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New Method To Determine Antioxidant Activity of Polyphenols

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The capacity of polyphenolic compounds to reduce the β-carotene−linoleic acid cooxidation enzymatically induced by soybean lipoxygenase was assayed to determine their comprehensive antioxidant ability. The inhibition of the coupled oxidation is a well-known spectrophotometric method for the antioxidant activity measurement. A modification of this method is proposed to reduce assay time and to gain simplicity. The antioxidant abilities of several polyphenols were determined, and quercetin and sinapic acid were most active. These results were compared to those obtained by the DPPH• procedure to evaluate the free radical scavenging contribution to the total protective action. The highest values of antiradical activity were found for ellagic and quercetin.

KEYWORDS: Antioxidant activity; β-carotene bleaching; lipoxygenase; polyphenols; flavonoids; phenolic acids; DPPH•

INTRODUCTION

In the last decades oxidation mechanisms and free radical role in living systems have gained increased attention (*1*). Oxygen uptake inherent to cell metabolism produces reactive oxygen species such as superoxide and hydroxyl radicals. The reaction of this species with lipid molecules originates peroxyl radicals and their interaction with nucleic acids and proteins conduces to certain alterations and, therefore, functional modifications (*2*). There are also other oxidative reactions of polyunsaturated fatty acids (PUFA) enzymatically catalyzed by the action of dioxygenases such as cyclooxygenases and lipoxygenases (*3*, *4*).

These processes are recognized as the cause of aging and participate in the pathogenesis of several serious disorders as cancer, atherosclerosis, inflammatory, and coronary heart diseases (*5*−*7*). Oxidative reactions are also concerned with food industry because lipids, natural constituents of cellular membranes, are oxidized during peroxidation, producing partial or total changes in food sensorial properties (color and flavor) and in its nutritional value because of vitamin, essential fatty acid, and protein losses (*8*).

There are substances that delay, inhibit, or even prevent oxidative reactions by different mechanisms: (1) chain breaking by donation of hydrogen atoms or electrons that convert free radicals in more stable species; (2) chelating metal ions which are involved in the generation of reactive oxygen species; (3) decomposing lipid peroxides into stable final products; (4) inhibiting the deleterious action of prooxidant enzymes (*9*).

Many natural substances such as carotenoids, tocopherols, and polyphenols are able to act as antioxidants and are widely spread in food and plants. Flavonoids and other polyphenols have the ability to scavenge free radicals and, therefore, delay lipid autoxidation (*10*). Several studies relate flavonoid radical scavenging and antioxidative potential to some structural features especially the presence of (i) a catechol group (*ortho*-dihydroxyl substitution pattern) in the B ring, (ii) a hydroxyl group in C3 in C ring, and (iii) a double bond conjugated with the *oxo* group (*11*, *12*). These characteristics are related to the stability of the aroxyl radical formed after the flavonoid donates an hydrogen atom to the radical (*13*). Other phenolic compounds present in plants, derived from cinnamic acid (ferulic, caffeic, coumaric, and sinapic acids) and from benzoic acid (ellagic, gallic, and 4-hydroxybenzoic acids), have this kind of antioxidant activity. Their action is associated with the number and substitution pattern of phenolic hydroxyl groups (*14*).

In parallel to their free radical scavenging properties, flavonoids are good inhibitors of lipoxygenases (LOX). LOX is a metal-bound protein with an Fe atom in its active center and oxidizes only fatty acids containing a 1-*cis*,4-*cis*-pentadiene system. Therefore, linoleic acid is one of the preferred substrates for this enzyme (*15*). Recent report correlated flavonoid activity against LOX with structural features. Factors i and iii enhace its inhibitory potency while factor ii diminishes instead of reinforces this activity. Besides, in the absence of a catechol arrangement there was an inverse correlation to total hydroxyl group number (*16*).

There are several different methods to determine the antioxidant ability by varying the oxidizing species and the spectroscopic or chromatographic methodology used for monitoring the reaction progress. There are also many forms to express the relative activity of the tested substances (*17*).

One of the most frequently used techniques for antiradical capacity measurements is following the depletion of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) by addition of scavenger compounds (*18*). It determines the ability of pure

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substances or crude extracts for trapping this unpaired-electron species by donating hydrogen atoms or electrons, producing in consequence the radical disappearance and the formation of less reactive species derived from the antioxidant (*19*). The inhibition of β-carotene bleaching in a coupled oxidation with linoleic acid is a well-known methodology used for evaluating the antioxidant activity (*18*). This spectrophotometric technique monitors the bleaching of the carotenoid caused by its interaction with peroxyl radicals produced during linoleic acid oxidation. The reaction that takes place is an addition of the peroxyl radical to the polyenic system of the carotenoid producing a resonance-stabilized radical (*20*, *21*). This carbon-centered radical is able to react with other radicals yielding nonradical products (termination step); otherwise, it is able to add molecular oxygen in a fast process producing a very reactive carotene−peroxyl radical in a propagation step of the chain reaction (*22*−*24*). With the addition of a radical scavenger, the consumption of the carotenoid is reduced as a function of the antioxidant concentration and its intrinsic potency (*25*).

This methodology is very time-consuming: each determination implies 2 h of reaction at 50 °C (*18*, *26*), being a real limitation for very numerous samples. Besides, it requires an arduous control of the experimental conditions for accomplishing reproducibility. This thermically induced oxidation is quite unspecific and involves even β-carotene thermal degradation. Thermal treatment produces carotenoid isomerization at the very first stage thus generating products with different reactivities even though their absorptivity is close to that of β-carotene (*27*). Carotenoid oxidation takes place during polyunsaturated fatty acid peroxidation also induced by LOX (*28*, *29*). In consequence, the peroxidation of linoleic acid can be initiated by LOX at room temperature in a specific reaction, generating the same oxidizing species as the thermally induced reaction. The initiation step of the peroxidation in the system can be modulated by modification of the pH (*30*). Soybean LOX catalyzes β-carotene bleaching in the presence of linoleic acid in aerobic conditions (*31*, *32*).

Consequently, from this preceding knowledge we propose to modify this widely used thermically induced method to simplify the experimental conditions and make it adequate for a large number of samples.

The main objective of this work was to determine the antioxidant capacity of a polyphenol series frequently present in food and natural products discriminating the contribution of radical scavenging action (DPPH• producedure) from enzymatic inhibition (modified β-carotene bleaching method).

MATERIALS AND METHODS

Materials. All commercially available chemicals were purchased from Sigma-Aldrich (Buenos Aires, Argentina): Aldrich (radical 2,2-diphenyl-1-picrylhydrazyl, gallic acid, 98%, coumaric acid, 98%; ferulic acid, 99%; caffeic acid, 97%; cinnamic acid, 99%; sinapic acid, 98%; chlorogenic, 4-hydroxy-3-methoxybenzoic acid, 97%); Sigma (ellagic, benzoic, salicylic, 4-hydroxybenzoic, and 3,4-dihydroxybenzoic acids; kaempferol, 98%; galangin, 98%; apigenin, 98%; chrysin, 98%; quercetin, 99%; soybean lipoxygenase type 1-S, β-carotene, 95%); Riedel de Haën (99% linoleic acid).

Solution Preparations. Buffer solution was prepared by dissolving 6.80 g of KH_2PO_4 and 2.00 g of NaOH in tridistilled water and adjusted to pH 7.0 with 1 mol L^{-1} NaOH with dilution to 1 L with tridistilled water. An aliquot of 500 μL off a saturated solution of β-carotene in chloroform was mixtured with the same amount of Tween 20. Chloroform was removed using a stream of nitrogen for 20 min. The final solution was obtained by adding pH 7 buffer to absorbance at 460 nm equal to 0.68. Linoleic acid solution was prepared by mixturing 50 μL of this compound and 500 μL of Tween 20 and diluting with pH 7 buffer to 10 mL. LOX solution was obtained by dissolving 10.0 mg of the enzyme in pH 7 buffer to a 10 mL solution. DPPH• was dissolved in methanol to absorbance (517 nm) equal to 1.00. All the solutions were prepared daily before use.

General Procedure. For the control-reaction measurements, 2.5 mL of the β-carotene solution and 200 μL of linoleic acid solution were mixtured in a 3 mL cuvette. Finally, 200 μL of LOX solution was added to initiate the reaction, which was measured by monitoring the absorbance at 460 nm in cycles of 30 s during 10 min.

For antioxidant activity determinations of polyphenolic compounds, the previous procedure was performed by adding an aliquot of 10 μL of 200 μg/mL methanolic solutions of the pure compounds. All the assays were carried out in triplicate at room temperature (25 ± 1 °C).

Antioxidant activity (AOA) is calculated as suggested by Burda and Oleszek as the percentage of inhibition of the β-carotene bleaching of the samples compared to that of the control (*26*):

$$\%\ \mathrm{AOA} = 100 \times \left[1 - \frac{A_s^{\,0} - A_s^{\,\infty}}{A_c^{\,0} - A_c^{\,\infty}}\right] \qquad (1)$$

Here A_s^0 is the absorbance of the sample at 0 min, A_c^0 is the absorbance of the control at 0 min, and A_c^∞ and A_s^∞ are the absorbances of the control and the sample, respectively, in the steady state.

The absorbance of the system in a steady state was estimated by mathematical fitting of kinetic curves performed with Origin 7.0 software.

DPPH• Procedure. For each compound an aliquot containing 20 μg of a methanolic solution was added to 3 mL of DPPH• solution. The decrease in absorbance at 517 nm was determined by spectrophotometric monitoring during 70 min. The percentage inhibition of the DPPH• radical by the samples was calculated according to the equation (*26*)

$$\%\ \mathrm{AAR} = 100 \times \left[1 - \frac{A_{SS}}{A_0}\right] \qquad (2)$$

where A_{SS} is the absorbance of the solution in a steady state and A_0 is the absorbance of DPPH• solution before adding the antioxidant. All determinations were performed in triplicate. The absorbance of the system in a steady state was estimated by mathematical fitting of kinetic curves performed with Origin 7.0 software.

RESULTS AND DISCUSSION

A modification of the previously mentioned thermally induced method is proposed. This consists in an enzymatic initiation of the reaction reducing assay time and gaining in simplicity and reproducibility. The assay is based on the protective ability of antioxidant substances or natural extracts to reduce β-carotene disappearance produced by an enzymatic initiation of the cooxidation system by LOX. **Figure 1a** shows conjugated diene spectral evolution as well as carotenoid bleaching in the cooxidation system of β-carotene and linoleic acid with enzymatic induction by LOX. The effect of an antioxidant compound addition to prevent the oxidation reaction is shown in **Figure 1b**.

AOA Determination of Pure Polyphenolic Compounds. This modified method was used for screening the AOA determination of a polyphenolic compound series including flavonoids and hydroxylated cinnamic and benzoic acids. The kinetic behavior of the system in the presence of some phenolic compounds (galangin, quercetin, and caffeic acid), expressed as absorbance at 460 nm vs time, is presented in **Figure 2**.

The AOA values determined for the 18 phenolic compounds analyzed are shown in **Table 1**. The greatest antioxidant activities were detected for quercetin, sinapic acid, and kaempferol (5.30, 3.7, and 3.00% $mmol^{-1}$, respectively) followed by caffeic, chlorogenic, and 3,4-dihydroxybenzoic acids (2.00, 2.0,

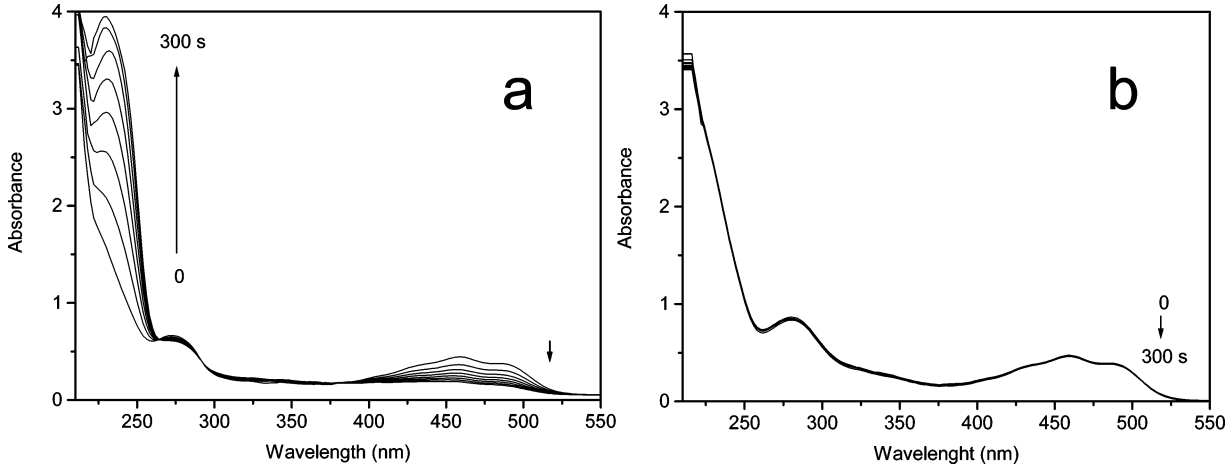

Figure 1. Spectral evolution in the cooxidation system of β-carotene and linoleic acid induced by LOX at room temperature. Experiments were carried out (**a**) without and (**b**) with the addition of an antioxidant compound aliquot.

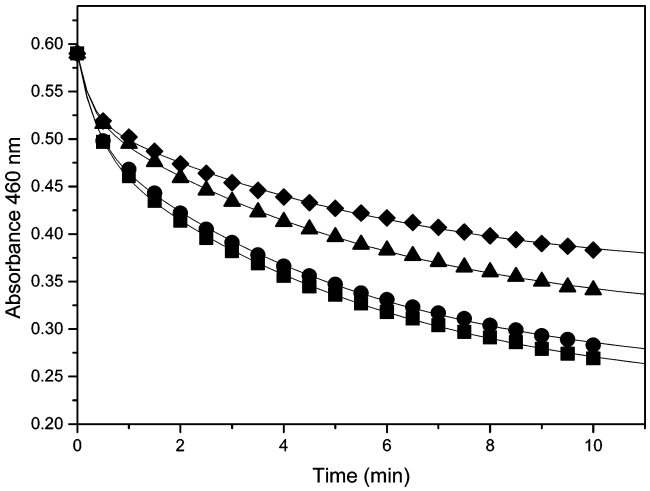

Figure 2. Kinetic behavior of β-carotene–linoleic acid oxidation system induced by LOX in the presence of phenolic antioxidants: galangin (●); quercetin (◆); caffeic acid (▲); control reaction (■).

Table 1. AOA of Phenolic Compounds Determined by β-Carotene–Linoleic Acid Cooxidation Induced by the LOX Method[a]

phenolic compds	% AOA mmol^{-1}
quercetin	5.30 ± 0.05
sinapic acid	3.7 ± 0.1
kaempferol	3.00 ± 0.01
caffeic acid	2.00 ± 0.05
chlorogenic acid	2.0 ± 0.5
3,4-dihydroxybenzoic acid	1.9 ± 0.1
gallic acid	1.5 ± 0.1
ellagic acid	1.5 ± 0.2
ferulic acid	1.30 ± 0.05
salicylic acid	1.00 ± 0.02
coumaric acid	0.90 ± 0.05
apigenin	0.90 ± 0.04
galangin	0.7 ± 0.1
4-hydroxy-3-methoxybenzoic acid	0.60 ± 0.01
chrysin	0.60 ± 0.01
4-hydroxybenzoic acid	0.50 ± 0.02
benzoic acid	0.20 ± 0.01
cinnamic acid	–0.100 ± 0.001

[a] Values indicate the mean ± standard deviation.

and 1.9% mmol^{-1}, respectively). Compounds exhibiting moderate activity were gallic, ellagic, ferulic, and salicylic acids (1.5, 1.5, 1.30, and 1.00% mmol^{-1}) as well as coumaric acid and apigenin (both with 0.90% mmol^{-1}). Lower activities were

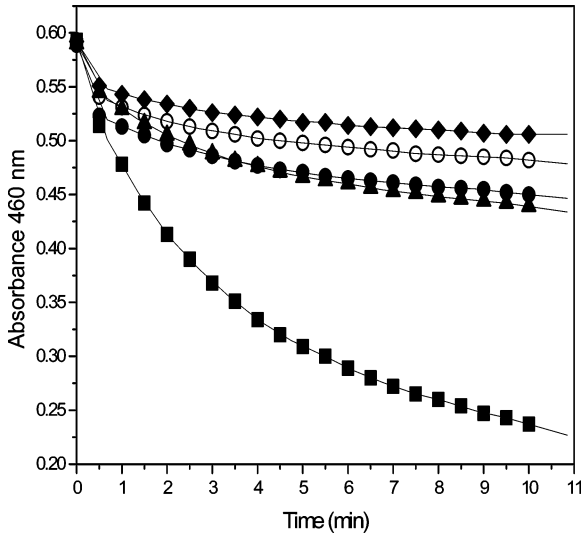

Figure 3. Kinetic behavior of β-carotene–linoleic acid oxidation system induced by LOX in the presence of propolis extracts from a different provenance: Villa Silípica (◆); Nueva Francia (▲); Arraga (●); La abrita (○); control reaction (■).

presented by galangin, 4-hydroxy-3-methoxybenzoic acid, chrysin, and 4-hydroxybenzoic acid (0.7, 0.60, 0.60, and 0.50% mmol^{-1}) and, finally, benzoic and cinnamic acids (0.20 and −0.100% mmol^{-1}). This negative value may represent a prooxidant effect of cinnamic acid in this system.

As an example of the determination of the comprehensive AOA of complex mixture of polyphenols, the analysis of propolis samples was carried out. For this purpose, the addition of the pure compound solution was replaced by an aliquot of ethanolic extract of propolis (bee glue) from different provenances and polyphenolic compound composition. **Figure 3** shows the results obtained from some of the analyzed samples.

Ruggedness. The reproducibility of this new method was evaluated by a ruggedness study, which was performed by analyzing quercetin AOA. The variability observed for these determinations was <5% day to day and <1% during the same day.

ARA Determination of Pure Polyphenolic Compounds. To estimate the contribution of free radical scavenging action in the comprehensive AOA of pure compounds, the results found by this modified method were compared with those obtained by the DPPH• procedure. The measurements of the consumption

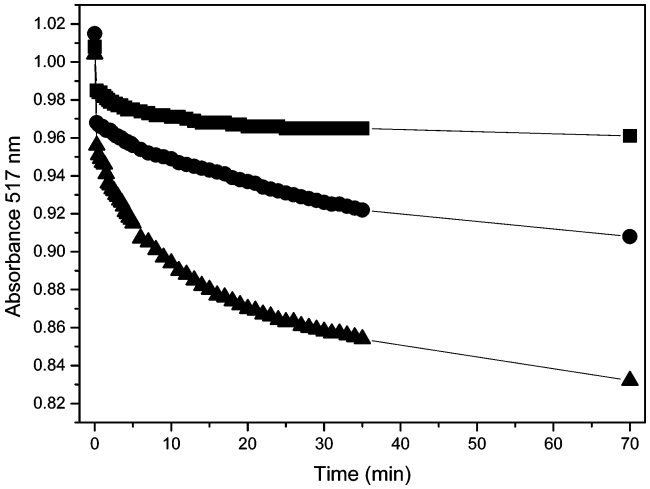

Figure 4. Kinetic behavior of DPPH• solution in the presence of phenolic antioxidants: galangin (■); quercetin (▲); caffeic acid (●)

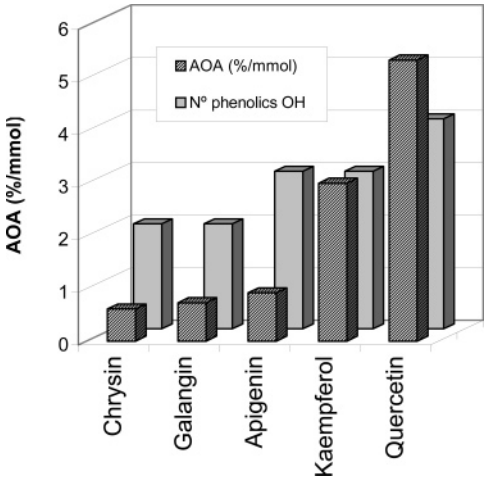

Figure 5. Comparative scheme of AOA of some flavonoids and their number of OH phenolic groups.

of DPPH• radical allow one to determine exclusively the intrinsic ability of a substance or a complex mixture to donate hydrogen atoms or electrons to this reactive species in a homogeneus system. This technique does not consider the partition in organized mediums or antioxidant mechanisms other than radical scavenging. These results cannot be directly extrapolated to biological systems, where the species are distributed in microheterogenous systems according to their lipophilicities.

The kinetic behavior of the radical disappearance in the presence of some phenolic compounds (galangin, quercetin, and caffeic acid), expressed as absorbance at 517 nm vs time, is presented in **Figure 4**.

The results obtained for the polyphenolic compound interaction toward DPPH• radicals show the highest scavenging activity for ellagic (3.5% $mmol^{-1}$) followed by quercetin, chlorogenic, 3,4-dihydroxybenzoic, and gallic acids, 2.9, 2.2, 2.2, and 2.1% $mmol^{-1}$, respectively. Moderate activities were presented by caffeic, ferulic, and sinapic acids and kaempferol (1.5, 0.9, 0.7, and 0.7% $mmol^{-1}$). Cinnamic, benzoic, 4-hydroxybenzoic, 4-hydroxy-3-methoxybenzoic, and coumaric acids and the flavonoids apigenin, galangin, and chrysin presented antiradical activity values $\leq$0.2% $mmol^{-1}$.

Chemical Structure−Activity Relation. Antioxidant activity of flavonoids strongly depends on their structure. In decreasing order of activity, they are as follows: quercetin > kaempferol > apigenin > galangin > chrysin. This order is related with the diminution of phenolic group number in the molecules (**Figure 5**), and it is consistent with the report of Burda and Oleszek measured by the thermally induced peroxidation (*26*). The most active compound is quercetin. This flavonol presents four phenolic hydroxyl groups (in the 5, 7, 3′, and 4′ positions) and a vinylic hydroxyl in position 3. This substitution pattern represents the three structural groups required for exhibiting this very high activity: the catechol group in the B-ring, the 2,3-double bond in conjugation with a 4-oxo functional group, and the presence of both 3- and 5-hydroxyl groups as informed by Benavente et al. (*11*).

Kaempferol, a flavonoid where the catechol present in quercetin is replaced by a single OH phenolic, has shown lower antioxidant activity confirming the importance of the catechol presence.

Although kaempferol and apigenin have the same number of phenolic hydroxyls, the former exhibits higher activity than the latter. This result suggests that the presence of the hydroxyl in C3 is also an important factor responsible for this behavior.

This methodology considers the inhibitory activity of LOX as well as the capacity of breaking the radical propagation chain; hence, this determined activity represents a comprehensive value.

According to Sadik et al. (*16*), a hydroxyl in C3 produces a diminution in the inhibitory activity toward an oxidative enzyme. In spite of its substitution pattern, our results suggest that the preponderant mechanism for the flavonoid kaempferol is the enzymatic inhibition and not the radical scavenging action considering the low ARA for this compound. Same behavior was found for sinapic where very high AOA and even low ARA values are observed. Finally, galangin and chrysin present the lowest activities attributed to the reduced degree of hydroxylation compared to the other analyzed flavonoids. These results are in agreement with Melo and Guerra report (*33*).

For the phenolic acids, in decreasing order the AOA found is as follows: sinapic > caffeic > chlorogenic > 3,4-dihydroxybenzoic > gallic > ellagic > ferulic > salicylic > coumaric > 4-hydroxy-3-methoxybenzoic > 4-hydroxybenzoic > benzoic > cinnamic acids. The highest antioxidant activity observed for sinapic acid is attributed to its substitution features: two methoxyl groups in positions 3 and 5 and a hydroxyl group in position 4. This is in accordance with the conclusions of Dziedzic and Hudson (*34*). Some evidence of the role of the double bond conjugated with the aromatic ring in the enhancement of the activity was found. The double bond characteristic of cinnamic derivatives (−HC═CH−COOH) are able to participate in the radical stabilization by resonance of the unpaired electron. Melo and Guerra (*33*) explain the higher activities shown by cinnamic derivatives compared with the benzoic acid group.

Figure 6 presents a comparative scheme of AOA and ARA for the compounds analyzed showing a general tendency where good free radical scavenging compounds have high comprehensive antioxidant capacity. ARA corresponds to one of the possible mechanisms consider in the AOA. The behavior of those compounds that do not follow this tendency can be explained in terms of their inhibitory activity toward LOX.

Ellagic and gallic acid present high free radical scavenging capacity in the homogeneous DPPH• solution but moderate activity in the micellar medium because of their limited distribution in the lipophilic phase having scarce participation in the lipid oxidation chain. Even though these polyphenols are

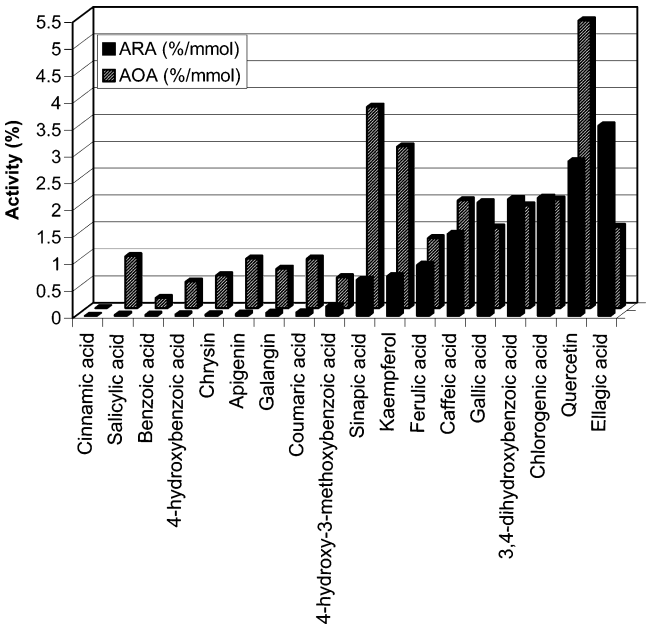

Figure 6. AOA and ARA determined for polyphenolic compounds.

distributed in the same phase as LOX, their inhibitory activity would be reduced by their structure limiting the access to the enzyme active site.

An enzymatic initiation by soybean lipoxygenase is proposed as an improvement of the β-carotene−linoleic acid cooxidation method. The modification proposed for this widely used technique has some advantages compared to the original thermally induced reaction. The main features of these improvements are there is a reduction of assay time, from 2 h to 10 min, the definition of the initial time of the reaction is very clear, and the reaction takes place at room temperature avoiding undesirable reactions making the experimental conditions more simple and gaining reproducibility. For these reasons the assay is a very useful technique for analyzing numerous samples in a short time. This determination also considers another possible mechanism of antioxidant action such as enzymatic inhibition closer to biological systems for other frequently used procedures.

ABBREVIATIONS USED

LOX, lipoxygenase; AOA, antioxidant activity; ARA, antiradical activity; DPPH•, 2,2-diphenyl-1-picrylhydrazyl.

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LITERATURE CITED

(1) Halliwell, B.; Gutteridge, J.M.C. In *Free Radicals in Biology and Medicine*, 2nd ed.; Oxford University Press: London, 1989.
(2) Gray, J. I. Measurements of lipid oxidation: a review. *J. Am. Oil Chem. Soc.* **1978**, *55*, 539−546.
(3) Chi, Y. S.; Jong, H. G.; Son, K. H.; Chang, H. W.; Kang, S. S.; Kim, H. P. Effects of naturally ocurring prenylated flavonoids on enzymes metabolizing arachidonic acid: cyclooxygenases and lipoxygenases. *Biochem. Pharmacol.* **2001**, *62*, 1185−1191.
(4) Yoon, S; Klein, B. P. Some properties of pea lipoxygenase isoenzymes *J. Agric. Food Chem.* **1979**, *27*, 955−962.
(5) Halliwell, B.; Gutteridge, J. M. C.; Cross, C. E. Free Radicals, Antioxidants and human diseases. Where are we now? *J. Lab. Clin. Med.* **1992**, *119*, 598−620.
(6) Trush, M. A.; Kensler, T. W. Role of free radicals in carcinogen activation. In *Oxidative stress. Oxidants and antioxidants*; Sies, H., Ed.; Academic Press: San Diego, CA, 1991; pp 277−318.
(7) Troll, W.; Wiesner, R. The role of oxygen radicals as a possible mechanism of tumour promotion. *Ann. Rev. Pharmacol. Toxicol.* **1985**, *25*, 509−528.
(8) Larson, R. A. Autooxidizable substances: weathering. In *Naturally Occurring Antioxidants*; Lewis Publishers: Boca Raton, FL, 1997; Chapter 1, pp 1−15.
(9) Rajalakshmi, D.; Narasimhan, S. Food antioxidants sources and methods of evaluation. In *Food antioxidants technological, toxicological and Health perspectives*; Madhavi, D. L., Deshpande, S. S., Salunkhe, D. K., Eds.; Marcel Dekker, Inc.: New York, 1996; Chapter 3, pp 65−73.
(10) Fauré, M.; Lissi, E.; Torres, R.; Videla, L. A. Antioxidant activities of lignans and flavonoids. *Phytochemistry* **1990**, *29*, 3773−3775.
(11) Benavente-García, O.; Castillo, J.; Marin F. R.; Ortuño, A.; Del Río, J. A. Uses and properties of *Citrus* flavonoids. Review. *J. Agric. Food Chem.* **1997**, *45*, 4505−4515.
(12) Foti, M.; Piattelli, M.; Baratta, M. T.; Ruberto, G. Flavonoids, coumarins, and cinnamic acids as antioxidants in a micellar system. Structure−activity relationship. *J. Agric. Food. Chem.* **1996**, *44*, 497−501.
(13) Potterat, O. Antioxidants and free radical scavengers of natural origin. *Curr. Org. Chem.* **1997**, *1*, 415−439.
(14) Chen, J. H.; Ho, C. T. Antioxidant activities of acid caffeic and its related hydroxycinnamic acid compounds *J. Agric. Food Chem.* **1997**, *45*, 2374−2378.
(15) Belitz, H. D.; Grosch, W. Lipids. In *Food Chemistry*, 2nd ed.; Springer-Verlag: Berlin, Heideberg, Germany, 1999; Chapter 3, pp 199−200.
(16) Sadik, C. D.; Sies, H.; Schewe, T. Inhibition of 15-lipoxygenases by flavonoids: structure-activity relations and mode of action. *Biochem. Pharmacol.* **2003**, *65*, 773−781.
(17) Kim, D. O.; Lee, K. W.; Lee, H. J.; L.; C. Y. Vitamin C equivalent antioxidant capacity of phenolic phytochemicals. *J. Agric. Food Chem.* **2002**, *50*, 3713−3717.
(18) Kulisic, T.; Radonic, A.; Katalinic, V.; Milos, M. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem.* **2004**, *85*, 633−640.
(19) Brand-Williams, W.; Cuvelier, M. E.; Berset, E. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25−30.
(20) Yamauchi, R.; Miyake, N.; Inoue, H.; Kato, K. Products formed by peroxyl radical oxidation of β-carotene *J. Agric. Food Chem.* **1993**, *41*, 708−713.
(21) Woodall, A. A.; Lee, S. W. M.; Weesie, R. J.; Jackson, M. J.; Britton, G. Oxidation of carotenoids by free radicals: relationship between structure and reactivity. *Biochim. Biophys. Acta* **1997**, *1336*, 33−42.
(22) Tsuchihashi, H.; Kigoshi, M.; Iwatsuki, M.; Niki, E. Action of β-carotene as an antioxidant against lipid peroxidation. *Arch. Biochem. Biophys.* **1995**, *323*, 137−147.
(23) Yamauchi, R.; Tsuchihashi, K.; Kato, K. Oxidation products of β-carotene during peroxidation of methyl linoleate in the bulk phase. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1301−1306.
(24) Kennedy, T. A.; Liebler, D. C. Peroxyl radical scavenging by β-carotene in lipid bilayers. *J. Biol. Chem.* **1992**, *267*, 4658−4663.
(25) Fukumoto, L. R.; Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 3597−3604.
(26) Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem.* **2001**, *49*, 2774−2779.
(27) Pesek, C. A.; Warthesen, J. J.; Taoukis, P. S. A Kinetic model for equilibration of isomeric β-carotenes. *J. Agric. Food Chem.* **1990**, *38*, 41−45.
(28) Robinson, D. S.; Wu, Z.; Casey, R.; Domoney, C. Lipoxygenases and the quality of foods. *Food Chem.* **1995**, *54*, 33−43.

(29) Yeum, K. J.; Lee-Kim, Y. C.; Yoon, S.; Lee, K. Y.; Park, I. S.; Lee, K. S.; Kim, B. S.; Tang, G. W.; Russell, R. M.; Krinsky, N. I. Similar metabolites formed from β-carotene by human gastric mucosal homogenates, lipoxygenase, or linoleic acid hydroperoxide. *Arch. Biochem. Biophys.* **1995**, *321*, 167−174.

(30) Skórzynska-Polit, E.; Krupa, Z. The Activity of Lipoxygenase in *Arabidopsis thaliana* (L.) Heynh-A Preliminary Study. *Cell. Mol. Biol. Lett.* **2003**, *8*, 279−284.

(31) Barimalaa, I. S.; Gordon, M. H. Cooxidation of β-carotene by soybean lipoxygenase *J. Agric. Food Chem.* **1988**, *36*, 685−687.

(32) Wu, Z.; Robinson, D. S.; Hughes, R. K.; Casey, R.; Hardy D.; West, S. I. Co-oxidation of β-carotene catalyzed by soybean and recombinant pea lipoxygenases. *J. Agric. Food Chem.* **1999**, *47*, 4899−4906.

(33) Melo, E.; Guerra, N. Açao antioxidante de compostos fenólicos naturalmente presentes em alimentos (Antioxidant action of polyphenolic compounds naturally occurring in food). *Boletin SBCTA* **2002**, *36*, 1−11.

(34) Dziedick, S. Z.; Hudson, B. J. F. Hydroxy isoflavones as antioxidants for edible oils. *Food Chem.* **1983**, *11*, 161−166.

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