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Food Chemistry 98 (2006) 599–608
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activities of various extracts and fractions of *Sorbus domestica* fruits at different maturity stages

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Received 7 March 2005; received in revised form 15 June 2005; accepted 15 June 2005

Abstract

Thirty four different extracts, fractions and residues of five different maturity stages of the Greek Service tree fruits (*Sorbus domestica*, fam. Rosaceae) were evaluated for their antioxidant activities (DPPH· and luminol-induced chemiluminescence methods) and in correlation with their total phenolic contents (Folin–Ciocalteau test).

Dichloromethane, diethyl ether and ethyl acetate fractions possessed significant radical-scavenging activities which were greater than the activity of trolox. This seemed to be correlated with their total phenolic content. Unripe yellow fruits, together with the fruit pulp, were the strongest antioxidants, while the well-matured brown fruits were the weakest ones. Results showed that the fractions of diethyl ether, ethyl acetate and dichloromethane, can be used as antioxidants in food and medicinal preparations.

Keywords: *Sorbus domestica*; Radical scavenging activity; DPPH·; Chemiluminescence; Total phenolic content

1. Introduction

The fruit of the Greek Service Tree (*Sorbus domestica*, family Rosaceae), although not very widespread, is widely consumed by the lower economically local community of Xanthi (Rodopi) and it is considered to be a vital component of their daily diet. The tree is self-sown in the mountainous regions of Rodopi; thus it is of a great importance, not only from a nutritional, but also from an economic point of view. Local people use this fruit, not only as nutritious food, but also as a traditional astringent, diarrheic and antidiabetic agent in pulp form.

S. domestica belongs to the same species as *Sorbus aucuparia*, for which there are reports on phenolic content (Gil-Izquierdo & Mellenthin, 2001; Hakkinen et al., 1999; Hakkinen, Karenlampi, Heinonen, Mykkanen, & Torronen, 1999; Maatta-Riihinen, Kamal-Eldin, Mattila, Gonzalez-Paramas, & Torronen, 2004) and antioxidant activity (Heinonen, Lehtonen, & Hopia, 1998; Kahkonen, Hopia, & Heinonen, 2001). However, very little is known about the antioxidant capacity of *S. domestica* fruits on its correlation with the phenolic content (Olschlager, Milde, Schempp, & Treutter, 2004).

Nowadays, food scientists and nutrition specialists agree that food antioxidants, consumed daily, contribute to the conservation of good health (Halliwell & Gutteridge, 1989). Furthermore, natural antioxidants can

Abbreviations: DPPH·, 1,1-diphenyl-2-picryl-hydrazyl; CL, chemiluminescence; AE, antiradical efficiency; GAE, gallic acid equivalents; TPC, total phenolic content; SD, standard deviation.

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doi:10.1016/j.foodchem.2005.06.025

be used, either as food additives, or as pharmaceutical supplements, reducing the risk of a number of chronic diseases (Nicoli, Anese, & Parpinel, 1999) and protecting essential molecules from damage (Saez Tormo et al., 1994).

Food industries pay much attention to natural antioxidants, especially in deterioration of high fat foods (Kanner, 1994). The safety of synthetic antioxidants, used by the food industry, is now questioned. Thus, the tendency today is toward their replacement with antioxidants of natural origin (Hudson, 1990).

The aim of this study was the detailed evaluation of the antioxidant capacity of *S. domestica* fruits from the region of Rodopi at five different maturity stages and well matured fruit pulp. Furthermore, a detailed comparative analysis of all antioxidant results of the various subfractions is done in order to classify them in accordance with their antioxidant power. Two assays were used to determine free and hydroxyl radical-scavenging activity of the fruit extracts: (i) DPPH$^{\cdot}$ test and (ii) luminol-enhanced chemiluminescence. Apart from this, the Folin–Ciocalteau method was applied to all samples in order to record their total phenolic content and estimate its relationship with each extract's, fraction's and residue's antioxidant capacity.

2. Materials and methods

2.1. Plant material

All fruit samples were collected in September, 2003. Five fruit categories were tested: A, unripe fruits (yellow colour); B, well-matured on tree (brown colour); C, collected unripe and matured for one week in dark, at room temperature; D, as in C, but prolonged maturation at three weeks (dark brown colour), form consumed by the local population; E, sterilized pulp from well-matured fruits (disposed at local drugstores). Fruits of category A, C and D were harvested on the 10th of September, while those of B were ten days later. All plant material was directly extracted with methanol.

2.2. Chemicals and reagents

The solvents used for the present work were purchased from Merck (Germany) and Panreac (Spain). Folin–Ciocalteau reagent, sodium carbonbate, $CoCl_2 \cdot 6H_2O$ and perhydrol, 30% H_2O_2, stabilized, were purchased from Merck (Germany). Gallic acid 1-hydrate was purchased from Panreac (Spain). DPPH (1,1-diphenyl-2-picryl-hydrazyl, 90%), EDTA, luminol (3-aminophtahydrazine) and boric acid were from Sigma (Germany).

2.3. Extraction procedure

The procedure followed was according to Mellidis, Papageorgiou, and Kokkalou (1993). The fruits were segmented and their seeds were carefully removed. Their extracted weights were 500 g for A, B and D, 700 g for C and 250 g for E. They were then directly put into a Soxhlet apparatus 1 l and extracted exhaustively with methanol. For the pulp extraction, the content of the sterilized bottle was directly put into methanol after its opening and filtered until discoloration of the solvent. The extracts obtained were evaporated under vacuum to dryness. Their weights were 102.6 g for A (20.5% of the initial fruit weight), 150.2 g for B (30% of the initial fruit weight), 195.2 g for C (27.9% of the initial fruit weight), 166 g for D (33.2% of the initial fruit weight) and 32.6 g for E (13% of the initial pulp weight). The dry residues of the methanolic extracts were dissolved in 1.5 l of boiling water and then directly filtered. The water solution was partitioned with dichloromethane, diethyl ether, ethyl acetate and butanol (three times × 500 ml each). (All the above solvents can be completely removed). The organic layers were dried with anhydrous sodium sulphate (Merck p.a.) and evaporated under vacuum to dryness to give the following weights: A. Dichoromethane fraction, 500 mg; diethyl ether fraction, 166.2 mg; ethyl acetate fraction, 700 mg; butanol fraction, 11 g; water fraction, 90 g. B. Dichoromethane fraction, 703.2 mg; diethyl ether fraction, 400 mg; ethyl acetate fraction, 720 mg; butanol fraction, 20.5 g; water fraction, 126.7 g. C. Dichoromethane fraction, 201.6 mg; diethyl ether fraction, 115.6 mg; ethyl acetate fraction, 1.38 g; butanol fraction, 49.2 g; water fraction, 144.32 g. D. Dichoromethane fraction, 383.1 mg; diethyl ether fraction, 11.7 mg; ethyl acetate fraction, 723.8 mg; butanol fraction, 16.34 g; water fraction, 144.38 g. E. Diethyl ether fraction, 117.8 mg; ethyl acetate fraction, 90.7 mg; butanol fraction, 8.2 g; water fraction, 23.9 g. All extracts, fractions and initial residues were kept at 0 °C in a nitrogen atmosphere.All samples tested were given a code number (1–34, Table 1).

2.4. Estimation of the phenolic content by the Folin–Ciocalteau test

The total concentration of the phenols in the extracts was determined according to the Folin–Ciocalteau method (Waterman & Mole, 1994). In a 1.5 ml Eppendorf tube, 790 μl of distilled water, 10 μl of diluted sample and 50 μl of Folin–Ciocalteau reagent were added and the mixture vortexed. After 1 min, 150 μl of aqueous sodium carbonate (20%) were added and the mixture was vortexed and allowed to stand at room temperature without light for 120 min. The absorbance

Table 1
Coding numbers of the different samples

Maturity stage/type of plant material	Type of partitioning						
	Residues	Dicloro-methane	Diethyl ether	Ethyl acetate	Butanol	Water	Crude/methanol
Unripe	1	6	10	15	20	25	30
Well matured on tree	2	7	11	16	21	26	31
Matured for 1 week at room temperature	3	8	12	17	22	27	32
Matured for 3 weeks at room temperature	4	9	13	18	23	28	33
Fruit pulp	5	–	14	19	24	29	34

Horizontally, the stages of partitioning appear, together with all the organic solvents. Squarely, the maturity stage and the type of all the initial plant material appear.

was read at 750 nm using an HP 8452a diode array spectrophotometer, in a 10 mm cuvette. The total phenol concentration was calculated from the calibration curve, using gallic acid as a standard and the results were expressed as mg of gallic acid equivalents (GAE).

2.5. Evaluation of the antioxidant activity using the DPPH· method

The antioxidant activity of all extracts was first determined using the DPPH· test, according to Brand-Williams, Cuvelier, and Berset (1995), Parejo, Codina, Petrakis, and Kefalas (2000) and Arnous, Makris, and Kefalas (2001). Different concentrations of all extracts (1–34) were prepared (Table 1). An aliquot of 25 μl of diluted sample was added to 975 μl DPPH· solution (2×10^{-5} M) and the mixture vortexed. The decrease in the absorbance was determined at 515 nm when the reaction reached a plateau, using an HP 8452 A diode array spectrophotometer, in a 10 mm quartz cuvette. For the samples well diluted in methanol, methanol was used to zero the spectrophotometer. For those not diluted in methanol, the apparatus was zeroed with methanol (975 μl) and dimethylsulphoxide (DMSO 25 μl). The absorbance of the DPPH· radical without any sample was measured. The DPPH· concentration in the reaction medium was calculated from the calibration curve, as determined by linear regression:

$$A_{515\,\mathrm{nm}} = 0.0248 \times [\mathrm{DPPH^{\bullet}}\ (\mu g/ml)] + 0.0138 (R^2 = 0.9968).$$

For each sample concentration tested, the percentage of DPPH· remaining in the steady state, was calculated in the following way:

Percentage of remaining $\mathrm{DPPH^{\bullet}} = [\mathrm{DPPH^{\bullet}}]_{\mathrm{at}\ t = T} / [\mathrm{DPPH^{\bullet}}]_{\mathrm{at}\ t = 0}$, where T is the time necessary to reach the steady state.

The antioxidant capacity of each sample was expressed as the amount of sample necessary to decrease the initial DPPH· concentration by 50% (EC_{50}). The antiradical efficiency (AE) is calculated as follows:

$$AE = 1/EC_{50}.$$

2.6. Estimation of the antioxidant activity using the Co(II)/EDTA – induced luminol chemiluminescence method

The antioxidant activity was also determined using the Co(II)/EDTA – induced luminol chemiluminescence method, according to Parejo et al. (2000), with some variations. The chemiluminescence measurements were carried out on a Model 6200 Fluorimeter, JENWAY (Jenway Gransmore Green Felster Dunmow Essex CM6 3 LB), keeping the lamp off and using only the photomultiplier of the apparatus.

At least three different dilutions of the extracts were prepared. 1 ml of borate buffer (0.05 M, adjusted to pH 9 with 1 M NaOH), containing 1 mg/ml EDTA and 0.2 mg/ml of $CoCl_2 \cdot 6H_2O$ was added to 100 μl of luminol solution (5.6×10^{-4} M) in borate buffer (0.05 M, adjusted to pH 9 with 1 M NaOH) in a test tube and the mixture vortexed for 15 s. Then, 25 μl of H_2O_2 aqueous solution (4.5×10^{-3} M) was deposited on the bottom of a 10×10 glass cuvette using precision pipettes. The luminol–buffer mixture was rapidly added to the cuvette, using a Pasteur pipette, and carefully mixed for 15 s in order to initiate the chemiluminescence reaction. When the reaction reached a plateau, the chemiluminescence (CL) intensity (I_o) was recorded. Immediately afterwards, 25 μl of the sample were added and the instantaneous decrease of the light emission was recorded (I). The ratio I_o/I was calculated. This ratio vs. μg extract/ml was plotted for three prepared dilutions of each extract and a linear regression was established in order to calculate IC_{50}. IC_{50} is the amount of sample needed to decrease, by 50%, the CL intensity (Parejo et al., 2000), according to the equation:

$$[I_o/I = a(\mathrm{mg\ extract/ml}) + b].$$

The antiradical efficiency ($AE = 1/IC_{50}$) was also calculated. Results were also expressed as standard equivalents using quercetin and trolox, on the basis of the IC_{50} value.

3. Results and discussion

3.1. Total phenolic content

The total phenolic content of the fruit extracts ranged from 2.27 to 324 μg of gallic acid/mg dry extract (Tables 2–8). Results were classified according to the solvents that were used for extraction. In general, the phenolic content of the six types of extracts decreased in the following order: ethyl acetate fractions > diethyl ether fractions > dichloromethane fractions > butanol fractions > residues > methanolic extracts > water fractions. Ethyl acetate fractions seemed to concentrate, together with diethyl ether fractions, the most phenolic substances. This is in accordance with findings of Parejo et al. (2002) and Chung et al. (1999). The fruit pulp and the unripe fruits are the categories with the highest phenolic contents and the fruits matured at room temperature for one week follow these. Fruits matured well on tree and those matured well at room temperature gave the lowest phenolic content (Table 8).

3.2. Radical scavenging activity (DPPH·, CL)

All antioxidant results are summarized in Tables 2–8. Radical-scavenging activity, expressed as EC_{50}, ranged from 0.341 to 39.5 mg dry extract/mg DPPH·. A wide range of antioxidant capacity among extracts was observed. The butanolic fractions of the pulp and the unripe fruits showed the greatest antiradical activity, followed by the ether and the ethyl acetate fractions. The initial methanolic extracts, the residues and the dichloromethane fractions followed. The weakest antioxidants were the water fractions. According to Table 8, which summarises the results for all methanolic extracts, fruit pulp possesses the greatest antioxidant capacity. Unripe fruits and fruits matured for only one week come next. Well-matured fruits, either on tree, or at room temperature, do not have marked antioxidant power. These conclusions also emerge from Tables 2–7.

A wide range of antioxidant capacity is also observed from the CL results. IC_{50} ranges from 0.675 to 352 μg dry extract/ml. As with the DPPH· results, ethyl ether and ethyl acetate fractions had high antioxidant power. On the other hand, butanolic fractions appeared to be weak, together with the residues and the initial methanolic extracts. Water fractions were even weaker. Dichloromethane fractions had strong antioxidant power, analogous to the ethyl ether and ethyl acetate fractions, as emerges from the statistical analysis of the results (Duncan's test). According to Table 8, fruit pulp again possesses the strongest antioxidant capacity, followed by the extracts of the unripe fruits. Fruits matured at room temperature for only one week and these matured well on the tree, had a slightly weaker antioxidant capacity, while those matured well at room temperature were the weakest ones.

Table 2
Total phenolic contents (GAE) and antioxidant capacities of the residues of all the maturity stages of the fruits, expressed as EC_{50}, IC_{50}, AE, quercetin and trolox equivalents

Sample	DPPH· radical scavenging method				Chemiluminescence method				GA[I] equivalents
	$EC_{50} \pm SD$[A]	AE[B]	EC_{50} quercetin[C] equivalent	EC_{50} trolox[D] equivalent	$IC_{50} \pm SD$[E]	AE[F]	IC_{50} quercetin[G] equivalent	IC_{50} trolox[H] equivalent	
1▲	4.829 ± 0.12	0.207 ± 0.005^{k}	0.0141	0.0371	36.7 ± 1.96	$0.027 \pm 0.001^{f,g}$	0.00873	0.0682	13.6 ± 0.3
2●	6.29 ± 0.20	0.159 ± 0.005^{l}	0.0108	0.0285	67.8 ± 0.84	0.015 ± 0.0002^{k}	0.00472	0.0369	25.4 ± 1.97
3■	3.72 ± 0.08	0.269 ± 0.006^{j}	0.0183	0.0482	35.4 ± 1.52	$0.028 \pm 0.001^{e,f,g}$	0.00903	0.0705	20.50 ± 1.19
4◆	2.73 ± 0.11	$0.367 \pm 0.02^{f,g,h,i}$	0.0249	0.0656	16.5 ± 2.13	$0.061 \pm 0.008^{b,c,d}$	0.0194	0.152	32.1 ± 0.42
5▼	1.81 ± 0.04	$0.553 \pm 0.01^{c,d,e}$	0.0376	0.0989	3.36 ± 2.05	0.298 ± 0.49^{a}	0.0954	0.745	30.2 ± 4.05

▲ Unripe fruits.
● Fruits well matured on tree.
■ Fruits collected unripe and left to mature for one week at room temperature.
◆ Fruits collected unripe and left to mature for three weeks at room temperature.
▼ Fruit pulp from well matured fruits.

Results are ±SD ($n = 3$). Values of the same column, and among Tables 2–8, followed by the same letter, are not statistically different ($P < 0.05$) as measured by Duncan's test.

[A] Efficient concentration (mg antioxidant/mg DPPH·): amount of antioxidant needed to decrease the initial DPPH· concentration by 50%.
[B] Antiradical efficiency: $1/EC_{50}$.
[C] Quercetin $EC_{50} = 0.068$ mg quercetin/mg DPPH·.
[D] Trolox $EC_{50} = 0.179$ mg trolox/mg DPPH·.
[E] Efficient concentration (μg antioxidant/ml): amount of antioxidant needed to decrease the initial chemiluminescence intensity by 50%.
[F] Antiradical efficiency: $1/IC_{50}$.
[G] Quercetin $IC_{50} = 0.32$ μg/ml.
[H] Trolox $IC_{50} = 2.5$ μg/ml.
[I] Gallic acid equivalants: μg gallic acid/1 mg dry extract.

Table 3
Total phenolic contents (GAE) and antioxidant capacities of the dichloromethane fractions of all the maturity stages of the fruits, expressed as EC_{50}, IC_{50}, AE, quercetin and trolox equivalents

Sample	DPPH· radical scavenging method				Chemiluminescence method				GA[I] equivalents
	$EC_{50} \pm SD$[A]	AE[B]	EC_{50} quercetin[C] equivalent	EC_{50} trolox[D] equivalent	$IC_{50} \pm SD$[E]	AE[F]	IC_{50} quercetin[G] equivalent	IC_{50} trolox[H] equivalent	
6▲	3.60 ± 0.24	0.278 ± 0.02^{j}	0.0189	0.0189	0.785 ± 0.02	1.27 ± 0.03^{a}	0.408	3.18	74.5 ± 0.62
7●	9.88 ± 1.63	0.104 ± 0.02^{n}	0.0069	0.0181	2.59 ± 0.56	0.387 ± 0.08^{a}	0.124	0.967	27.0 ± 0.93
8■	3.82 ± 0.06	0.262 ± 0.004^{j}	0.0178	0.0469	0.877 ± 0.03	1.14 ± 0.04^{a}	0.365	2.85	97.0 ± 11.79
9◆	6.01 ± 0.10	0.166 ± 0.003^{l}	0.0113	0.0298	1.20 ± 0.02	0.836 ± 0.01^{a}	0.267	2.09	66.5 ± 2.13

▲ Unripe fruits.
● Fruits well matured on tree.
■ Fruits collected unripe and left to mature for one week at room temperature.
◆ Fruits collected unripe and left to mature for three weeks at room temperature.
Results are ±SD ($n = 3$). Values of the same column, and among Tables 2–8, followed by the same letter, are not statistically different ($P < 0.05$) as measured by Duncan's test.
[A] Efficient concentration (mg antioxidant/mg DPPH·): amount of antioxidant needed to decrease the initial DPPH· concentration by 50%.
[B] Antiradical efficiency: $1/EC_{50}$.
[C] Quercetin $EC_{50} = 0.068$ mg quercetin/mg DPPH·.
[D] Trolox $EC_{50} = 0.179$ mg trolox/mg DPPH·.
[E] Efficient concentration (μg antioxidant/ml): amount of antioxidant needed to decrease the initial chemiluminescence intensity by 50%.
[F] Antiradical efficiency: $1/IC_{50}$.
[G] Quercetin $IC_{50} = 0.32$ μg/ml.
[H] Trolox $IC_{50} = 2.5$ μg/ml.
[I] Gallic acid equivalants: μg gallic acid/1 mg dry extract.

Table 4
Total phenolic contents (GAE) and antioxidant capacities of the diethyl ether fractions of all the maturity stages of the fruits, expressed as EC_{50}, IC_{50}, AE, quercetin and trolox equivalents

Sample	DPPH· radical scavenging method				Chemiluminescence method				GA[I] equivalents
	$EC_{50} \pm SD$[A]	AE[B]	EC_{50} quercetin[C] equivalent	EC_{50} trolox[D] equivalent	$IC_{50} \pm SD$[E]	AE[F]	IC_{50} quercetin[G] equivalent	IC_{50} trolox[H] equivalent	
10▲	0.997 ± 0.07	$1.01 \pm 0.07^{a,b,c}$	0.0682	0.180	0.872 ± 0.03	1.15 ± 0.03^{a}	0.367	2.87	245 ± 1.74
11●	1.74 ± 0.15	$0.580 \pm 0.05^{c,d,e}$	0.0392	0.103	1.63 ± 0.05	0.615 ± 0.02^{a}	0.197	1.54	151 ± 0.59
12■	0.825 ± 0.02	$1.12 \pm 0.03^{a,b}$	0.0824	0.217	0.675 ± 0.01	1.48 ± 0.03^{a}	0.474	3.71	324 ± 51.7
13◆	3.28 ± 0.23	$0.307 \pm 0.02^{i,j}$	0.0207	0.0546	1.57 ± 0.03	0.635 ± 0.01^{a}	0.203	1.59	148 ± 1.84
14▼	2.97 ± 0.04	$0.337 \pm 0.005^{g,h,i,j}$	0.0229	0.0603	1.24 ± 0.06	0.806 ± 0.04^{a}	0.258	2.02	143 ± 3.65

▲ Unripe fruits.
● Fruits well matured on tree.
■ Fruits collected unripe and left to mature for one week at room temperature.
◆ Fruits collected unripe and left to mature for three weeks at room temperature.
▼ Fruit pulp from well matured fruits.
Results are ±SD ($n = 3$). Values of the same column, and among Tables 2–8, followed by the same letter, are not statistically different ($P < 0.05$) as measured by Duncan's test.
[A] Efficient concentration (mg antioxidant/mg DPPH·): amount of antioxidant needed to decrease the initial DPPH· concentration by 50%.
[B] Antiradical efficiency: $1/EC_{50}$.
[C] Quercetin $EC_{50} = 0.068$ mg quercetin/mg DPPH·.
[D] Trolox $EC_{50} = 0.179$ mg trolox/mg DPPH·.
[E] Efficient concentration (μg antioxidant/ml): amount of antioxidant needed to decrease the initial chemiluminescence intensity by 50%.
[F] Antiradical efficiency: $1/IC_{50}$.
[G] Quercetin $IC_{50} = 0.32$ μg/ml.
[H] Trolox $IC_{50} = 2.5$ μg/ml.
[I] Gallic acid equivalants: μg gallic acid/1 mg dry extract.

3.3. Correlation between total phenolic content and radical scavenging activity

A low correlation was found between total phenolic content and antiradical efficiency, measured by the DPPH· method ($R^2 = 0.2532$, Fig. 1), while a higher correlation was found when antioxidant activity was estimated by the CL method ($R^2 = 0.6347$, Fig. 2).

More analytically (Table 9), there is a high correlation between the gallic acid equivalents of the different extracts

Table 5
Total phenolic contents (GAE) and antioxidant capacities of the ethyl acetate fractions of all the maturity stages of the fruits, expressed as EC_{50}, IC_{50}, AE, quercetin and trolox equivalents

Sample	DPPH· radical scavenging method				Chemiluminescence method				GA[I] equivalents
	$EC_{50} \pm SD$[A]	AE[B]	EC_{50} quercetin[C] equivalent	EC_{50} trolox[D] equivalent	$IC_{50} \pm SD$[E]	AE[F]	IC_{50} quercetin[G] equivalent	IC_{50} trolox[H] equivalent	
15▲	1.78 ± 0.07	$0.563 \pm 0.02^{c,d,e}$	0.0383	0.101	1.26 ± 0.11	0.793 ± 0.08^{a}	0.254	1.99	285 ± 6.83
16●	1.75 ± 0.03	$0.571 \pm 0.01^{c,d,e}$	0.0388	0.102	1.91 ± 0.05	0.523 ± 0.01^{a}	0.167	1.31	137 ± 5.55
17■	1.84 ± 0.05	$0.543 \pm 0.01^{c,d,e}$	0.0369	0.0971	1.44 ± 0.04	0.695 ± 0.02^{a}	0.222	1.74	198 ± 5.43
18◆	3.17 ± 0.13	$0.317 \pm 0.01^{h,i,j}$	0.0215	0.0566	2.44 ± 0.05	0.410 ± 0.009^{a}	0.131	1.02	64.0 ± 0.28
19▼	0.899 ± 0.08	$1.12 \pm 0.09^{a,b}$	0.0756	0.199	0.869 ± 0.03	1.15 ± 0.05^{a}	0.368	2.88	341 ± 4.95

▲ Unripe fruits.
● Fruits well matured on tree.
■ Fruits collected unripe and left to mature for one week at room temperature.
◆ Fruits collected unripe and left to mature for three weeks at room temperature.
▼ Fruit pulp from well matured fruits.
Results are ±SD ($n = 3$). Values of the same column, and among Tables 2–8, followed by the same letter, are not statistically different ($P < 0.05$) as measured by Duncan's test.
[A] Efficient concentration (mg antioxidant/mg DPPH·): amount of antioxidant needed to decrease the initial DPPH· concentration by 50%.
[B] Antiradical efficiency: $1/EC_{50}$.
[C] Quercetin $EC_{50} = 0.068$ mg quercetin/mg DPPH·.
[D] Trolox $EC_{50} = 0.179$ mg trolox/mg DPPH·.
[E] Efficient concentration (μg antioxidant/ml): amount of antioxidant needed to decrease the initial chemiluminescence intensity by 50%.
[F] Antiradical efficiency: $1/IC_{50}$.
[G] Quercetin $IC_{50} = 0.32$ μg/ml.
[H] Trolox $IC_{50} = 2.5$ μg/ml.
[I] Gallic acid equivalants: μg gallic acid/1 mg dry extract.

Table 6
Total phenolic contents (GAE) and antioxidant capacities of the butanol fractions of all the maturity stages of the fruits, expressed as EC_{50}, IC_{50}, AE, quercetin and trolox equivalents

Sample	DPPH· radical scavenging method				Chemiluminescence method				GA[I] equivalents
	$EC_{50} \pm SD$[A]	AE[B]	EC_{50} quercetin[C] equivalent	EC_{50} trolox[D] equivalent	$IC_{50} \pm SD$[E]	AE[F]	IC_{50} quercetin[G] equivalent	IC_{50} trolox[H] equivalent	
20▲	0.588 ± 1.7	1.70 ± 0.01^{a}	0.116	0.304	11.2 ± 0.84	$0.089 \pm 0.007^{a,b,c}$	0.0287	0.224	94.0 ± 10.5
21●	8.00 ± 0.34	0.125 ± 0.005^{m}	0.0085	0.0224	32.6 ± 5.50	$0.031 \pm 0.005^{e,f}$	0.00982	0.0767	16.1 ± 1.05
22■	3.75 ± 0.18	0.268 ± 0.01^{j}	0.0182	0.0478	26.2 ± 1.62	$0.038 \pm 0.002^{d,e}$	0.0122	0.0953	25.1 ± 2.09
23◆	13.2 ± 0.21	0.076 ± 0.001^{o}	0.0052	0.0136	37.5 ± 1.51	$0.027 \pm 0.001^{f,g}$	0.00853	0.0666	12.5 ± 1.43
24▼	0.341 ± 0.01	2.94 ± 0.10^{a}	0.200	0.525	6.71 ± 0.34	$0.149 \pm 0.008^{a,b}$	0.0477	0.373	140 ± 13.9

▲ Unripe fruits.
● Fruits well matured on tree.
■ Fruits collected unripe and left to mature for one week at room temperature.
◆ Fruits collected unripe and left to mature for three weeks at room temperature.
▼ Fruit pulp from well matured fruits.
Results are ±SD ($n = 3$). Values of the same column, and among Tables 2–8, followed by the same letter, are not statistically different ($P < 0.05$) as measured by Duncan's test.
[A] Efficient concentration (mg antioxidant/mg DPPH·): amount of antioxidant needed to decrease the initial DPPH· concentration by 50%.
[B] Antiradical efficiency: $1/EC_{50}$.
[C] Quercetin $EC_{50} = 0.068$ mg quercetin/mg DPPH·.
[D] Trolox $EC_{50} = 0.179$ mg trolox/mg DPPH·.
[E] Efficient concentration (μg antioxidant/ml): amount of antioxidant needed to decrease the initial chemiluminescence intensity by 50%.
[F] Antiradical efficiency: $1/IC_{50}$.
[G] Quercetin $IC_{50} = 0.32$ μg/ml.
[H] Trolox $IC_{50} = 2.5$ μg/ml.
[I] Gallic acid equivalants: μg gallic acid/1 mg dry extract.

and the AE from the DPPH· test for dichloromethane fractions ($R^2 = 0.7695$), diethyl ether fractions ($R^2 = 0.8795$), butanol fractions ($R^2 = 0.9954$), water fractions ($R^2 = 0.9922$) and initial methanolic extracts ($R^2 = 0.739$), and a lower one for the residues ($R^2 = 0.6531$) and the ethyl acetate fractions ($R^2 = 0.6934$). It is note-

Table 7
Total phenolic contents (GAE) and antioxidant capacities of the water fractions of all the maturity stages of the fruits, expressed as EC_{50}, IC_{50}, AE, quercetin and trolox equivalents

Sample	DPPH· radical scavenging method				Chemiluminescence method				GA[I] equivalents
	$EC_{50} \pm SD$[A]	AE[B]	EC_{50} quercetin[C] equivalent	EC_{50} trolox[D] equivalent	$IC_{50} \pm SD$[E]	AE[F]	IC_{50} quercetin[G] equivalent	IC_{50} trolox[H] equivalent	
25▲	4.95 ± 0.15	0.202 ± 0.006^{k}	0.0137	0.0362	116 ± 5.26	0.009 ± 0.0004^{l}	0.00276	0.0216	14.8 ± 1.05
26●	39.1 ± 0.47	0.026 ± 0.0003^{q}	0.0017	0.0046	289 ± 18.63	0.003 ± 0.0002^{o}	0.00111	0.00865	3.03 ± 0.15
27■	5.57 ± 0.04	$0.180 \pm 0.001^{k,l}$	0.0122	0.0322	150 ± 11.6	0.007 ± 0.0005^{m}	0.00214	0.0167	11.3 ± 0.5
28◆	39.5 ± 1.24	0.025 ± 0.0008^{q}	0.0017	0.0045	352 ± 11.2	0.003 ± 0.00009^{p}	0.000909	0.00711	2.27 ± 0.7
29▼	2.17 ± 0.04	$0.460 \pm 0.01^{d,e,f,g}$	0.0313	0.0823	68.6 ± 7.78	0.015 ± 0.002^{k}	0.00466	0.0364	34.4 ± 1.62

▲ Unripe fruits.
● Fruits well matured on tree.
■ Fruits collected unripe and left to mature for one week at room temperature.
◆ Fruits collected unripe and left to mature for three weeks at room temperature.
▼ Fruit pulp from well matured fruits.
Results are ±SD ($n = 3$). Values of the same column, and among Tables 2–8, followed by the same letter, are not statistically different ($P < 0.05$) as measured by Duncan's test.
[A] Efficient concentration (mg antioxidant/mg DPPH·): amount of antioxidant needed to decrease the initial DPPH· concentration by 50%.
[B] Antiradical efficiency: $1/EC_{50}$.
[C] Quercetin $EC_{50} = 0.068$ mg quercetin/mg DPPH·.
[D] Trolox $EC_{50} = 0.179$ mg trolox/mg DPPH·.
[E] Efficient concentration (μg antioxidant/ml): amount of antioxidant needed to decrease the initial chemiluminescence intensity by 50%.
[F] Antiradical efficiency: $1/IC_{50}$.
[G] Quercetin $IC_{50} = 0.32$ μg/ml.
[H] Trolox $IC_{50} = 2.5$ μg/ml.
[I] Gallic acid equivalants: μg gallic acid/1 mg dry extract.

Table 8
Total phenolic contents (GAE) and antioxidant capacities of the initial methanolic extracts of all the maturity stages of the fruits, expressed as EC_{50}, IC_{50}, AE, quercetin and trolox equivalents

Sample	DPPH· radical scavenging method				Chemiluminescence method				GA[I] equivalents
	$EC_{50} \pm SD$[A]	AE[B]	EC_{50} quercetin[C] equivalent	EC_{50} trolox[D] equivalent	$IC_{50} \pm SD$[E]	AE[F]	IC_{50} quercetin[G] equivalent	IC_{50} trolox[H] equivalent	
30▲	2.55 ± 0.11	0.393 ± 0.02^{e}	0.0267	0.0702	45.4 ± 1.78	$0.022 \pm 0.0009^{g,h}$	0.00706	0.0551	32.5 ± 3.63
31●	10.6 ± 0.34	0.094 ± 0.003^{n}	0.0064	0.0169	57.9 ± 0.38	$0.017 \pm 0.0001^{i,j}$	0.00553	0.0432	10.3 ± 2.08
32■	1.89 ± 0.06	$0.530 \pm 0.02^{d,e,f}$	0.0360	0.0948	60.5 ± 3.90	$0.017 \pm 0.001^{j,k}$	0.00529	0.0414	26.3 ± 5.65
33◆	20.0 ± 0.12	0.050 ± 0.0003^{p}	0.0034	0.0090	160 ± 2.14	0.006 ± 0.00008^{h}	0.0020	0.0156	5.58 ± 0.673
34▼	1.45 ± 0.02	$0.682 \pm 0.01^{b,c,d}$	0.0464	0.122	25.5 ± 1.16	$0.039 \pm 0.002^{d,e}$	0.0126	0.09812	28.1 ± 3.34

▲ Unripe fruits.
● Fruits well matured on tree.
■ Fruits collected unripe and left to mature for one week at room temperature.
◆ Fruits collected unripe and left to mature for three weeks at room temperature.
▼ Fruit pulp from well matured fruits.
Results are ±SD ($n = 3$). Values of the same column, and among Tables 2–8, followed by the same letter, are not statistically different ($P < 0.05$) as measured by Duncan's test.
[A] Efficient concentration (mg antioxidant/mg DPPH·): amount of antioxidant needed to decrease the initial DPPH· concentration by 50%.
[B] Antiradical efficiency: $1/EC_{50}$.
[C] Quercetin $EC_{50} = 0.068$ mg quercetin/mg DPPH·.
[D] Trolox $EC_{50} = 0.179$ mg trolox/mg DPPH·.
[E] Efficient concentration (μg antioxidant/ml): amount of antioxidant needed to decrease the initial chemiluminescence intensity by 50%.
[F] Antiradical efficiency: $1/IC_{50}$.
[G] Quercetin $IC_{50} = 0.32$ μg/ml.
[H] Trolox $IC_{50} = 2.5$ μg/ml.
[I] Gallic acid equivalants: μg gallic acid/1 mg dry extract.

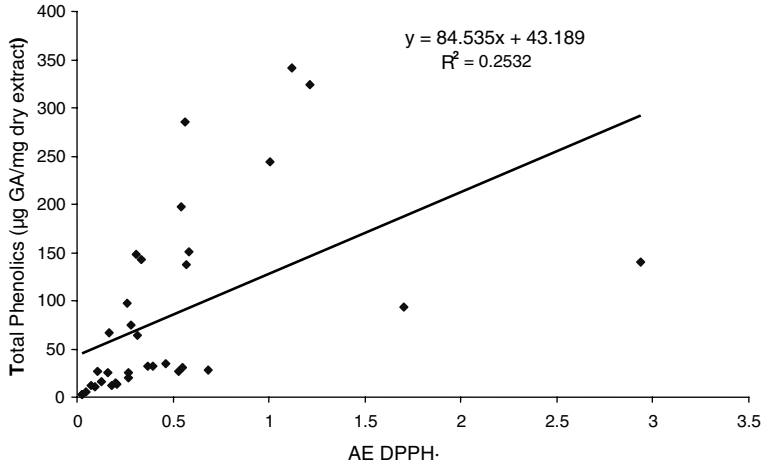

Fig. 1. Correlation between total phenolics (μg gallic acid/mg of dry extract) and DPPH· results, expressed as antiradical efficiencies; $AE = 1/EC_{50}$; EC_{50}: efficient concentration (mg antioxidant/mg DPPH·): amount of antioxidant needed to decrease the initial DPPH· concentration by 50%.

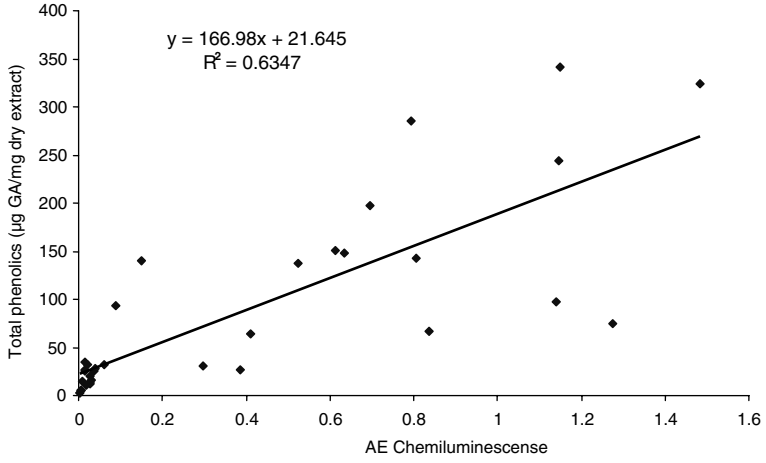

Fig. 2. Correlation between total phenolics (μg gallic acid/mg of dry extract) and Chemiluminescence results, expressed as antiradical efficiencies; $AE = 1/IC_{50}$; IC_{50}: efficient concentration (μg antioxidant/ml): amount of antioxidant needed to decrease the initial chemiluminescence intensity by 50%.

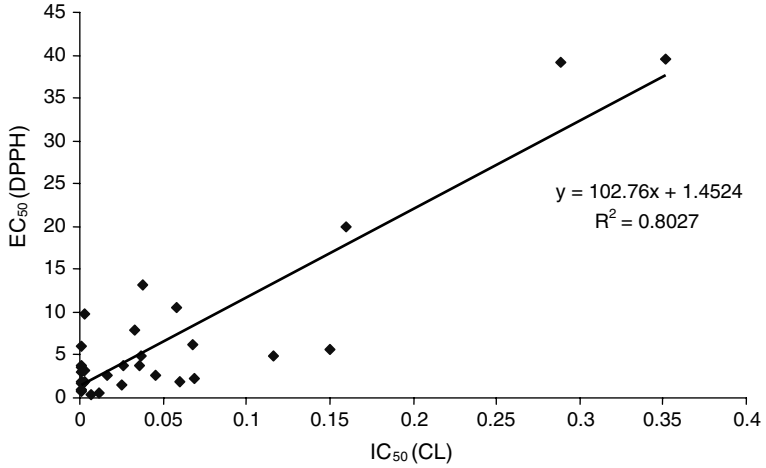

Fig. 3. Correlation between the DPPH· results and the chemiluminescence results, expressed as antiradical efficiencies. $AE = 1/EC_{50}$ and $AE = 1/IC_{50}$, respectively. EC_{50}: efficient concentration (mg antioxidant/mg DPPH·): amount of antioxidant needed to decrease the initial DPPH· concentration by 50%; IC_{50}: efficient concentration (μg antioxidant/ml): amount of antioxidant needed to decrease the initial chemiluminescence intensity by 50%.

Table 9
Correlation coefficient of gallic acid equivalents versus antioxidant capacities and correlation coefficients of EC_{50} versus IC_{50} for samples of all fruit categories

	R^{2A}	R^{2B}	R^{2C}
Residues (samples 1–5)	0.6531	0.3219	0.9539
Dichloromethane fractions (samples 6–9)	0.7695	0.7891	0.9698
Diethyl ether fractions (samples 10–14)	0.8795	0.7703	0.5129
Ethyl acetate fractions (samples 15–19)	0.6934	0.9187	0.8279
Butanol fractions (samples 20–24)	0.9954	0.9837	0.8675
Water fractions (samples 25–29)	0.9922	0.9836	0.9361
Initial methanolic extracts (samples 30–34)	0.739	0.469	0.8311
Unripe fruits (samples 1,6,10,15,20,25,30)	0.1317	0.4156	0.4418
Fruits well matured on tree (samples 2,7,11,16,21,26,31)	0.9868	0.806	0.9255
Fruits collected unripe and left to mature for one week (samples 3,8,12,17,22,27,32)	0.7808	0.765	0.44
Fruits collected unripe and left to mature for three weeks (samples 4,9,13,18,23,28,33)	0.4031	0.6252	0.9631
Fruit pulp (samples 5,14,19,24,29,34)	0.0772	0.7594	0.0524

[A] Correlation coefficient between GAE and AE (DPPH·).
[B] Correlation coefficient between GAE and AE (CL).
[C] Correlation coefficient between EC_{50} and IC_{50}.

worth mentioning that the correlation coefficients were very low for the unripe fruits ($R^2 = 0.1317$) and pulp ($R^2 = 0.0772$). These two categories in general had the strongest antioxidant capacities.

For the AE that emerges from the CL method, the correlation with the GAE is high : dichloromethane fractions ($R^2 = 0.7891$), diethyl ether fractions ($R^2 = 0.7703$), ethyl acetate fractions ($R^2 = 0.9187$), butanol fractions ($R^2 = 0.9837$) and water fractions ($R^2 = 0.9836$). On the other hand, for the same category, residues' and initial methanolic extracts', AE versus GAE, had $R^2 = 0.3219$ and $R^2 = 0.469$, respectively. For all maturity stages of the fruits, correlation coefficient was relatively high with the exception of the unripe fruits ($R^2 = 0.4156$).

3.4. Comparison between the two methods for radical-scavenging activity

A direct correlation between the two methods for radical-scavenging activity (EC_{50} for DPPH· test vs. IC_{50} for CL test), was demonstrated by linear regression analysis (Fig. 3). The two methods showed a high correlation coefficient (0.8027). This means that, generally, extracts, fractions and residues showed similar trends both in the free radical-and hydroxyl radical-scavenging activities. However, this observation is partially invalid for the fruit pulp ($R^2 = 0.0524$) and the unripe fruits ($R^2 = 0.4418$) (Table 9).

3.5. Extracts, fractions and reference antioxidants

From the results of the CL test and according to the statistical analysis (Duncan's test), it emerges that the dichloromethane, diethyl ether and ethyl acetate fractions exhibited the best radical-scavenging capacity and that was in correlation with the phenolic content (Tables 3–5). The butanol fraction of the unripe fruits and the pulp and the pulp residue came next (Tables 2 and 6). According to the results from the DPPH· test, the most powerful antioxidants were the butanol fractions of unripe fruits and pulp, followed by the diethyl ether fractions of unripe fruits and the ethyl acetate fraction of pulp (Tables 3,5 and 6). However, these DPPH· results are not always in correlation with the total phenolic content.

Two standards, known for their good antioxidant activity, trolox (weaker) and quercetin (stronger), were used. These standards were also used in the past (Parejo et al., 2000). Evaluating the IC_{50}, all the dichloromethane, diethyl ether and ethyl acetate fractions (samples 6–19, Table 1) were found to be stronger or equal antioxidants to trolox (Tables 2–4). Sample 12 was found to be 3.7 times stronger than trolox, and sample 7, almost equal (Table 1). Trolox though, was stronger when using EC_{50} (at least 2 times). Quercetin was at least 2 times stronger than samples (IC_{50}) and at least 5 times stronger than samples (EC_{50}).

4. Conclusions

Results showed that dichloromethane, diethyl ether and ethyl acetate fractions possessed significant radical-scavenging activity, which was greater than the activity of trolox when examined by the chemiluminescence test. This seemed to be correlated with the total phenolic content. Among the initial methanolic extracts of the five different categories of the fruits, the raw yellow fruits, together with the fruit pulp, were the strongest antioxidants, while the brown, well-matured fruits, which are the ones consumed, were the weakest

ones. It is interesting that the total phenolic content did not correlate well with the results from the DPPH· test. This may indicate a specific mechanism of antiradical activity of the extracts, probably due to the physicochemical and structural characteristics of the components contained. This is to be explored when the extensive phytochemical (on preparative scale) analysis of each fraction is accomplished.

All this information may be useful for the promotion of use of *S. domestica* fruit extract as a natural antioxidant in food and medicinal products, justifying the traditional use of the fruit as food with beneficial health properties.

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