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Antioxidant capacity of the Spanish Mediterranean diet

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Abstract

The objective of this work was to determine the total dietary antioxidant capacity (TDAC) of the Spanish Mediterranean diet. The antioxidant capacity of plant foods and beverages included in National food consumption data was determined. TDAC of the Spanish diet was estimated at 6014 and 3549 μmol trolox equivalents by FRAP (ferric reducing antioxidant power) and ABTS (free radical-scavenging capacity) procedures, respectively. About 68% of TDAC came from beverages and 20% from fruits and vegetables, with a very low contribution from cereals. The capacity to inhibit *in vitro* LDL oxidation of plant foods and beverages was consistent with their antioxidant capacity. The recommended daily intakes of antioxidant vitamins, C and E, represent about 10% of TDAC. Total phenolics intake was estimated as 1171 mg gallic acid/person/day by the Folin–Ciocalteau method. TDAC may be a parameter to be considered in nutritional and epidemiological studies.

Keywords: Food antioxidant capacity; Total dietary antioxidant capacity; Total phenolics intake; Dietary antioxidants; Spanish Mediterranean diet

1. Introduction

There is growing scientific evidence that dietary antioxidants may be a critical mediator of the beneficial effects of the Mediterranean diet (Trichopoulou & Lagiou, 2001). Dietary antioxidants are able to neutralise oxygen free radicals and inhibit LDL oxidation, and they may protect against coronary heart disease, cancer and neurodegenerative diseases. The abundance of seasonally fresh and minimally processed beverages and plant foods provides a wide variety of dietary antioxidants, such as vitamins C and E, carotenoids, flavonoids and other phenolic compounds. The additive and synergistic effects of these antioxidant compounds may contribute to the health benefits of the diet (Liu, 2003). In the past two decades, numerous biochemical and clinical studies have provided consistent evidence of the healthy properties of foods such as olive oil, red wine, fish, citrus and legumes (Stark & Madar, 2002). Indeed, there is a wealth of articles dealing with the antioxidant capacities (AC) of individual foods and isolated food antioxidants in the literature. In this connection, the contribution of beverages to the intake of antioxidants in the Spanish diet was recently reported (Pulido, Hernández-García, & Saura-Calixto, 2003). However, to our knowledge, there are no studies on AC of whole diets. We believe that a more comprehensive view of this field may be gained by examining the antioxidant capacity of diets rather than of single nutrients or foods.

The objective of this work was to determine the total dietary antioxidant capacity (TDAC) of the Spanish Mediterranean diet.

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2. Materials and methods

2.1. Food intake and samples

Estimates of plant food and beverage intakes in the Spanish diet were based on National consumption data (MAPA, 2001). These data are obtained annually from daily budget questionnaires. Six thousand households are surveyed, along with 700 hotels and restaurants and 200 institutions, such as schools, hospitals and the armed forces (confidence level 95%; error range 2% in amount of food). Dietary intakes included in Table 1 correspond to the average Spanish diet.

Two purchases of each individual plant food and beverage listed in the National dietary survey (Table 1) (MAPA, 2001) were acquired at different local supermarkets. Individual items selected in this study are representative of plant food and beverage common in the Spanish diet.

The edible portion of the daily amount consumed, *per capita*, for each plant food as eaten (Table 1) was weighed and grouped into five duplicate samples, one for each of the five types of plant foods: cereals (total: 221.6 g), vegetables (total: 280.8 g), legumes (total: 22.3 g), nuts (total: 5.9 g) and fruits (total: 200.8 g). These five samples correspond to the total *per capita* daily intake of solid plant foods in the Spanish diet. Each duplicated sample was freeze-dried, ground and stored *prior to* analysis.

Beverage samples were the following: red wine (Bodegas Felix Solis, Valdepeñas, Spain); white and rose wine (Bodegas N^a S^a Concepción, Madrid, Spain); beer (*Aguila-Amstel*, 5% alcohol, Heineken Spain); coffee: Colombian chicory coffee (*Cafés la Mexicana*, Rodriguez y Mateus S.A., Madrid, Spain); tea (*Lipton yellow label* quality no. 1 from Unilever Belgium N.V., London, England); cola, *Coca-Cola*, (Coca-Cola S.A., Madrid, Spain); orange juice, 100% orange juice (Juver Alimentación S.A., Murcia, Spain); vegetable oils were olive oil (*Carbonell*, Córdoba, Spain) and sunflower oil (*Koipesol*, Koipe, S.A., Jaen, Spain).

Beverages and vegetable oils were individually analysed. Only coffee and tea infusions required a previous preparation. Commercially available infusions were prepared as follows: one tea bag (1.5 g) was infused for 5 min in 250 ml of hot water; soluble coffee was prepared with 26.2 g of ground coffee in 325 ml of hot water.

2.2. Food antioxidant extraction

One gramme of ground plant food sample (duplicate) was placed in a test tube; 40 ml of methanol/water (50:50, v/v) and 2 N HCl, to obtain a pH 2.0, were added, and the tube was thoroughly shaken at room temperature for 1 h. The tube was centrifuged at 2500*g* for 10 min, and the supernatant was recovered. Forty millilitres of acetone/water (70:30, v/v) were added to the residue, followed by shaking and centrifugation. Both methanol and acetone extracts were combined.

Two grammes of the oils were mixed with 2 ml of methanol and the mixture was vigorously stirred for 30 min and further centrifuged at 2500*g*. The methanolic phase was removed and the extraction was repeated with 2 ml of methanol. Both methanol extracts were combined.

Methanol–acetone extracts of plant food, methanol extracts of oils and aliquots of beverages were used as test samples to determine total phenolic and antioxidant activity (triplicate).

2.3. Antioxidant activity assays

2.3.1. FRAP (ferric reducing antioxidant power) assay (Pulido, Bravo, & Saura-Calixto, 2000)

FRAP reagent (900 μl), freshly prepared and warmed at 37 °C, was mixed with 90 μl distilled water and either 30 μl of test sample or standard or appropriate reagent blank. Readings, at the absorption maximum (595 nm), were taken every 15 s. The readings at 4 and 30 min were selected for calculation of FRAP values. Methanolic solutions of known trolox concentrations were used for calibration.

2.3.2. ABTS assay (Re et al., 1999)

ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{+\cdot}$ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 658 nm. After addition of 100 μl of sample or trolox standard to 3.9 ml of diluted $ABTS^{+\cdot}$ solution, absorbance readings were taken every 20 s. Reaction was monitored during 6 min. The percentage inhibition of absorbance vs. time was plotted and the area below the curve (0–6 min) was calculated. Methanolic solutions of known trolox concentrations were used for calibration.

2.3.3. In vitro copper-induced oxidation of human low-density lipoprotein assay (Sánchez-Moreno, Jiménez-Escrig, & Saura-Calixto, 2000)

Low-density lipoprotein (LDL) was obtained from Ramón y Cajal Hospital, Madrid, Spain. The plasma was collected from a patient with homozygous familial hypercholesterolemia. LDL hydroperoxidation was estimated on the basis of formation of conjugated dienes by measuring the change in absorbance at 234 nm. This assay was carried out with extract from solid plant foods (1 g dry edible portion/100 ml).

Table 1
Intake of plant foods, oils and beverages in the Spanish diet (2000 year)

	g fresh matter/ day/person	g edible proportion/ day/person
Cereals		
Rice[a]	16.7	16.7
White bread	148.2	148.2
White bread sliced	11.5	11.5
Spaghetti[a]	11.2	11.2
Biscuits	17.0	17.0
Croissants	17.0	17.0
Total	221.6	221.6
Vegetables		
Potatoes[a]	132.8	119.5
Tomatoes	44.3	41.6
Tomatoes transformed	18.4	18.4
Onions	23.5	20.2
Garlic	4.6	3.5
Cabbage[a]	5.1	4.0
Green beans[a]	8.1	7.4
Cucumber	6.8	5.2
Capsicum	14.1	11.5
Mushrooms	3.8	3.0
Lettuce	22.1	13.3
Asparagus	1.8	1.1
Spinach[a]	3.5	2.8
Chard[a]	3.5	2.4
Others[b]	38.4	26.4
Total	330.9	280.8
Nuts		
Almonds	0.8	0.8
Peanuts	1.1	1.1
Walnuts	1.1	1.1
Others[b]	3.8	2.9
Total	6.8	5.9
Fruits		
Oranges	63.9	46.6
Mandarin oranges	16.2	11.7
Bananas	26.6	17.6
Apples	35.4	29.7
Pears	20.9	18.4
Peaches	13.5	12.4
Apricots	3.3	3.1
Strawberries	6.3	6.0
Melon	22.0	13.2
Watermelon	15.9	8.4
Plums	3.9	3.3
Cherries	3.32	2.9
Grapes	6.9	6.2
Kiwi	7.2	6.2
Olives	8.8	7.0
Others[b]	11.2	8.1
Total	265.7	200.8
Legumes		
Chickpeas[a]	7.9	7.9
Beans[a]	6.8	6.8
Lentils[a]	7.6	7.6
Total	22.3	22.3
Oils (ml)		
Olive	31.2	31.2
Sunflower	20.8	20.8
Others[b]	4.4	4.4
Total	56.4	56.4
Beverages (ml)		
Coffee	119.1	119.1
Tea	16.7	16.7
Wine		
Red	48.5	48.5
White	26.0	26.0
Rose	15.1	15.1
Beer	150.4	150.4
Fruit juices	47.4	47.4
Cola drinks	81.7	81.7
Total	504.9	504.9

[a] Boiled.
[b] Others: Vegetables: artichoke[a], carrot, tender pumpkin[a], celery[a], aubergine[a], turnip[a], leek[a], pumpkin[a], beet root[a], avocado; Nuts: hazelnuts, pistacho; Fruits: pomegranate, mango, pineapple, grapefruit, caqui, chirimoya; Oils: corn, soya.

2.4. Total phenolics

Total phenolics were estimated in methanol–acetone extracts from solid samples, in methanol extracts from vegetable oils and in aliquots of beverages by the Folin–Ciocalteau method (Montreau, 1972). Test sample, (0.5 ml) was mixed with 1 ml of Folin–Ciocalteau reagent and swirled. After 3 min, 10 ml of sodium carbonate solution (75 g/l) were added and mixed. Additional distilled water was mixed throughly by inverting the tubes several times. After 1 h, the absorbance at 750 nm was recorded. The results were expressed as gallic acid equivalents.

2.5. Statistical analysis

Results were expressed as mean values ± standard deviation. Comparison of the means of three measurements using a significance level of $P < 0.05$ was performed by one-way analysis of variance (ANOVA) using the Statgraphics Computer System, version 5.1.

3. Results and discussion

The intake of plant foods, beverages and vegetable oils in the Spanish diet is shown in Table 1. The Spanish diet is especially rich in a wide range of fruits and vegetables. The most widely consumed alcoholic beverages are wine and beer, while coffee, fruit juices and colas are the main non-alcoholic beverages. Olive oil is the major source of fat. These foods and beverages provide

a significant amount and variety of antioxidants. The synergistic action of these food antioxidants may be a significant factor in biological effects and may have added benefits.

The influence of different factors on the effectiveness of antioxidants in complex heterogeneous foods and biological systems cannot be evaluated using only a one-assay protocol. The two systems chosen to evaluate the TDAC (FRAP and ABTS) measure the total reducing power and the free radical-scavenging activity, respectively. Both methods are widely used to evaluate AC in foods and biological systems (Frankel & Meyer, 2000). In addition, a model of copper-induced oxidation of lipoprotein was selected to measure the prevention of lipid peroxidation.

The AC and total phenolics content of plant foods, beverages and vegetable oils are shown in Table 2. All types of plant foods, except cereals, presented a high AC per g of dry edible matter by both FRAP and ABTS methods. The highest values were registered for nuts and fruits. Beverages also exhibited high AC, measured by these methods, while the AC of vegetable oils was comparatively low; coffee and red wine presented the highest AC.

The capacities to inhibit LDL oxidation of plant foods and beverages, included in Table 1 were consistent with their AC. Fig. 1 shows the delay in LDL oxidation for food extracts (1 g dry edible matter/100 ml). The lag time was highest for fruits, was lower but significant for vegetables and not significant for cereals. Legumes, nuts and beverages were also efficient LDL oxidation inhibitors.

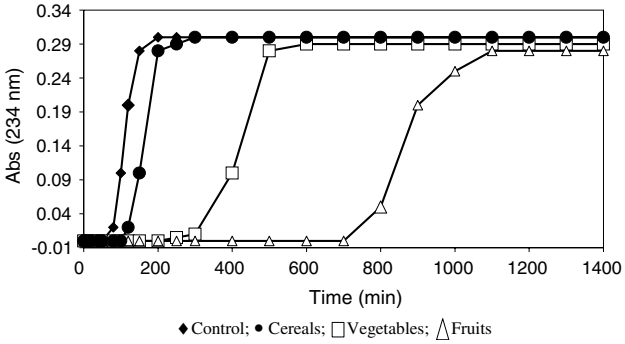

Fig. 1. Copper mediated LDL oxidation (formation of conjugated dienes, Abs 234 nm) in the presence of vegetable, fruit and cereal extracts (1 g dry edible part/100 ml). Each sample contains a mixture of foods (listed in Table 1) that correspond to the *per capita* daily intake in the Spanish diet. Vegetables: potatoes, tomatoes, onions, garlic, cabbage, green beans, cucumber, capsicum, mushrooms, lettuce, asparagus, spinach, chard and others. Fruits: oranges, bananas, apples, pears, peaches, apricots, strawberries, melon, watermelon, plums, cherries, grapes, kiwi, olives and others. Cereals: rice, bread, spaghetti, biscuits and croissants.

Total dietary antioxidant capacity (TDAC) can be defined as the antioxidant capacity of all plant foods and beverages (alcoholic and non alcoholic) consumed daily in a diet. TDAC may represent the amount of antioxidant units (trolox equivalents) present daily in the human gut.

TDAC, in the Spanish diet, was estimated to be 6014 and 3549 μmol trolox equivalents by FRAP and ABTS, respectively (Table 3). The contribution of each specific food to the TDAC was dependent on both food intake and food AC. The largest contributors to

Table 2
Antioxidant capacity and total polyphenol content of plant foods and beverages

Plant foods	Antioxidant capacity (μmol trolox equivalents/g dry matter edible part)		Total polyphenol content (mg/100 g dry matter edible part)
	FRAP	ABTS	
Nuts	44.8 ± 1.4	33.6 ± 0.8	894 ± 48.2
Fruits	25.5 ± 0.5	10.2 ± 0.4	538 ± 20.27
Vegetables	10.3 ± 0.1	6.7 ± 0.7	287 ± 13.5
Legumes	9.0 ± 0.2	6.4 ± 0.5	155 ± 20.4
Cereals	2.2 ± 0.1	0.2 ± 0.01	107 ± 2.9
Beverages	(μmol trolox equivalents/100 ml)		(mg/100 ml)
Coffee[a]	2267 ± 18.9	1328 ± 5.1	343 ± 4.4
Tea[b]	601 ± 5.5	631 ± 8.0	76.3 ± 0.1
Red wine	1214 ± 24.5	1093 ± 54.2	160 ± 2.6
White wine	154 ± 36.8	181 ± 22.2	21.3 ± 5.5
Rose wine	286 ± 39.2	261 ± 23.7	40.9 ± 5.8
Beer	108 ± 9.9	77.2 ± 1.7	56.1 ± 8.3
Orange juice	515 ± 41.5	249 ± 3.4	50.1 ± 5.2
Cola	20.7 ± 0.7	<10	6.7 ± 0.2
Vegetable oils			
Olive	152 ± 10.3	40.9 ± 2.9	17.3 ± 0.7
Sunflower	65.3 ± 1.7	11.6 ± 0.1	14.0 ± 0.9

Each value is the mean ± SD of three replicate experiments of each purchase.
[a] Infusion 80.6 g powder/l.
[b] Infusion 6 g powder/l.

Table 3
Total antioxidant capacity and polyphenol *per capita* daily intake in the Spanish diet

Plant foods (g fresh matter)	Intake	Antioxidant capacity (μmol trolox equivalents)		Polyphenols (mg)
		FRAP	ABTS	
Nuts	6.8	235	176	47.1
Fruits	266	850	342	178
Vegetables	331	418	272	117
Legumes	22.3	189	134.7	33.0
Cereals	222	367	33.4	174
Beverages (ml)	505	3894	2576	614
Vegetable oils (ml)				
Olive	31.2	47.6	12.8	5.4
Sunflower	20.8	13.6	2.4	2.9
Total		6014	3549	1171

the TDAC were beverages (about 68% – mean of FRAP and ABTS values) and fruits and vegetables (about 20%), while the contribution of cereals and vegetable oils was very low. Nuts and legumes accounted for nearly 8% of TDAC, despite low consumption (6.8 and 22.3 g, respectively).

Our findings suggest that whole diets ought to be considered when addressing the role of dietary antioxidants in health. Studies on individual foods may overestimate their potential effects within a whole diet. Individual foods known to have a high antioxidant capacity (Lee, Kim, Lee, & Lee, 2003; Moschandreas, Vissers, Wiseman, van Putte, & Kafatos, 2002; Serra-Majem, Cruz, Ribas, & Tur, 2003) may contribute very little to the AC of whole diets. For example, the contributions of tea and olive oil account for just 2.7% and 0.6% of the TDAC in the Spanish diet. Wine consumption represents about 14% of the TDAC but, surprisingly, coffee was the single greatest contributor (44.5%). The high contribution of coffee to the intake of antioxidants in the Spanish diet was previously reported (Pulido et al., 2003) and again recently demonstrated (Svilaas et al., 2004) in a nationwide Norwegian survey.

With regard to food microconstituents, vitamins C and E, polyphenolic compounds and carotenoids are recognized as the main dietary antioxidants. In assessing the contribution of these food microconstituents to the TDAC, the following points must be considered:

- The daily intake of total phenolics in the Spanish diet was estimated to be 1171 mg/person/day (Table 3) that is considerably higher than the daily intake of carotenoids (4 mg) and vitamins C (126 mg) and E (13 mg) (Granado, Olmedilla, Blanco, & Rojas-Hidalgo, 1996).
- Phenolic compounds present higher *in vitro* and *ex vivo* AC than vitamins and carotenoids (Pulido et al., 2000; Sánchez-Moreno et al., 2000). Phenolics appear to be quantitatively the main dietary antioxidant in fruits (Vinson, Su, Zubik, & Bose, 2001). Vitamin C has been reported to be a minor contributor to the AC of common fruits, the contribution of carotenoids being negligible (Gardner, White, McPhail, & Duthie, 2000).
  The recommended daily intake of vitamin C (60 mg) plus vitamin E (12 mg) is equivalent to about 580 μmol trolox equivalents (FRAP) and 400 μmol trolox equivalents (ABTS). It represents only about 10% of TDAC in the Spanish Mediterranean diet (Pulido et al., 2003).
- The AC of foods and beverages correlates closely with the polyphenol content. There was a high correlation between polyphenol content and AC for plant food ($r = 0.9924$, FRAP method; $r = 0.9446$, ABTS method) and beverages ($r = 0.9809$, FRAP method; $r = 0.9046$, ABTS method).

These data suggest that polyphenols are quantitatively the main dietary antioxidants. However, the biological effects of these substances will depend on their bioavailability (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). In spite of low bioavailability, significant metabolic effects can be expected because of the high intake and the AC of these compounds.

Fruit appears to be an essential item in dietary disease prevention, either alone or in some cases in combination with, vegetables, nuts and other plant foods. One possible reason for the beneficial effects of fruit consumption can be its AC and LDL oxidation inhibition capacity. In this connection, Martínez-González et al. (2002) found an inverse association between the first acute myocardial infarction and the consumption of fruits in the Spanish Mediterranean diet, but not of vegetables or legumes.

A recent population study, based on prospective investigation involving 22 043 adults in Greece, showed that adherence to a Mediterranean Diet was associated with significantly lower total mortality from coronary heart disease and mortality from cancer. However, as far as specific foods are concerned only the intake of

fruits plus nuts was predictive of total mortality (Trichopoulou, Costacou, Bamia, & Trichopoulos, 2003).

Our data point in the same direction, but they further suggest that beverages are an important factor in the beneficial effects of dietary antioxidants. For instance, if we extrapolate our AC values to data reported in the MONICA epidemiological study – ''French paradox'' – (Renaud & Lorgeril, 1992), the wine intake (267–383 ml/day) in the French regions can be estimated to be around 3500 μmol trolox equivalents (ABTS method), while the consumption of fruits (160–238 g/day) account for about 260 μmol trolox equivalents.

In summary, total dietary antioxidant capacity may be a parameter to be considered in nutritional and epidemiological studies. Further studies on potential correlations between this parameter and disease incidence in different European regions and countries are needed.

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