Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 102 (2007) 1352–1356
Food Chemistry
www.elsevier.com/locate/foodchem

The use of photochemiluminescence for the measurement of the integral antioxidant capacity of baobab products

Elena Besco [b], Elena Braccioli [a], Silvia Vertuani [b], Paola Ziosi [a], Francesca Brazzo [b], Renato Bruni [c], Gianni Sacchetti [d], Stefano Manfredini [a,*]

[a] *Department of Pharmaceutical Sciences, University of Ferrara, Via Fossato di Mortara 17/19, 44100 Ferrara, Italy*
[b] *Ambrosialab srl, University of Ferrara, Via Fossato di Mortara 17/19, 44100 Ferrara, Italy*
[c] *Department of Functional and Evolutive Biology, University of Parma, Parco Area delle Scienze 11A, 43100 Parma, Italy*
[d] *Department of Natural and Cultural Resources, University of Ferrara, Corso Porta Mare 2, 44100 Ferrara, Italy*

Received 7 March 2005; accepted 18 May 2006

Abstract

The number of methods to measure antioxidants in botanicals, foods, nutraceuticals and other dietary supplements are increased considerably in the last 10 years. However most techniques require long experimental times and high costs to determine antioxidant capacity of hydrophilic or lipophilic compound in a food mixture. By means of a photochemiluminescence method, we assessed the Integral Antioxidant Capacity (IAC) which represents the sum of the antioxidant capacity of hydrophilic and lipophilic antioxidants. In this study the IAC of extracts from *Adansonia digitatata* (i.e. red fiber, fruit pulp and leaves), was assessed in comparison to those deriving from other natural sources of antioxidants (i.e. orange, kiwi, apple and strawberry). When compared, IAC values for the examined product resulted as follows: *Adansonia digitata* red fibre $\ggg$ *Adansonia digitata* fruit pulp $\gg$ *Adansonia digitata* fresh leaves $\gg$ *Adansonia digitata* seeds $\geqslant$ *Adansonia digitata* radix cuticle $\geqslant$ orange fresh pulp $\gg$ strawberry fresh fruit pulp $>$ *Adansonia digitata* radix $>$ bilberry fresh pulp $\geqslant$ kiwi fruit pulp. Results clearly indicate the interesting antioxidants properties of *Adansonia digitata* red fibre, in particular the IAC value of baobab red fibre was 66 time higher than that of orange pulp, with value of 1617.3 μmol/g and 24.3 μmol/g, respectively.

Keywords: *Adansonia digitata*; Antioxidant capacity; Ascorbic acid

1. Introduction

Clinical trials and epidemiological studies have established an inverse correlation between the intake of fruits and vegetables and the occurrence of diseases such as inflammation, cardiovascular disease, cancer and aging-related disorders (Willet, 2001). Dietary antioxidants, including polyphenolic compounds, vitamins E and C, and carotenoids, are believed to be the effective nutrients in the prevention of these oxidative stress related diseases (Kaur & Kapoor, 2001). Taken this into account it is easy to understand the considerably increase of researches on antioxidants during the last 10 year. The number of methods to measure antioxidants in botanicals, foods, nutraceuticals and other dietary supplements has also increased considerably. Due to the complexity of the composition of foods, the investigation of each single antioxidant compound is costly and inefficient, moreover possible synergistic interactions among the antioxidant compounds in a food mixture are not taken into account (Prior, Wu, & Schaich, 2005).

A number of assays has been developed for the detection of both general and specific antioxidant action of complex mixtures.

* Corresponding author. Tel.: +39 0532 291292; fax: +39 0532 291296.
E-mail addresses: s.manfredini@unife.it, mv9@unife.it (S. Manfredini).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.05.067

TEAC assay was first reported by Miller and Rice-Evans (Miller et al., 1993), and is based on the scavenging ability of antioxidants on the long-life radical anion $ABTS^{\cdot+}$. In this assay, ABTS is oxidized by peroxyl radicals or other oxidants to its radical cation, $ABTS^{\cdot+}$, which is intensely colored and reacts rapidly with antioxidants, typically within 30 min. Antioxidant capacity, referred to Trolox, is measured as the ability of test compounds to decrease the color reacting directly with the $ABTS^{\cdot+}$ radical. Sanchez-Moreno suggested the 2,2-di(4-*tert*octylphenyl)-1-picrylhydrazyl (DPPH) assay as an easy and accurate method for the measurement of the antioxidant capacity of fruit and vegetable juices or extracts (Sanchez-Moreno, 2002). The assays is based on measurement of the loss of DPPH color at 515 nm after reaction with test compounds (Bondet, Brand-Williams, & Berset, 1997), and the reaction is monitored by a spectrometer. The percentage of remaining $DPPH^{\cdot}$ ($DPPH^{\cdot}$ REM) is proportional to the antioxidant concentration, and the concentration that causes a decrease in the initial $DPPH^{\cdot}$ concentration by 50% is defined as EC50. The test is simple and rapid and needs only a UV–Vis spectrophotometer to be performed, however, interpretation is complicated when the test compounds have spectra that overlap DPPH at 515 nm.

The total radical-trapping antioxidant parameter (TRAP) assay has also been widely used (Ghiselli, Serafini, Natella, & Scaccini, 2000). This method monitors the ability of antioxidant compounds to interfere with the reaction between peroxyl radicals generated by AAPH or ABAP [2,2′-azobis(2-amidinopropane) dihydrochloride] and a target probe. Requirements for the assay are that the probe must be reactive with $ROO^{\cdot}$ at low concentrations, there must be a dramatic spectroscopic change between the native and oxidized probe (to maximize sensitivity), and no radical chain reaction beyond probe oxidation should occur. Typically, oxidation of the probe is followed optically (Bartosz, Janaszewska, Ertel, & Bartosz, 1998) or by fluorescence (Ghiselli, Serafini, Maiani, Azzini, & Ferro-Luzzi, 1995). TRAP values are usually expressed as a lag time or reaction time of the sample compared to corresponding times for Trolox. The TRAP assay involves the initiation of lipid peroxidation by generating water-soluble peroxyl radicals and is sensitive to all known chain breaking antioxidants, but it is relatively complex and time-consuming to perform, requiring a high degree of expertise and experience.

The oxygen radical absorbance capacity (ORAC) assay has found even broader application for measuring the antioxidant capacity of botanical samples (Prior & Cao, 2000) and biological samples (Cao & Prior, 1998). The ORAC measures antioxidant inhibition of peroxyl radical induced oxidations and thus reflects classical radical chain breaking antioxidant activity by H atom transfer (Ou, Hampsch-Woodill, & Prior, 2001). In the basic assay, the peroxyl radical reacts with a fluorescent probe to form a nonfluorescent product, which can be quantitated easily by fluorescence. Antioxidant capacity is determined by a decreased rate and amount of product formed over time. To be made more broadly applicable, the ORAC assay has been adapted to measure lipophilic as well as hydrophilic antioxidants using a solution of 50% acetone/50% water (v/v) containing 7% randomly methylated α-cyclodextrin (RMCD) to solubilize the antioxidants (Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002; Wu et al., 2004.). The lipophilic and hydrophilic components are selectively extracted before assay (Prior et al., 2003).

Therefore researchers are looking for a method that allow a quick analyse of total antioxidant capacity.

Taking this into account, we have further investigated antioxidant properties of *Adansonia digitata* products, by the use of Photochemiluminescence (PCL) (Vertuani, Braccioli, Buzzoni, & Manfredini, 2002). This assay involves the photochemical generation of superoxide ($O2^{\cdot-}$) free radicals combined with chemiluminescence detection. The integral antioxidant capacity (IAC) of *Adansonia digitata* products in comparison with that of other, vitamin C rich, known fruits in was evaluated in a comparative study. By this assay was possible to evaluate antioxidant capacity of both hydrophilic and lipophilic compounds and their possible synergistic interactions.

2. Photochemiluminescence method (PCL)

In the PCL assay (photochemiluminescence) the photochemical generation of free radicals is combined with the sensitive detection by using chemiluminescence. The PCL is based on the photo-induced autoxidation inhibition of luminol by antioxidants, mediated from the radical anion superoxide ($O_2^{\cdot-}$) and is suitable to measure the radical scavenging properties of single antioxidants as well as more complex systems in the nanomolare range. Luminol works as photosensitiser as well as oxygen radical detection reagent. The antioxidant potential is measured by means of the lag phase at different concentrations, calculated by a Trolox calibration curve and expressed as mmol equivalents in antioxidant activity of a reference compound (i.e. Trolox). The PCL method was carried out with the procedure described by Popov and Lewin (Popov, Lewin, & Baehr, 1987; Popov & Lewin, 1999) and can be conducted by two different protocols ACW and ACL that consent to measure the antioxidant capacity of the water- and lipid-soluble components respectively. In the water soluble fraction antioxidants such are flavonoids, ascorbic acid, aminoacids, etc. are detected, while in the lipid soluble fraction tocopherols, tocotrienols, carotenoids, etc. are measured. The most widely used methods for measuring antioxidant activity involve the generation of radical species and the presence of antioxidants determining the disappearance of these radicals. Most of the assays determine the antioxidant activity in the micromolar range needing minutes or hours. The PCL assay, which is easy and rapid to perform, and although less reliable with lipohilic substrates, presents numerous advantages: it does

not requires high temperatures to generate radicals and it is more sensitive to measure, in few minutes, the scavenging activity of antioxidants against the superoxide radical which is one of the most dangerous reactive oxygen species (ROS) also occurring in human body (Schlesier, Harwat, Bohm, & Bitsch, 2002).

2.1. The Adansonia digitata fruit

The baobab fruit pulp is contained in a woody epicarp; the internal ripe fruit, endocarp, is split in small floury, dehydrated and powdery slices that enclose multiple seeds and filaments, the red fibers, that subdivide the pulp in segments (Nour, Magboul, & Kheiri, 1980). The ripe fruit pulp appears as naturally dehydrated, powdery, whitish colored and with a slightly acidulous taste, and its separation from the shell only needs of a single mechanical process without any extraction, concentration or chemical treatment (Obizoba et al., 1994). This ensure to the pulp the characteristic of a slightly processed food.

3. Materials and methods

ACW (Antioxidant Capacity of Water soluble substance) and ACL (Antioxidant Capacity of Liposoluble substance) kits (no. 400.801) were purchased from, Analytik Jena AG, Jena, Germany; Trolox ((*S*)-(2)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) (no. 39,192-1) was purchased from Aldrich, Sigma–Aldrich, Taufkirchen, Germany. Sample of Baobab red fibre, fruit pulp, dry leaves, seeds, radix, were purchased from Baobab Fruit Co., Verona, Italy. Several varieties of each fruit and vegetable, depending on their availability, were purchased at local supermarkets and then processed through dispersion by sonication in water (ACW) or methanol (ACL), centrifuged and the supernatant filtered by a 0.45 μ filter before measurements.

3.1. Preparation of samples for PCL analysis

3.1.1. Preparation of the Trolox standard solution (ACL protocol)

Five hundred microlitre of Reagent 1 (Kit ACL, AnalytikJena) were added to the vial containing Trolox (Reagent 4, Kit ACL, AnalytikJena) and mixed by vortex for 20–30 s. The obtained stock solution was then diluted 1:100 with Reagent 1. Ten μl of this working solution contain 1 nmol Trolox as calibration standard.

Measurements were done using 5, 10, 20 and 25 μl volumes of the sample, and were repeated two times.

3.1.2. Preparation of the ascorbic acid standard solution (ACW protocol)

Four ninety microlitre of Reagent 1 (Kit ACW, AnalytikJena) and 10 μl H_2SO_4 were added to the vial containing ascorbic acid (Reagent 4, Kit ACW, AnalytikJena) and mixed by vortex for 20–30 s. The obtained stock solution was then diluted 1:100 with Reagent 1. 10 μl of this working solution contain 1 nmol ascorbic acid as calibration standard.

Measurements were done using 5, 10, 20 and 25 μl volumes of the sample, and were repeated two times.

3.1.3. ACW and ACL sample preparation – general procedure

An exact quantity of *Adansonia digitata* products or fruit sample (0.5 g) was accurately suspended in 10 mL methanol (ACL) or water (ACW) HPLC grade, sonicated, centrifuged and the surnatant was then filtered through HPLC filter (Chemtek Analitica, Bologna, Italy) by a syringe and diluted with Reagent 1 of ACL or ACW kit (AnalytikJena, Jena, Germany).

Results are expressed as μmol equivalents, in antioxidant activity, of Trolox for each gram of product under examination.

4. Results and discussion

In view of the ever increasing importance of health promotion and of the benefits related to the use of antioxidant rich preparations, we have undertaken the present work to determine the integral antioxidant capacity of several fruits products: *Adansonia digitata* and other, vitamin C rich, known fruits in order to conduce a comparative evaluation. All fruit products were examined in their natural form as fresh wet preparation, avoiding any further chemical–physical process. To this end we have used a simple and quick assay and we have introduced a new parameter termed as IAC (Integral Antioxidant Capacity) which represents the sum of the antioxidant capacity of hydrophilic and lipophilic antioxidants, calculated as μmol equivalents in activity of Trolox, determined in the best experimental conditions, for each kind of plant product. In our opinion, it is important to conduce separated determinations because of the different nature of the single antioxidants contained in the product under examination. Thus, potency of lipophilic antioxidants cannot be properly measured in the same experimental conditions as for the hydrophilic ones. As a consequence, the true antioxidant capacity of a sample will be then better described by the sum of the two separated values. Indeed, the products we have tested, might have both lipid- and water-soluble antioxidant capacities at the same time. In these regards, the IAC value better describe the whole antioxidant capacity, a higher IAC means a wider spectrum of activity because the ROS species will be counteracted by a mixture of antioxidant compounds, typically contained in the products under examination, some of them able to work, in a concerted manner, in hydrophilic and others in lipophilic body compartments.

Among the different methods available for the determination of antioxidant capacity, the photochemiluminescence method (PCL) has been chosen for its sensitivity and reliability. Moreover, it gives a measure of the protec-

Table 1
Ascorbic acid contents in some fruits, expressed as mg of vitamin each 100 g of product[a]

Fruit	Latin name	mg ascorbic acid/100 g
Baobab	*Adansonia digitata*	150–499
Kiwi, green	*Actinidia deliciosa*	98
Orange	*Citrus sinensis*	53
Strawberry	*Fragaria x ananassa*	61
Bilberry	*Vaccinium myrtillus*	1

[a] Data taken from the US Dept. of Agricolture (USDA) Nutrient database for standard reference.

tive capacity of a plant product against ROS which are, among the many, the most dangerous species of free radicals for leaving beings.

As it can be seen in Table 2, and taking into account the water content of each product, the highest water-soluble antioxidant capacity was observed for some of baobab products, in particular for red fibre that resulted endowed with a potent capacity, followed by baobab fruit pulp, orange fresh pulp and baobab fresh leaves. In comparison to the *Adansonia digitata* products: strawberry, bilberry and kiwi all resulted endowed with a lower capacity (Table 2).

Concerning the lipid-soluble antioxidant capacity, once again taking into account the relative water content, baobab red fibre resulted the most interesting among those tested. Also in this case it showed the highest capacity followed by baobab fruit pulp and fresh leaves (Table 3). The other plant products considered were all endowed with a very limited capacity, this might be explained on the light of a low content in lipid-soluble antioxidants.

When comparing water- to lipid-soluble antioxidant capacity of plant products, it can be observed that the red fiber and fresh fruit pulp from *Adansonia digitata*, showed the highest values in both cases. Concerning other products, an high ACW and very low ACL value were observed for orange fresh pulp, thus suggesting in ascorbic acid the major contributor to the activity. These data are in agreement with known values reported in literature (Table 1), for example orange fruit that contains about six times less ascorbic acid than baobab fruit pulp, showed a lower water-soluble antioxidant capacity.

As it can be seen in Table 4, the results of the study can be easily understood by the reading of the IAC values for the evaluated plant products. Taken together, data obtained clearly shows that products from *Adansonia digitata* are endowed with very interesting antioxidant capacity. In particular, best capacity was found for red fiber with a IAC as high as 1617.3 μmol/g of Trolox. Also very interesting were fresh pulp (240.5 μmol/g) and fresh leaves (89 μmol/g). Moreover, in the case of the IAC of *Adansonia digitata* fruit pulp and leaves it is very interesting to note that the activity was related to just a plant component, very slightly processed (trituration in the case of leaves, mechanical separation in the case of fruit pulp), thus conferring to the product the as much as possible natural characteristics and lowering at the same time its manufacturing costs. In conclusion, when compared together, and taking into account relative water content of samples, IAC values for the examined product resulted as follows: *Adansonia digitata* red fibre $\ggg$ *Adansonia digitata* fruit pulp $\gg$ *Adansonia digitata* fresh Leaves $\gg$ *Adansonia digitata* seeds $>$ *Adansonia digitata* radix cuticle $>$ orange fresh pulp $>$ strawberry fresh fruit pulp $\geqslant$ *Adansonia digitata* radix $>$ bilberry fresh pulp $\geqslant$ kiwi fruit pulp.

Table 2
Water-soluble antioxidant capacity, corresponding to the activity expressed as μmol equivalents of ascorbic acid for each gram of tested product

Products	Ascorbic acid equivalents (μmol/g)	% Water/dry extract
Baobab fruit pulp	75.0 ± 0.005	11
Baobab leaves	23.0 ± 0.002	10
Baobab seeds	16.0 ± 0.0007	18
Baobab red fibre	386.0 ± 0.058	8
Baobab radix	1.2 ± 0.0001	88
Baobab radix cuticle	8.5 ± 0.0003	88
Kiwi fruit pulp	0.73 ± 0.015	84
Orange fruit pulp	17.0 ± 0.015	86
Strawberry fruit pulp	1.72 ± 0.006	90
Bilberry fruit pulp	1.95 ± 0.017	82

The value are mean of 3 measures ± SD.

Table 3
Lipid soluble antioxidant capacity, corresponding to the activity expressed as μmol equivalents of Trolox for each gram of tested product

Products	Trolox equivalents (μmol/g)	% Water/dry extract
Baobab fruit pulp	25.0 ± 0.0015	11
Baobab leaves	24.5 ± 0.003	10
Baobab seeds	6.5 ± 0.00004	18
Baobab red fibre	508.0 ± 0.008	8
Baobab radix	1.0 ± 0.00002	88
Baobab radix cuticle	11.0 ± 0.0003	88
Kiwi fruit pulp	0.27 ± 0.015	84
Orange fruit pulp	0.29 ± 0.015	86
Strawberry fruit pulp	0.36 ± 0.006	90
Bilberry fruit pulp	2.00 ± 0.017	82

The values are mean of 3 measures ± SD.

Table 4
Integral antioxidant capacity (IAC) corresponding to the sum of the corresponding water- and lipid-soluble antioxidants capacity

Products	IAC
Baobab fruit pulp	240.5
Baobab leaves	89.0
Baobab seeds	51.4
Baobab red fibre	1617.3
Baobab radix	4.3
Baobab radix cuticle	35.3
Kiwi fruit pulp	2.4
Orange fruit pulp	24.3
Strawberry fruit pulp	5.3
Bilberry fruit pulp	2.6

5. Conclusions

This current study reports about an analytical method, the PCL assay, that is rapid, relatively simple, and reproducible, making it an attractive biomonitoring tool especially for food supplement, nutrition and food technologies. In this study we have introduced a novel concept based on the Integral Antioxidant Capacity (IAC), expressed as the sum of the water and lipid antioxidant capacity referred to a common reference compounds, Trolox. This value resulted to be a useful index to describe the capacity of complex samples, such are those of natural origin, to counteract reactive oxygen species and in particular the superoxide anion, very harmful for human health. It is confirmed that the health benefits of fruits and vegetables are mediated through their antioxidant content: in virtue of high antioxidant capacity, high concentration in nutrients, bounded to the characteristic of a slightly processed food, it seems reasonable to consider the baobab fruit pulp and leaves as interesting foods for diet supplements (Gruenwald & Galizia, 2005).

In this study we determine the antioxidant capacity of products deriving from *Adansonia digitata*, a plant up to date only known for the high content of vitamin C of the fruit, and for the centenary use in traditional african medicine. This investigation, until all the active components of this plant will be clearly established, was conducted as an initial step to elucidate the therapeutical, nutriceutical and cosmeceutical potential of *Adansonia digitata* plant products. Moreover, in view of the very high antioxidant capacity, we propose the red fiber as new valuable ingredient for food preparation and/or nutriceutical application in the promotion of health.

References

Bartosz, G., Janaszewska, A., Ertel, D., & Bartosz, M. (1998). Simple determination of peroxyl radical-trapping capacity. *Biochemistry and Molecular Biology International, 46*, 519–528.

Bondet, V., Brand-Williams, W., & Berset, C. (1997). Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. *Lebensmittel-Wissenschaft & Technologie, 30*, 609–615.

Cao, G., & Prior, R. L. (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry, 44*, 1309–1315.

Ghiselli, A., Serafini, M., Maiani, G., Azzini, E., & Ferro-Luzzi, A. (1995). A fluorescence-based method for measuring total plasma antioxidant capability. *Free Radical Biology and Medicine, 18*, 29–36.

Ghiselli, A., Serafini, M., Natella, F., & Scaccini, C. (2000). Total antioxidant capacity as a tool to assess redox status: critical review and experimental data. *Free Radical Biology and Medicine, 29*, 1106–1114.

Gruenwald, J., & Galizia, M. (2005). Market brief in the European union for selected natural ingredients derived from native species, *Adansonia digitata L.* Baobab. In The United Nations conference on trade and development (UNCTAD) BioTrade Initiative / BioTrade Facilitation Programme (BTFP) UNCTAD /DITC/TED/2005/.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J., & Deemer, E. (2002). Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated â-cyclodextrin as the solubility enhancer. *Journal of Agricultural and Food Chemistry, 50*, 1815–1821.

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

Miller, N. J., Diplock, A. T., Rice-Evans, C., Davies, M. J., Gopinathan, V., & Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science, 84*, 407–412.

Nour, A. A., Magboul, B. I., & Kheiri, N. H. (1980). Chemical composition of baobab fruit (*Adansonia digitata* L). *Tropical Science, 22*, 383–388.

Obizoba, I. C., & Anyika, J. U. (1994). Nutritive value of baobab milk (gubdi) and mixtures of baobab (*Adansonia digitata* L.) and hungry rice, acha (*Digitaria exilis*) flours. *Plant Foods for Human Nutrition, 46*, 157–165.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*, 4619–4926.

Popov, I., & Lewin, G. (1999). Oxidants and Antioxidants Part B – Antioxidative homeostasis: characterization by means of chemiluminescent technique. *Methods in Enzymology, 300*, 437–456.

Popov, I., Lewin, G., & Baehr, R. (1987). Photochemiluminescent detection of antiradical activity. I. Assay of superoxide dismutase. *Biomedica Biochimica Acta, 46*, 775–779.

Prior, R. L., & Cao, G. H. (2000). Analysis of botanicals and dietary supplements for antioxidant capacity: a review. *Journal of AOAC International, 83*(4), 950–956.

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., et al. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry, 51*, 3273–3279.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Sanchez-Moreno, C. (2002). Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International, 8*(3), 121–137.

Schlesier, K., Harwat, M., Bohm, V., & Bitsch, R. (2002). Assessment of antioxidant activity by using different in vitro methods. *Free Radical Research, 36*, 177–187.

Vertuani, S., Braccioli, E., Buzzoni, V., & Manfredini, S. (2002). Antioxidant capacity of Adansonia digitata fruit pulp and leaves. *Acta Phytotherapeutica, 2*, 2–7.

Willet, W. C. (2001). *Eat, drink, and be healthy. The Harvard Medical School guide to healthy eating*. New Work: Simon and Schuster.

Wu, X., Gu, L., Holden, J., Haytowitz, D., Gebhardt, S. E., Beecher, G., et al. (2004). Factors in the development of a database of food total antioxidant capacity using lipophilic and hydrophilic oxygen radical absorbance capacity (ORACFL): a preliminary study of 28 foods. *Journal of Food Composting Analysis, 17*, 407–422.