietary factors. Hence, as a consequence of the emerging need to estimate the functional profile of foods in relation to their contents of natural antioxidants, a huge number of methodologies have been developed for the assessment of antioxidant capacity. The majority of them are kinetic methods, based on the measurement of the quenching ability of antioxidants toward a reference radical (Frankel & Meyer, 2000; Kaur & Kapoor, 2001), while the measurement of a thermodynamic quantity, such as the redox potential, which gives information on the ability of a redox couple to be oxidized or reduced, has been scarcely considered (Buettner, 1993; Dikanovic-Lucan & Palic, 1995). In fact, although the redox potential is generally recognized to be an important hurdle for extending the shelf-life of foods, it is mainly used in the microbiological area, for instance, to follow the evolution of fermentation processes or to estimate whether aerobic or anaerobic micro-organisms will grow in a given foodstuff (Bogh-Sorensen, 1994; Leistner & Gorris, 1995).

As is well known, any compound able to donate a hydrogen atom, or to promote an electron transfer to a radical, can be considered an antioxidant. In general terms, the antioxidant activity may be displayed through two pathways. The first can be regarded as the donation of a hydrogen atom by the antioxidant molecule (Reaction 1) to afford a secondary radical, which may be responsible for the propagation of a radical reaction

$$R\text{–}H + OH^{\cdot} \rightarrow H_2O + R^{\cdot} \qquad \text{(Reaction 1)}$$

The second pathway involves electron transfer from a reducing substrate to the oxidizing radical species (Reaction 2 and Reaction 3).

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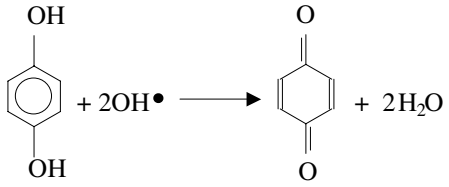
$$Fe^{2+} + OH^{\cdot} \rightarrow Fe^{3+} + OH^{-} \quad \text{(Reaction 2)}$$



(Reaction 3)

In other words, redox potential measurements are suitable to evaluate the ability of reducing compounds to promote electron transfer. On the other hand, the mentioned kinetic methods allow us to measure the chain-breaking activity of both electron-transferring and hydrogen-donating antioxidants. It must be pointed out, however, that the latter approaches can estimate the antioxidant efficiency only of those compounds which can react with the reference radical at sufficiently high reaction rates; i.e., kinetic measurements allow the rate of the radical decay to be determined, but do not give information about the mechanisms involved. Such information, that could be of importance from a biological point of view, does not take into account the presence of other less efficient antioxidants, which may greatly contribute to the functional properties as well as to the shelf-life of foods. In addition, as already pointed out, after the primary reaction with the radical species, the resulting antioxidant radical can promote the development of further radical chain reactions. For this reason, the assessment of the antioxidant activity by means of kinetic tests, may give results which are in general due to contributions of both antioxidant and pro-oxidant activities.

A redox potential value gives information about the real oxidation/reduction ability of a molecule and its prevalent form (oxidized or reduced) in the system. Because of its thermodynamic nature, a redox potential value does not give any information about the rate of the reaction which may occur. Nevertheless, in a system where at least two redox couples are present, its measurement may be useful to predict whether, and in which direction, an oxidation/reduction reaction will take place (Buettner, 1993; Shwartz, 1996). Thus, for simplicity and reliability, the measurement of the redox potential is an interesting way of estimating the antioxidant capacity of a product. Until now, the possibility of extending the use of the redox potential to the assessment of the antioxidant properties of food matrices has been hampered by several limitations, some of which are summarized as follows:

(a) It does not give information about the ability of molecules to donate hydrogen atoms.
(b) The standard potential of the redox couples present in food matrices is generally difficult to identify.
(c) Technological operations may modify the redox potential of processed foods by causing changes in pH or ratio of the molar concentrations of the oxidized and reduced forms, and also by leading to the formation of novel redox couples. This is the case for heat-induced antioxidants, namely Maillard reaction products (MRPs), as well as for oxidized polyphenols (Hayase, Hirashima, Okamoto, & Kato, 1989; Namiki, 1990; Nicoli, Calligaris, & Manzocco, 2000).

Despite these limitations, the redox potential could be a suitable tool which, once coupled with the kinetic methods, can give additional information about the antioxidant status of food products.

On the basis of these considerations the aim of this study was to investigate the relationships between the redox potential and the chain-breaking activities of some foods and model systems when they undergo changes of their oxidative status. In particular, foods and model systems, selected on the basis of their composition in natural antioxidants and/or their technological history, were considered. In particular, tea extracts and tomato purees were chosen for their high contents of naturally occurring antioxidants. A glucose-glycine model system was considered for its susceptibility to develop MRPs when heated. A coffee beverage was chosen for its high content of MRPs with antioxidant capacity (Nicoli, Anese, Manzocco, & Lerici, 1997). The samples were subjected to processing conditions responsible for changes in the oxidative status due to oxidation of the natural antioxidants and/or formation of heat-induced antioxidants (i.e., MRPs), and analyzed for the chain-breaking activity and the redox potential.

2. Materials and methods

2.1. Model system

An aqueous solution (0.1 M glucose and 0.03 M glycine) was used. Aliquots of this the solution were placed in 20 ml capacity vials, which were subsequently sealed with butyl septa and metallic caps and heated at 90 °C up to 50 h. After heating, the samples were rapidly cooled in cold running water. As a consequence of heating, pH changed from an initial value of 5 to 4.

2.2. Coffee extracts

Coffee brews were prepared from medium-roasted coffee powder, supplied by the Nestlè Research Centre of Lousanne. The roasting degree of coffee powder corresponded to a weight loss during the roasting process of 16.2%. The coffee powder had a Neuhaus (CTN)

colour value of 85, which corresponded to L^*, a^*, b^* Hunter scale values of 26.0, 8.8 and 5.8, respectively. The coffee brews were obtained by solid-liquid extraction with deionized water (100 °C) for 10 min (1:10 w/w ratio of coffee powder and water). After extraction, samples were rapidly cooled in cold running water and then filtered through Whatman No. 4 filter paper. The beverages were bottled in 20 ml capacity vials in the presence of nitrogen, which was flushed into the vials for 3 min. The vials were then hermetically sealed with butyl septa and metallic caps and stored at 30 °C for 17 days. During storage, the pH change was less than 0.5.

2.3. Black tea extracts

Black tea extracts were prepared according to Manzocco, Anese, and Nicoli (1998). Tea extracts were obtained from black dried leaves by solid-liquid extraction with deionized water (100 °C) for 5 min (1:100 w/w ratio of tea leaf and water). The cooled and filtered beverages were bottled in 50 ml capacity screw-capped flasks in the presence of air and pasteurized at 105 °C for 20 min in a laboratory autoclave (FVS 13, Federgari, Italy). The tea extracts were then stored at 25 °C for 30 days.

2.4. Tomato purees

Tomato purees were prepared according to Anese, Falcone, Fogliano, Nicoli, and Massini (2002). Aliquots of 200 g of peeled tomato purees, under vacuum, and hermetically sealed in 200 g capacity aluminium pouches, were sterilized in a simulator steam retort (Stork, The Netherlands) by applying different time-temperature combinations (90, 110 and 120 °C for 110, 1.1 and 0.11 min, respectively). After retorting, the samples were rapidly cooled to room temperature by a spray of water. All treatments were carried out in order to achieve the same sterilizing effect (F value), equivalent to 11 min at 100 °C with a 10 °C z-value, which is generally applied in industrial processing for inactivating spores of spoilage-causing microorganisms in tomato products with pH 4.5.

2.5. Redox potential determination

Measurements were made using a platinum indicator electrode and a silver/silver chloride reference electrode, connected to a digital voltmeter (Hanna Instruments, model 8417, Milano, Italy) according to the methodology proposed by Manzocco et al. (1998). Calibration was performed against a redox standard solution (Reagecon, Shannon, Co. Clare, Ireland) having a redox potential value of 220 mV at 25 °C. Electrodes were placed in a 50 ml 3-neck flask, containing 15 ml volume of each sample. Prior to analysis, oxygen was removed from the system by continuous nitrogen flushing for 10 min. The redox potential was monitored for at least 20 min at 25 °C, until a stable potential was achieved, arbitrarily defined as that changing less than 2 mV in a 3 min period. The analyses were performed in triplicate and the variation coefficients, expressed as the percentage ratio between the standard deviation (SD) and the relevant mean values, were below 8%.

2.6. Chain-breaking activity determination

The chain-breaking activity of the coffee extracts was measured by means of the DPPH· (2,2-diphenyl-1-picrylhydrazyl) method (Brand-Williams, Cuvelier, & Berset, 1995). A volume of 1.9 ml of 6.0×10^{-5} M DPPH· methanol solution was used. The reaction was started by the addition of 100 μl of sample, previously diluted 1:200 (w/w), with deionized water. The bleaching rate was followed at 515 nm (Beckman DU 640; Beckman Instruments, Inc., Fullerton, CA, USA) at 25 °C for at least 20 min. Reaction rates were calculated using equation (1) (Manzocco et al., 1998):

$$\mathrm{Abs}^{-3} - \mathrm{Abs}_0^{-3} = -3kt, \quad (1)$$

where k is the DPPH· bleaching rate, Abs_0 is the initial absorbance value and Abs is the absorbance at increasing time t. The antioxidant activity was expressed as $-\mathrm{Abs}^{-3}$/min/mg dry matter. The results are the average of at least three measurements, and the variation coefficients were below 10%.

3. Results and discussion

Fig. 1 shows the chain-breaking activity values, as a function of the redox potential, measured for the glucose–glycine model systems heated at 90 °C for up to 50

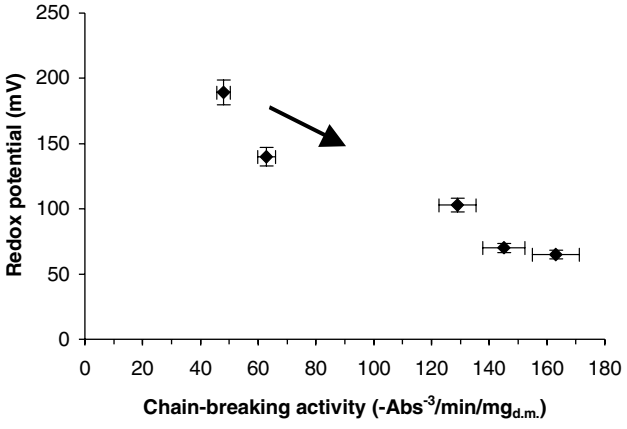


Fig. 1. Chain-breaking activity values as a function of redox potentials of a 0.1 M glucose and 0.3 M glycine solution heated at 90 °C for 50 h. The arrow indicates the direction of redox potential and chain-breaking activity changes. The mean values of at least three replicates are reported ± standard deviation.

h. A good agreement between the kinetic and thermodynamic measurements can be observed, as indicated by the fact that an increase in the radical-scavenging activity corresponded to a decrease in the redox potential. As already pointed out, this result can be ascribed to the formation of high molecular weight, brown, nitrogen-containing compounds, named MRPs. They develop in most foods submitted to heat treatments (e.g., roasted coffee and cocoa, bakery foods) and are known to possess strong radical scavenging and reducing properties (Hayase et al., 1989; Lingnert & Eriksson, 1980; Namiki, 1990). The intensity of the heating process greatly affects concentration, chemical structure and antioxidant properties of the MRPs (Kim & Harris, 1989; Lingnert & Eriksson, 1980). In our experimental conditions the antioxidant efficiency, as well as the reactivity of the heat-induced antioxidants towards the reference radical (DPPH·), progressively increased with the increasing the intensity of the heat treatment. In other words, heating caused a progressive enrichment of the solution's reservoir of antioxidants.

Fig. 2 shows the chain-breaking activity values as a function of the redox potential of pasteurized black tea extracts during storage at 25 °C for 30 days. In this case, progressively higher chain-breaking activity values corresponded to higher redox potential values. According to previous observations (Cheigh, Um, & Lee, 1995; Nicoli et al., 2000), the increase in the radical scavenging activity is attributable to the partial oxidation of some polyphenols, which can exhibit stronger antioxidant properties than the corresponding non-oxidized forms as a consequence of increased resonance delocalization as well as stability of the aryloxyl radicals. However, the increase in the polyphenol reactivity did not correspond to a gain in antioxidant efficiency. In fact, the increase in the redox potential value could reasonably indicate that the reservoir of these compounds was progressively reduced during storage. This means that the antioxidants present in the product can react "rapidly" but the reservoir of antioxidant efficiency, which is dispatched during storage does not last for long. This is quite interesting for the estimation of the shelf-life of a product and also in relation to its technological history and, perhaps for the prediction of its health promoting capacity.

Figs. 3 and 4, respectively show the chain-breaking activity values as a function of redox potentials of medium-roasted coffee brews, stored at 30 °C under nitrogen, and of tomato purees subjected to different heat treatments with equivalent thermal effect. In the case of the coffee brews no significant changes in the redox potential values were detected during storage, while the

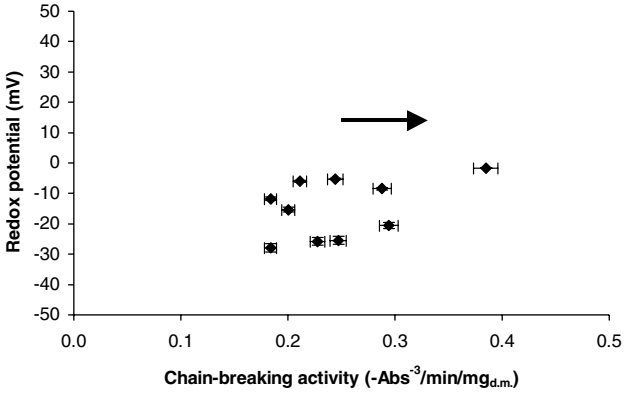


Fig. 3. Chain-breaking activity values as a function of redox potentials of medium-roasted coffee brews, packaged under nitrogen and stored at 30 °C. The arrow indicates the direction of chain-breaking activity change. The mean values of at least three replicates are reported ± standard deviation.

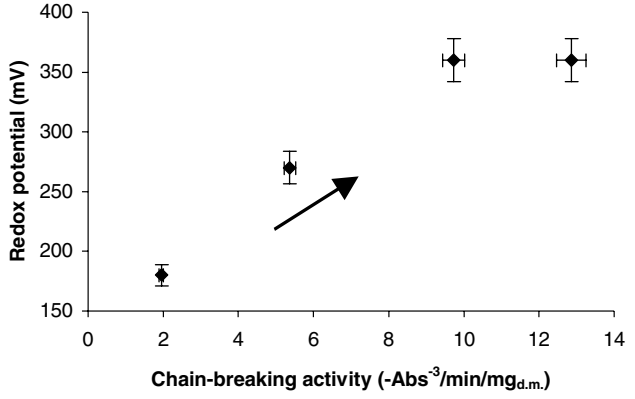


Fig. 2. Chain-breaking activity values as a function of redox potentials of pasteurized black tea extracts stored at 25 °C for 30 days. The arrow indicates the direction of redox potential and chain-breaking activity changes. The mean values of at least three replicates are reported ± standard deviation.

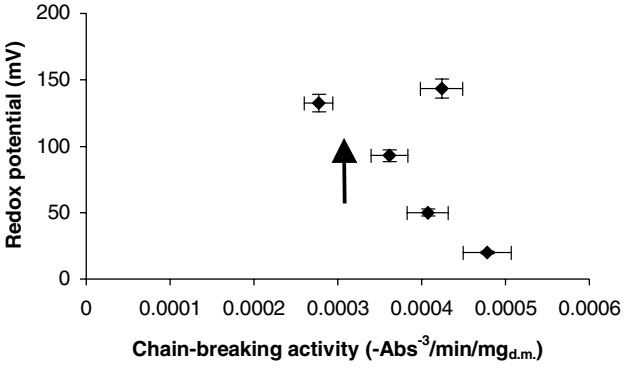


Fig. 4. Chain-breaking activity values as a function of redox potentials of tomato purees subjected to different heat treatments with equivalent thermal effect. The arrow indicates the direction of redox potential change. The mean values of at least three replicates are reported ± standard deviation.

chain-breaking activity increased. This means that, in the absence of oxygen, coffee maintained the original reducing power over the storage time. On the other hand, the redox potential values of tomato samples increased with the temperature, while no changes in the chain breaking-activity were detected. It was hypothesized that when high-temperature treatments were applied, the formation of heat-induced antioxidants could balance the likely depletion in tomato hydrophilic antioxidants (namely, flavonoids and ascorbic acid), thus contributing to retain the original chain-breaking activity (Anese et al., 2002). In other words, the partial loss of naturally occurring antioxidants in tomatoes, due to the thermal process, is counter-balanced by the formation of MRPs with radical scavenging properties (Nicoli, Anese, & Parpinel, 1999). However, the redox potential data seem to be quite contradictory to those found for the glucose–glycine system. Nevertheless, this can be explained by the fact that the intensity of the heat treatments applied to the tomato purees was greatly lower than that of the model system. As a result, the reducing power of the so formed MRPs was not high enough to compensate that of the thermally degraded natural antioxidants. Therefore, while the application of equivalent sterilizing effects did not affect the rate of the chain-breaking activities of processed tomatoes, the reservoir of efficient antioxidants decreased with the temperature, thus affecting the shelf-life of the product.

4. Conclusion

The assessment of the chain-breaking activity, together with the redox potential, represents an interesting way for assessing the antioxidant capacity of a food product. In fact, while kinetic methods estimate the antioxidant capacity by referring only to the most reactive compounds, redox potential measurements give indications of the overall reducing properties. The latter are due to the contribution of all the food antioxidants, including the ''slowest'' but highly efficient ones, which represent the antioxidant reservoir of the product. These compounds play an important role in the maintenance of the chemical stability of foods during storage and probably in maintenance of their functional properties.

On the basis of these considerations, the redox potential may be an interesting indicator of the antioxidant efficiency of food products and may allow a better estimate of the influence of possible oxidative damage caused by processing.

References

Anese, M., Falcone, P., Fogliano, V., Nicoli, M. C., & Massini, R. (2002). Effect of equivalent thermal treatments on the color and the antioxidant activity of tomato purees. *Journal of Food Science, 67*, 3442–3446.

Bogh-Sorensen, L. (1994). Description of hurdles. In L. Leistner & L. G. M. Gorris (Eds.), *Food Preservation by Combined Processes* (pp. 7–24). Brussels: European Commissions.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate the antioxidant activity. *Lebensmittel Wissenshaft und Technologie, 28*, 25–30.

Buettner, G. R. (1993). The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol and ascorbate. *Archives of Biochemistry & Biophysics, 300*, 535–543.

Cheigh, H. S., Um, S. H., & Lee, C.Y. (1995). Antioxidant characteristics of melanin-related products from enzymatic browning of catechin in a model system. In *ACS symposium series 600* (pp. 200–208). Washington, DC: American Chemical Society.

Dikanovic-Lucan, Z., & Palic, A. (1995). Redox potential in wine making process: A review. *Bulletin de l' O.I.V., 68*, 762–779.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*, 1925–1941.

Hayase, F., Hirashima, S., Okamoto, G., & Kato, H. (1989). Scavenging of active oxygens by melanoidins. *Agricultural and Biological Chemistry, 53*, 3383–3385.

Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables – the millenium's health. *International Journal of Food Science and Technology, 36*, 703–725.

Kim, N. S., & Harris, N. D. (1989). Antioxidant effect of non-enzymatic browning reaction products on linoleic acid. In A. H. Ghee (Ed.), *Trends in Food Science* (pp. 19–24). Singapore: Singapore Institute of Food Science and Technology.

Leistner, L., & Gorris, L. G. M. (1995). Food preservation by hurdle technology. *Trends in Food Science and Technology, 6*, 41–46.

Lingnert, H., & Eriksson, C. E. (1980). Antioxidative Maillard reaction products. I. Products from sugars and free amino acids. *Journal of Food Processing and Preservation, 4*, 161–167.

Manzocco, L., Anese, M., & Nicoli, M. C. (1998). Antioxidant properties of tea extracts as affected by processing. *Lebensmittel Wissenshaft und Technologie, 31*, 694–698.

Namiki, M. (1990). Antioxidants/antimutagens in food. *Critical Reviews in Food Science and Nutrition, 29*, 273–300.

Nicoli, M. C., Anese, M., Manzocco, L., & Lerici, C. R. (1997). Antioxidant properties of coffee brews in relation to the roasting degree. *Lebensmittel Wissenshaft und Technologie, 30*, 292–297.

Nicoli, M. C., Anese, M., & Parpinel, M. (1999). Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in Food Science and Technology, 10*, 94–100.

Nicoli, M. C., Calligaris, S., & Manzocco, L. (2000). Effect of enzymatic and chemical oxidation on the antioxidant capacity of catechin model systems and in apple derivatives. *Journal of Agriculture and Food Chemistry, 48*, 4576–4580.

Shwartz, J. L. (1996). The dual rate of nutrients and antioxidants and pro-oxidants: Their effects on tumor cell growth. *Journal of Nutrition, 126*, 1221–1227.

Various authors (2001). In W. Pfannhauser, G. R. Fenwick, & S. Khokhar (Eds.), *Biologically-active phytochemicals in food*. Cambridge: Royal Society of Chemistry.