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Commercial Dietary Antioxidant Supplements Assayed for Their Antioxidant Activity by Different Methodologies

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Seven commercial dietary antioxidant supplements were evaluated for their in vitro antioxidant capacity by different methodologies: antiradical activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH•), inhibition of methyl linoleate (MeLo) autoxidation, and resistance to ion-dependent oxidation of human low-density lipoprotein (LDL). Great variability in antioxidant activity was seen among the supplements, as well as different patterns of antioxidant capacity depending on the method used. The same orders of activity were found for the MeLo autoxidation and LDL-induced oxidation methods, but the differences recorded by the former were wider. "Food/compound intake equivalents" of the doses recommended by the manufacturers were calculated in terms of quercetin, strawberries, and red wine for each of the supplements studied. Food/compound intake equivalents varied between 7.9 and 190 mg of quercetin according to the DPPH• scavenging method, between 0.20 and 98 mg according to the MeLo autoxidation method, and between 3.4 and 83 mg according to LDL-induced oxidation. In equivalent terms of red wine, the food/compound intake equivalents varied between 7 and 159 mL, between 3 and 1354 mL, and between 4 and 89 mL for the same three methods. In terms of equivalents of strawberry, they varied between 14 and 343 mg according to the DPPH• scavenging method and between 57 g and 26 kg according to the MeLo autoxidation method. These results show the need to standardize dietary supplements in terms of their antioxidant capacity to match required doses to the oxidative status of consumers.

KEYWORDS: Dietary antioxidant supplements; DPPH•; methyl linoleate; LDL; polyphenols

INTRODUCTION

The homeostatic imbalance between the production of reactive oxygen species (ROS) and antioxidant defense systems determines the degree of oxidative stress suffered by cells. The production of too many ROS can result in damage to cell proteins, lipids, and DNA, whereas too few can interrupt the necessary physiological effects of oxidants on cell proliferation and host defenses (*1*, *2*). ROS have been implicated in the etiology of degenerative diseases including cardiovascular disease, cancer, neurodegenerative disorders, and aging (*3*). Several studies indicate that high consumption of fruits, vegetables, and beverages of vegetal origin (e.g., tea, wine, and cocoa) appears to reduce the risk of cancer and cardiovascular diseases (*4*). Fruits and vegetables are a rich source of antioxidant vitamins (C and E) and other phytochemicals with antioxidant properties (carotenoids, polyphenols, glutathione constituents, etc.). Apart from their direct antioxidant role, these compounds might also modify oxidant-regulated changes in gene expression (*5*).

Many new commercial products based on fruits, vegetables, or other plants rich in antioxidant compounds have been developed over the past decade and are sold as dietary antioxidant supplements. The marketing of these products claims "protection against free radicals", but most manufacturers offer scant information about their composition, and their labels include no data on their antioxidant power. Unfortunately, nutritional supplements are generally not subject to strict regulations; for example, European Union (EU) law sets no composition standards for nutritional supplements; it is up to each member state to decide how they are regulated in its own territory (*6*). In the United States, the ingredients used in dietary supplement may be sold without having to undergo the formal FDA-approval process required for drugs and food additives (*7*). However, authorities are aware of the lack of regulation in this area, and steps are being taken to solve the problem (*6*).

Because dietary supplements are formulated from different plant species, have different compositions, and have different concentrations of active ingredients, it is to be expected that they will vary widely in their antioxidant powers. However, because they are used by man to supplement the intake of natural antioxidants, their activity should be known and standardized. Standard analytical methods for determining antioxidant capacities should be adopted by both manufacturers—to standardize their products and to allow them to recommend an efficacious dose—and authorities—to ensure these products are controlled.

The literature contains many studies on the antioxidant activity of foods and ingredients of plant origin that could be

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Table 1. Composition of the Commercial Dietary Antioxidant Supplements and the Other Samples Studied

sample	recommended dose	composition	total polyphenols[a]
		Dietary Antioxidant Supplements	
1	1.2 g/day (3 tablets)	concentrate of grape seeds and rosemary	73 ± 1
2	1.2 g/day (3 tablets)	black grape concentrate supplemented with *trans*-resveratrol (0.025%)	7.3 ± 0.1
3	0.85 g/day (1 capsule)	*Vitis vinifera* L. grape (cv. Garnacha) extract (16.5%), wheat germ oil, and others	33 ± 2
4	1.2–1.8 g/day (2–3 tablets)	*Vitis vinifera* L. leave extract (50%), *Vitis vinifera* L. grape seed extract (11.7%)	101 ± 2
5	14.5–29 g/day (1–2 vials)	tomato concentrate (31 mg/vial), vitamin C (30 mg/vial), grape seed extract (25 mg/vial), yeast rich in selenium (13 mg/vial), vitamin E (4 mg/vial), and β-carotene (1.2 mg/vial) and others	0.98 ± 0.01
6	1.3–2.6 g/day (2–4 tablets)	citrus flavonoids (36%), *Ruscus aculeatus* L. (27%), *Ginkgo biloba* (18%), *Equisetum arvense* (9%), *Hamamelis virginiana* L. (4.5%), vitamin C (5.4%), and vitamin B_1 (0.13%)	27 ± 1
7	0.6 g/day (1 tablet)	vitamin C (10.3%), β-carotene (10.3%), citrus flavonoids (8.6%), L-cistein (8.6%), *Ginkgo biloba* extract (8.3%), manganese gluconate (5.8%), grape seed extract (3.4%), yeast rich in selenium (3.4%), vitamin E (3.4%), ZnO (2.1%), cooper gluconate (1.2%), and coenzyme Q10 (0.9%)	99 ± 1
		Other Samples	
strawberry extract			0.50 ± 0.01
white wine			232 ± 8
young red wine			1374 ± 3
oak-aged red wine			1835 ± 26

[a] Expressed as mg of gallic acid equiv (GAE)/g of sample for the dietary supplements and the strawberry extract and as mg of GAE/L for wines. Results are presented as the mean (n =3) ± SD.

used in supplement preparations. However, few references exist on the antioxidant activity of supplements already on the market (*8*), and most of these have used only one method to determine this potential (*9*). The purpose of this work was to evaluate the total antioxidant capacity of commercial dietary antioxidant supplements by different methodologies: antiradical activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH•), inhibition of lipid (methyl linoleate, MeLo) autoxidation, and resistance to in vitro ion-dependent oxidation of human low-density lipoprotein (LDL). The "food/compound intake equivalents" of the doses recommended by the manufacturers were calculated in terms of quercetin, a fruit (strawberry), and a fermented beverage of vegetable origin (red wine) for each of the products studied. Quercetin was chosen as a reference compound because its antioxidant properties have been widely studied. Strawberry was chosen as a red fruit widely consumed and red wine as a fruit-based product because of the increasing scientific interest in the "health-giving" properties of these products, especially wine.

MATERIALS AND METHODS

Chemicals. (+)-α-Tocopherol, quercetin, gallic acid, anhydrous cupric sulfate, and MeLo were purchased from Sigma (St. Louis, MO), 2,2-Diphenyl-1-picrylhydrazyl and 2,2,4-trimethylpentane (isooctane, spectrophotometric grade) were obtained from Aldrich (Milwaukee, WI), and methanol (HPLC grade) was from Lab-Scan (Dublin, Ireland).

Samples. A young red wine (var. *Graciano*) and an oak-aged red wine (var. *Tempranillo*) were provided by EVENA (Navarra, Spain). A *Rueda* white wine (*appelation controlé*, Valladolid, Spain), strawberries, and seven dietary antioxidant supplements were obtained from local markets. **Table 1** shows the composition of the dietary antioxidant supplements as indicated by the manufacturer.

Sample Preparation. Different quantities of dietary supplements were dissolved in methanol and centrifuged (2000 rpm, 10 min), and the supernatant was collected for analysis. Two different batches of each supplement were sampled. The strawberries (160 g) were washed, mashed, and macerated with 250 mL of methanol/formic acid (95:5, v/v) at room temperature for 1 day. The mixture was then filtered through a Whatman no. 2V filter and concentrated to a final volume of 25 mL. For analysis of the antioxidant activity according to the three methods, samples were diluted with methanol.

Determination of Total Polyphenols. Total polyphenol (TP) content was determined according to the Folin−Ciocalteu method (*10*), using gallic acid as a standard. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample for the dietary antioxidant supplements and the strawberry extract and as milligrams of GAE per liter for the wine samples.

Free Radical Scavenging Activity. The radical scavenging activity of the samples against DPPH• was measured using the method of Brand-Williams et al. (*11*), slightly modified as follows. Two milliliters of a methanolic solution of DPPH• (25 mg/L) was mixed with 50 μL solutions of different antioxidant concentrations in a 1 cm cuvette. Controls containing methanol instead of the antioxidant solution were also made. Absorbance at 515 nm was measured at 1 min intervals until the reaction reached a plateau (steady state). For each antioxidant concentration tested, the percentage of remaining DPPH• (% $DPPH_{rem}$) was calculated as follows:

$$\%\ DPPH_{rem} = [(A_{515nm})_{sample}/(A_{515nm})_{control}] \times 100$$

The percentage of remaining DPPH• was plotted against the sample concentration to obtain the C_{50}, defined as the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%. C_{50} was expressed as micrograms per milligram of DPPH• for the supplements, the pure compounds, and the strawberry extract and as micrograms of GAE per milligram of DPPH• for the wines. Three replicate experiments were performed for each sample.

Autoxidation of Methyl Linoleate. Modifications were made to the method proposed by Heinonen et al. (*12*), which has been used for antioxidant activity studies of berries, fruit, wines, and liquors. Autoxidation of MeLo was performed in open Eppendorf tubes in an oven at 40 °C, without stirring. To determine the optimum incubation time, the time course of the autoxidation of MeLo was initially examined. Sample aliquots (10 μL) were removed at times from 0 to 150 h, added to 990 μL of isooctane, and mixed vigorously. Ten microliters of this mixture was diluted with 2 mL of isooctane, and conjugated diene absorption was measured at 234 nm. The amount of

hydroperoxides formed was calculated using an absorptivity of 26000 AU (*13*). For autoxidation inhibition studies, 50 μL of methanolic antioxidant solutions was added to 50 mg of MeLo, and the methanol was evaporated under nitrogen. After 96 h of incubation, sample aliquots (10 μL) were removed and assayed for hydroperoxides as described above. For each antioxidant concentration tested, antioxidant activity was expressed as percentage inhibition of MeLo hydroperoxide formation. This was plotted against antioxidant concentration and adjusted to a polynomic equation. From this equation, the C_{50} parameter, defined as the amount of antioxidant necessary to inhibit hydroperoxide formation by 50%, was calculated and expressed as micrograms per milligram of MeLo for commercial supplements and standards and as micrograms of GAE per milligram of MeLo for wines. Three replicate experiments were performed for each sample.

To avoid interference, dietary supplement 5 and the strawberry extract were subjected to sugar elimination by solid-phase extraction (SPE) (*12*). SPE tubes (C_{18} Sep-Pak cartridge, Waters Associates, Milford, MA) were preconditioned with 2 mL of methanol and 2 mL of water. Samples (1 mL) were transferred to the SPE tube, and the tube was then washed with 5 mL of an aqueous solution of formic acid (2%). Phenolic antioxidants were eluted with methanol (5 mL).

LDL Isolation and Preparation. Blood was taken from a hypercholesterolemic patient by venopuncture and stored in tubes containing EDTA. Plasma was prepared by centrifuging the blood at 5000 rpm and 5 °C for 25 min. LDL was separated by vertical ultracentrifugation (50000 rpm, 10 °C, 2 h and 45 min) using a Beckman L70 with a VTi 50 vertical rotor (Beckman, Fullerton, CA).

For each set of oxidation experiments, EDTA was removed using an Econo-Pack 10 DG column (Bio-Rad, Hercules, CA) filled with P6 gel. LDL was eluted with 0.01 M PBS (0.15 M NaCl), pH 7.4. LDL protein concentration was determined according to the method of Bradford (*14*), using bovine serum albumin as a standard.

LDL Oxidation. The kinetics of lipid oxidation of human LDL (assessed by conjugated diene lipid hydroperoxide formation) were determined by monitoring the absorbance at 234 nm during copper-induced oxidation (*15*). Salt-free LDL, diluted with 0.01 M PBS (0.15 M NaCl), pH 7.4, to give a final concentration of 50 mg of protein/L, was mixed with the methanolic antioxidant solutions (10 μL) in a 1 cm capped cuvette. Oxidation was initiated by the addition of 10 μL of freshly prepared cupric sulfate at a final concentration of 20 μM. The final volume of the mixture was 1400 μL. A control, using methanol instead of the methanolic antioxidant solutions, was also tested. Incubations were performed at 37 °C and absorbance at 234 nm, measured every 5 min for a period of 12 h.

The time course of LDL oxidation shows three consecutive phases: a lag-phase during which the diene absorption increases slowly, a propagation phase with a rapid increase in diene absorption, and a decomposition phase (*15*). From this curve, several parameters can be determined: (a) *lag time*, the interval (minutes) between the intercept of the linear least-squares slope of the curve and the initial absorbance axis (a parameter determined graphically); (b) *maximum rate of oxidation* (V_{max}), calculated from the slope of the absorbance curve during the propagation phase (expressed as micromoles of dienes per minute × grams of LDL protein); (c) *maximum quantity of dienes formed* (CD_{max}) during the propagation phase (expressed as micromoles of dienes per gram of LDL protein); and (d) *maximum time* (t_{max}) (minutes) needed to reach CD_{max} during the propagation phase (*16*). **Figure 1** illustrates these parameters. The quantity of conjugated diene lipid hydroperoxides was calculated using an absorptivity of 29500 AU (*17*). Two replicate experiments were performed for each sample.

Calculation of the "Food/Compound Intake Equivalents". The food/compound intake equivalent of each dietary supplement, that is, the amount of a reference compound or food with the same antioxidant activity (assayed by the DPPH• scavenging and MeLo autoxidation methods), was determined for the daily doses recommended by their manufacturers:

$$\text{food/compound intake equivalents}_{(\text{suppl})} = [C_{50(\text{ref})}/C_{50(\text{suppl})}] \times \text{dose}_{(\text{suppl})}$$

For the LDL-induced oxidation method, the food/compound intake equivalent was determined by the equation

$$\text{food/compound intake equivalents}_{(\text{suppl})} = [C_{(\text{ref})}/C_{(\text{suppl})}] \times \text{dose}_{(\text{suppl})}$$

where C is the concentration of antioxidant that increases the lag time by 50%.

For comparative purposes, the food/compound intake equivalent of each commercial supplement was expressed as milligrams of quercetin, milliliters of wine, and grams of strawberries.

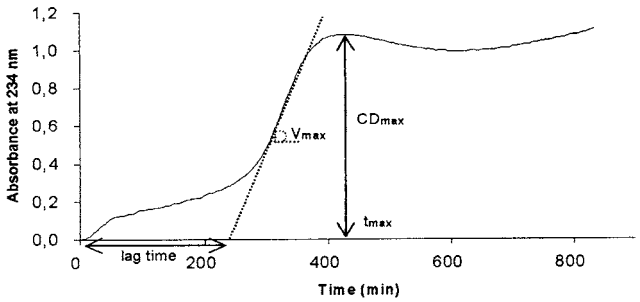

Figure 1. Definition of kinetic parameters determined in the LDL oxidation curve.

RESULTS AND DISCUSSION

Total Polyphenol Content. Dietary antioxidant supplements are made from different fruits, vegetables, and other plants rich in antioxidant phytonutrients and, as a result, their composition and the concentrations of their components differ. The antioxidant supplements studied showed large differences with respect to TP content: a 100-fold difference was recorded between sample 5 (0.98 mg of GAE/g) and samples 4 (101 mg of GAE/g) and 7 (99 mg of GAE/g) (**Table 1**). It should be noted that the Folin−Ciocalteu method, although widely used with plant extracts, is not specific for phenolic compounds and does suffer interference from other compounds (e.g., proteins).

Antioxidant Activity As Assessed by the DPPH• Scavenging Method. Although the antioxidant supplements may well have other biological activities, this study evaluated only their direct antioxidant capacity. **Table 2** reports this measured as free radical scavenging capacity against DPPH•. The higher the C_{50}, the lower the antiradical activity. The supplements showed large differences in their C_{50} values, ranging from 740 (sample 7) to 227,913 μg/mg of DPPH• (sample 5). The antiradical activities of the supplements followed the order $7 \geq 4 > 1 > 6 \geq 3 > 2 \gg 5$. The reference standards quercetin and (+)-α-tocopherol, respectively, showed C_{50} values of 82 and 375 μg/mg of DPPH•, in agreement with that indicated by other authors (*18*). The wines showed C_{50} values between 94 and 162 μg of GAE/mg of DPPH•, their antiradical activity ranking being oak-aged red wine > young red wine > white wine. These values are also in agreement with those reported by other authors (*19*). The greater antioxidant activity of red wines can be explained by the presence of anthocyanins, which are reported to have strong antioxidant power (*20*).

The wines and quercetin showed slow DPPH• neutralization kinetics [according to those leading up to the steady state (*18*)]. The dietary supplements showed different kinetics—slow for samples 1 and 3−5, intermediate for samples 6 and 7, and rapid for sample 2. Vitamin C, present in considerable concentration in samples 6 and 7, might be responsible for this behavior; rapid kinetics have been recorded for this compound by this method (*11*).

Table 2. Antiradical and Lipid Autoxidation Inhibitory Capacities of the Commercial Dietary Antioxidant Supplements and the Other Samples Studied

sample	DPPH• concn range (μg/mg of DPPH•)	DPPH• C_{50}[a] (μg/mg of DPPH•)	MeLo concn range (μg/mg of MeLo)	MeLo C_{50}[a] (μg/mg MeLo)
		Dietary Antioxidant Supplements		
1	500–5000	1958 ± 91	0.008–0.4	0.089 ± 0.013
2	2500–17500	12394 ± 186	1–100	15 ± 1
3	400–8000	2993 ± 61	0.08–4	0.56 ± 0.06
4	100–1000	775 ± 35	0.0125–0.2	0.050 ± 0.007
5	62500–500000	227913 ± 3484	22–438	333 ± 25
6	500–4000	2520 ± 396	1–20	4.0 ± 0.2
7	200–1000	740 ± 5	0.025–2	0.26 ± 0.05
		Others		
strawberry extract	63760–318800	147626 ± 9211	128–1275	724 ± 60
white wine	23–232	162 ± 18	0.019–0.926	0.17 ± 0.02
young red wine	28–197	118 ± 6	0.014–0.275	0.058 ± 0.008
oak-aged red wine	31–184	94 ± 6	0.009–0.184	0.061 ± 0.002
quercetin	15–121	82 ± 3	0.00125–0.0375	0.0026 ± 0.0002
(+)-α-tocopherol	70–560	375 ± 15	0.0005–0.02	0.0047 ± 0.0005

[a] C_{50} is the sample amount that produces a 50% reduction of the initial DPPH concentration and inhibits MeLo hydroperoxide formation by 50%. Results are presented as the mean ($n = 3$) ± SD. For wines, C_{50} is expressed as μg of gallic acid equiv (GAE)/mg of DPPH and μg of GAE/mg of MeLo.

Antioxidant Activity As Assessed by the MeLo Autoxidation Method. The kinetics of MeLo autoxidation at 40 °C were determined in the dark, and the formation of hydroperoxides was monitored until linear production was reached (between 400 and 800 mmol/kg of MeLo) (**Figure 2a**). On the basis of these results, an incubation time of 96 h was selected for autoxidation inhibition experiments. Repeatability of the control experiment (without antioxidant) was >95%. The antioxidant activities of some plant materials against MeLo autoxidation have been studied (*12*, *21*). In these investigations, antioxidant activity is expressed as percent inhibition of hydroperoxide formation at a particular concentration of antioxidant. However, this might limit or even prevent comparison of antioxidants in the same or different surveys, especially if the percent inhibition is close to 0 or 100%. To solve this problem, different antioxidant concentrations were tried and the results expressed as the amount of antioxidant necessary to inhibit hydroperoxide formation by 50% (C_{50}). Sugars have been reported to interfere with the MeLo autoxidation method (*12*). In the present study it was not necessary to remove sugars from the wines, but it was required for dietary supplement 5 and the strawberry extract.

Panels b−h of **Figure 2** show MeLo autoxidation inhibition curves for the antioxidant supplements. Different trends for the percent inhibition versus concentration curves can be seen, due to variation in the composition of these products. For supplements 1, 2, and 4−7, the curves fit a second-degree polynomial equation, whereas the curve for supplement 3 fits a third-degree polynomial equation. These results underscore the fact that samples cannot be accurately compared if antioxidant activity is tested at only one concentration. For the supplements and the reference standards, C_{50} was expressed as micrograms per milligram of MeLo; for the wines, it was expressed as micrograms of GAE per milligram of MeLo (**Table 2**). Among the dietary supplements, the C_{50} varied from 0.05 (supplement 4) to 333 (supplement 5) and the inhibitory capacity decreased in the following order: $4 > 1 > 7 > 3 > 6 > 2 \gg 5$. Among the wines, the oak-aged ($C_{50} = 0.061$) and the young ($C_{50} = 0.058$) red wines showed activity similar to and greater than that of the white wine ($C_{50} = 0.17$ μg of GAE/mg of MeLo). As mentioned above, these differences between the red and white wines might be due to the presence of anthocyanins and other compounds extracted from the grape skins during red wine fermentation. The slight differences observed between young and oak-aged red wines may indicate the contribution of antioxidants from the oak barrels during aging (gallic acid, gallotannins, and guaiacyl compounds), although this should be checked with wines aged for different times in the same barrels and for different oak barrels during the same time. Quercetin and (+)-α-tocopherol showed C_{50} values of 0.0026 and 0.0047 μg/mg of MeLo, respectively.

Antioxidant Activity As Assessed by the LDL-Induced Oxidation Method. The formation of hydroperoxides during the oxidation of LDL with Cu^{2+} in the absence and in the presence of an antioxidant is shown in **Figure 3**. In addition to the lag time (*15*), other parameters or indices also obtained from the time course kinetics of ion-induced LDL peroxidation (**Figure 1**) describe the potential for LDL oxidization and may allow differences in the efficiency of antioxidant capacity to be assessed (*16*): V_{max}, CD_{max}, t_{max} (see Materials and Methods for definition). In the absence of any antioxidant, the average values during LDL oxidation were 236 ± 7 min for lag time, 5.7 ± 0.3 for V_{max}, 764 ± 34 for CD_{max}, and 430 ± 16 for t_{max}. **Table 3** shows the results for the antioxidant supplements at three different concentrations that increase the lag time by approximately 0, 50, and 100%. **Table 4** shows results for the strawberry extract, wines, and reference standards, also at different concentrations. Although slight pro-oxidant effects were observed at the lower concentration for supplements 1−5, oak-aged red wine, quercetin, and α-tocopherol, increasing antioxidant concentration resulted in a dose-dependent increase of lag time and t_{max}, as well as a progressive shortening of V_{max} and CD_{max}. The trend in the variation of some LDL oxidation kinetic parameters (lag time, V_{max}, and CD_{max}) provides information on the mechanism of the inhibitory action of a given antioxidant (*22*). If the antioxidant prolongs the lag time and reduces the maximum rate (V_{max}) without modifying the maximum accumulation of oxidation products (CD_{max}), the mechanism involves either the binding of copper ions or the blocking of LDL copper-binding sites. If the antioxidant prolongs the lag time without affecting the V_{max} and CD_{max}, the mechanism involves the scavenging of free radicals. A reduction in CD_{max} indicates that the antioxidant catalyzes nonradical decomposition of hydroperoxides. Accordingly, the trend observed in the kinetic parameters of quercetin LDL oxidation (**Table 4**) indicates that this antioxidant acts as scavenger of free radicals. However, the trends observed in the

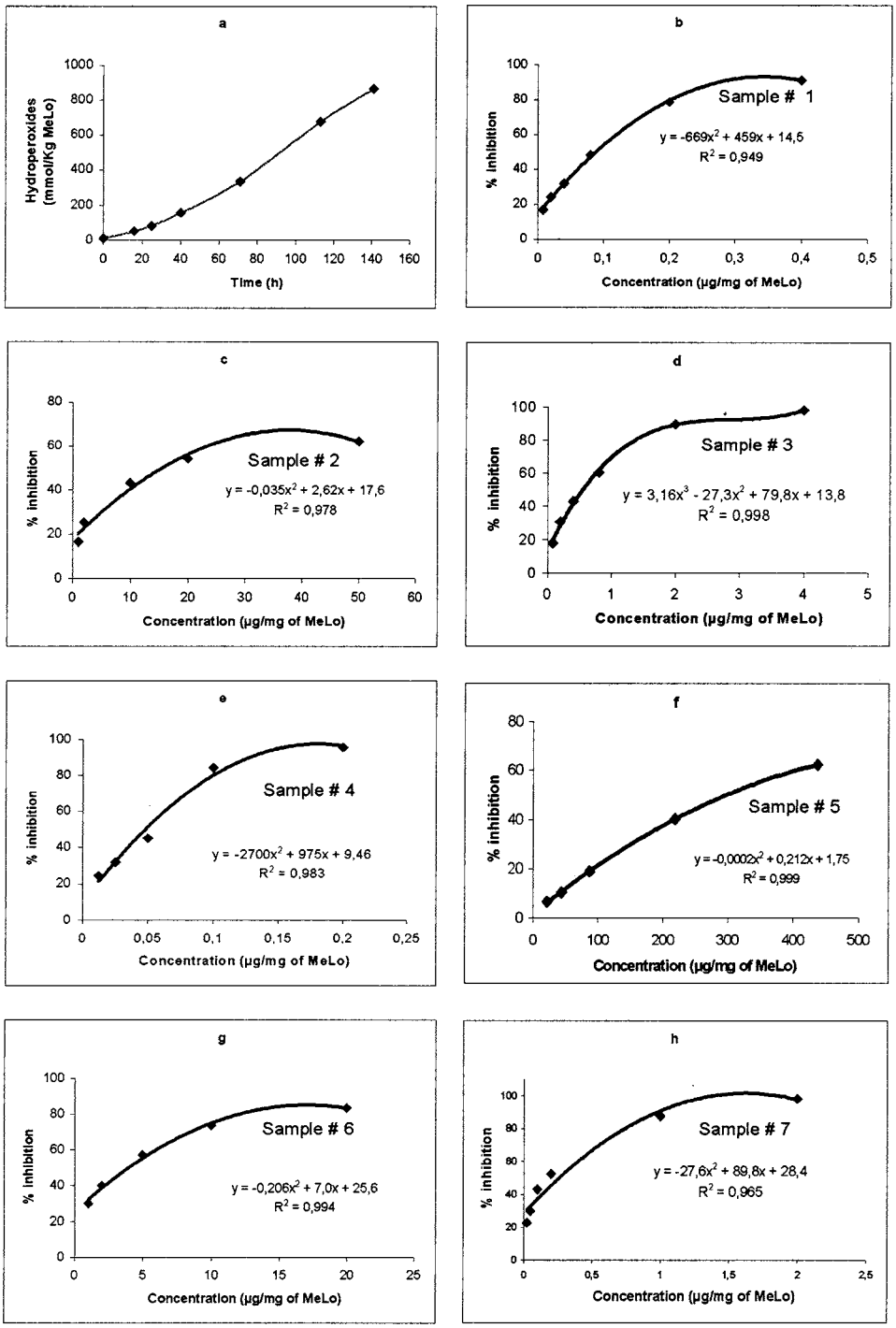

Figure 2. Autoxidation of methyl linoleate (MeLo): (a) time course in the dark at 40 °C; (b–h) lipid autoxidation inhibition curves (percent inhibition versus antioxidant concentration) for the commercial antioxidant supplements studied. Values are the mean ($n = 3$).

kinetic parameters of the supplements, wines, and strawberry extract (**Tables 4** and **5**) followed none of the specific patterns mentioned above, indicating that inhibition occurs through mixed mechanisms of free radical scavenging, binding of metals, and catalysts of nonradical decomposition of the oxidation products. These samples contain many active substances with different antioxidant mechanisms able to act synergistically.

The antioxidant activity of the dietary supplements, measured as the concentration that delays the lag time by ~50%, decreased in the order 4 > 1 > 7 > 3 > 6 > 2 ≫ 5 (**Table 3**). Quercetin prevented LDL oxidation at concentrations similar to those reported by other authors (**Table 4**) (*16*). Besides inhibiting LDL oxidation, flavonoids such quercetin may protect (+)-α-tocopherol from being consumed during LDL oxidation (*23*). Prooxidant activity at low concentrations of flavonoids has been reported (*24*). Although (+)-α-tocopherol plays an important role in inhibiting lipid peroxidation (*25*), the present results show no activity at the concentration tested (20 μM). This agrees with results reported by Vinson et al. (*26*) and Sánchez-Moreno et al. (*16*). The lipophilic characteristics of (+)-α-tocopherol make it difficult for it to incorporate into the LDL (a protein), which is isolated in an aqueous solution. The antioxidant activity of the wines, measured as the concentration that delays the lag time by ~50%, decreased in the order young red wine ≥ oak-aged red wine > white wine. These results are also in agreement with those published by Sánchez-Moreno et al. (*16*).

Table 3. LDL Oxidation Inhibitory Capacity of the Commercial Dietary Antioxidant Supplements

sample	concn[a] (mg/mg of LDL)	lag time increase[b] (min)	V_{max}[c] (μmol/min·g of LDL)	CD_{max}[d] (μmol/g of LDL)	t_{max} increase[e] (min)
1	0.14	−6.2 ± 23	5.5 ± 0.6	738 ± 72	−2.5 ± 46
	0.20	56 ± 17	4.8 ± 0.1	657 ± 3	75 ± 1
	0.29	132 ± 33	4.2 ± 0.1	634 ± 14	185 ± 50
2	3.57	−58 ± 8	9.9 ± 0.1	723 ± 7	−60 ± 6
	4.76	120 ± 38	8.9 ± 0.7	716 ± 17	128 ± 4
	7.14	299 ± 17	7.7 ± 0.5	715 ± 4	305 ± 42
3	0.29	−16 ± 5	4.6 ± 0.3	710 ± 12	−5 ± 2
	0.57	65 ± 5	3.8 ± 0.1	675 ± 14	123 ± 4
	1.14	341 ± 79	2.3 ± 0.3	nd[f]	nd
4	0.07	−8 ± 21	5.6 ± 0.1	727 ± 29	25 ± 14
	0.14	52 ± 12	5.1 ± 0.3	698 ± 5	95 ± 21
	0.29	183 ± 45	3.9 ± 0.2	656 ± 6	248 ± 67
5	7.7	−44 ± 13	6.7 ± 0.4	720 ± 28	−65 ± 19
	28.6	105 ± 32	6.1 ± 0.3	627 ± 10	172 ± 29
	38.0	358 ± 46	5.0 ± 0.5	579 ± 18	380 ± 45
6	0.71	6 ± 3	4.5 ± 0.1	697 ± 16	73 ± 32
	0.95	129 ± 33	3.9 ± 0.1	682 ± 6	215 ± 21
	1.43	304 ± 7	2.1 ± 0.1	647 ± 8	475 ± 10
7	0.29	30 ± 6	5.6 ± 0.2	693 ± 16	68 ± 4
	0.48	189 ± 32	4.3 ± 0.2	696 ± 4	235 ± 35
	0.71	405 ± 21	2.9 ± 0.2	671 ± 30	470 ± 24

[a] Concentration is expressed as mg of supplement/mg of protein. [b] Lag time delay with respect to the control. [c] Maximum rate of oxidation. [d] Maximum quantity of dienes formed. [e] Maximum time delay with respect to the control. Results are presented as mean ($n = 2$) ± SD. [f] nd = not determined.

Table 4. LDL Oxidation Inhibitory Capacity of the Strawberry Extract, Wines, and Standards Studied

sample	concn[a] (mg/mg of LDL)	lag time increase[b] (min)	V_{max}[c] (μmol/min·g of LDL)	CD_{max}[d] (μmol/g LDL)	t_{max} increase[e] (min)
strawberry extract	18.2	16 ± 5	5.8 ± 0.2	637 ± 14	100 ± 21
	22.8	31 ± 8	6.3 ± 0.2	691 ± 20	20 ± 5
white wine	0.033	59 ± 6	9.7 ± 1.6	723 ± 53	23 ± 3
young red wine	0.0098	8 ± 2	4.7 ± 0.3	716 ± 25	50 ± 12
	0.0196	121 ± 41	3.7 ± 0.3	709 ± 59	223 ± 4
	0.0280	307 ± 29	2.6 ± 0.2	596 ± 15	420 ± 38
oak-aged red wine	0.0131	−9 ± 2	4.9 ± 0.1	768 ± 22	15 ± 3
	0.0262	148 ± 23	3.3 ± 0.3	674 ± 16	235 ± 32
	0.0328	251 ± 55	2.3 ± 0.1	580 ± 33	408 ± 25
quercetin	0.0091	47 ± 4	6.2 ± 0.1	724 ± 2	40 ± 21
	0.0133	151 ± 39	5.8 ± 0.1	722 ± 7	188 ± 103
(+)-α-tocopherol	0.1723	−30 ± 8	3.1 ± 0.1	679 ± 20	60 ± 7

[a] For wines, concentration is expressed as mg of GAE/mg of protein. [b] Lag time delay with respect to the control. [c] Maximum rate of oxidation. [d] Maximum quantity of dienes formed. [e] Maximum time delay with respect to the control. Results are presented as mean ($n = 2$) ± SD.

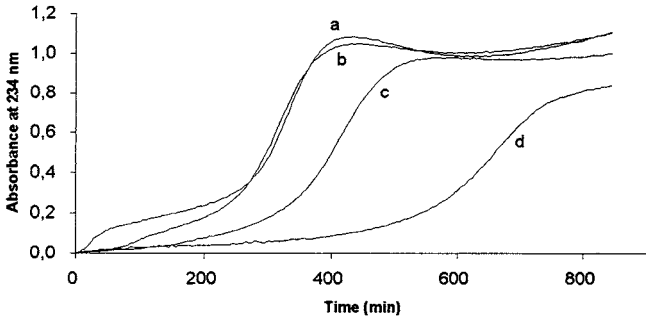

Figure 3. Oxidation of LDL with Cu^{2+} in the absence (a) and presence of 0.29 (b), 0.57 (c) and 1.14 (d) mg of supplement 3/mg of LDL.

Increasing evidence suggests that oxidative modification of lipoproteins is involved in the pathogenesis of atherosclerosis (*27*, *28*). Although in vitro studies with antioxidants can provide useful initial evidence of the potential biological effects on LDL oxidation and coronary heart disease (CHD), the biological importance of in vitro LDL oxidation is uncertain. The natural environment in which LDL oxidation may be expected to take place is difficult to reproduce, and it is questionable whether the concentrations used in in vitro studies can be reached in human plasma.

The relative antioxidant activity of a compound may vary according to different testing methods and the substrates to be protected (*29*). Dietary supplements composed of natural antioxidants and plant extracts are complex and multifunctional, so the measurement of their antioxidant activity by more than one method (based on different antioxidant mechanisms) is highly recommended. In the present study, the MeLo autoxidation and the LDL-induced oxidation methods gave the same antioxidant order for the seven dietary supplements studied (see above), although larger differences among supplements were recorded by the former. This order also corresponds to the polyphenol content of the supplements (**Table 1**). The DPPH• scavenging method gave a different antioxidant order, assigning relatively greater activity to supplement 7 than to supplement 4 or 1 and to supplement 6 compared to supplement 3. This discrepancy was attributed to the presence, in samples 7 and 6, of vitamin C, which exhibits strong antiradical action in the DPPH• method (*11*, *18*) but is almost insoluble in lipid systems.

Calculation of the Food/Compound Intake Equivalents. In an attempt to compare the potential beneficial effects of the

Table 5. Food/Compound Intake Equivalents of the Commercial Dietary Antioxidant Supplements Studied

	DPPH• scavenging				MeLo autoxidation				LDL-induced oxidation		
sample	quercetin[a]	young red wine[b]	oak-aged red wine[b]	straw-berries[c]	quercetin[a]	young red wine[b]	oak-aged red wine[b]	straw-berries[c]	quercetin[a]	young red wine[b]	oak-aged red wine[b]
1	50	53	31	91	35	566	445	9762	55	59	59
2	7.9	8	5	14	0.20	3	3	57	3.4	3.6	3.6
3	23	24	14	42	4.0	64	50	1099	10	11	11
4	190	200	118	343	98	1512	1188	26064	83	89	89
5	10	11	6	19	0.23	4	3	63	13	14	14
6	85	89	53	152	1.7	28	22	477	36	39	39
7	67	70	41	120	6.0	96	76	1658	17	18	18

[a] Amount of quercetin (mg) that exhibits the same antioxidant activity as the daily recommended dose of the dietary supplement. [b] Amount of wine (mL) that exhibits the same antioxidant activity as the daily recommended dose of the dietary supplement. [c] Amount of strawberries (g) that exhibits the same antioxidant activity as the daily recommended dose of the dietary supplement.

dietary supplements to those provided by known antioxidant compounds and foods of plant origin, their food/compound intake equivalents were calculated. The food/compound intake equivalent was defined as the amount of pure antioxidant compound or food that exhibits the same antioxidant activity (assayed by the three methods) as the daily dose of the supplement recommended by the manufacturer. Quercetin, a phenolic compound for which antioxidant properties and bioavailability have been most thoroughly studied, was chosen as a reference antioxidant. Epidemiological studies indicate that this flavonoid may reduce the risk of death from CHD in elderly men (*30*). Strawberries were selected as a reference food because other epidemiological observations have associated the consumption of fruit with low rates of chronic disease. Wine, a fermented beverage of grapes, was also selected for comparison because of its importance in a healthy diet and its richness in polyphenols. Samples of the types of wine most consumed (white wine, young red wine, and oak-aged red wine) were analyzed. The red wines showed a better antioxidant activity than the white wine, which agrees with the results of previous studies (*31*) and might be attributed to their phenolic composition (*32*). The low CHD mortality observed in France, even though the French consume high amounts of fat (the "French paradox"), is commonly attributed to the consumption of red wine (*33*). Moderate alcohol consumption is also associated with a reduction of CHD (*34*, *35*).

Supplement 4 showed the highest food/compound intake equivalents and supplement 2 the lowest for the three methods tested, although differences were seen depending on the method used [~25-fold for the DPPH• scavenging and LDL-induced oxidation methods and almost 500-fold for the MeLo autoxidation method (**Table 5**)]. According to the DPPH• scavenging method, the food/compound intake equivalents decreased in the following order: 4 > 6 > 7 > 1 > 3 > 5 > 2. For the MeLo autoxidation method, the ranking was 4 > 1 > 7 > 3 > 6 > 5 > 2. For the LDL-induced oxidation method, supplement antioxidant capacity decreased in the order 4 > 1 > 6 > 7 > 5 > 3 > 2.

The food/compound intake equivalents of supplement 4 were 190 mg of quercetin, as assessed by the DPPH• scavenging method, 98 mg by the MeLo autoxidation method, and 83 mg by the LDL-induced oxidation method (**Table 5**). The food/compound intake equivalents of supplement 4 were 159 mL of red wine (mean of oak-aged and young red wines) as assessed by the DPPH• scavenging method, 1354 mL as assessed by the MeLo autoxidation method, and 89 mL as assessed by the LDL-induced oxidation method (**Table 5**). The food/compound intake equivalent of supplement 4 was 343 mg of strawberries, as assessed by the DPPH• scavenging method, or 26 kg as assessed by the MeLo autoxidation method (**Table 5**). Differences in the composition and concentrations of compounds making up the supplements may have been responsible for the large differences recorded for them by the different methods. These results also emphasize the importance of using more than one method to evaluate the antioxidant activity of supplements that might show different biological activities.

Final Remarks. Although many researchers have found that diets rich in antioxidants such as vitamins and phenolic compounds (such as flavonoids) (*30*, *36*) reduce the risk of CHD, some randomized studies (HOPE, GISSI, PPP, HATS, and MPS) indicate no benefit from treatment with certain vitamins (reviewed in ref *37*). Because recommendations to take dietary antioxidant supplements require convincing proof of a positive effect, and considering that lowering ROS levels below the homeostatic set point may interrupt the physiological role of oxidation in cell proliferation and host defenses (*1*, *2*), patient oxidant status should be studied before any recommendations are made.

In conclusion, this paper shows that the in vitro antioxidant activity of commercial dietary antioxidant supplements varies considerably from one product to another—something expected given the different botanical origins of their components, and their different preparation processes, formulations, and concentrations. These supplements showed different antioxidant activity patterns depending on the assay method. Although they showed the same overall pattern, MeLo and LDL did demonstrate differences in the range of variability. The food/compound intake equivalent provided by most of the supplements was, in most cases, higher than what a typical Western diet could contribute in terms of flavonoids [17−50 mg/day (*38*, *39*)], although bioavailability has to be taken into consideration. The need to standardize dietary antioxidant supplements with respect to their antioxidant capacity is critical if effective doses are to be recommended for a person's current oxidative status.

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