Food Chemistry 111 (2008) 816–823

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Total phenolic compounds and antioxidant capacities of major fruits from Ecuador

Catalina Vasco [a,b,*], Jenny Ruales [a], Afaf Kamal-Eldin [b]

[a] Department of Food Science and Biotechnology, Escuela Politécnica Nacional, P.O. Box 17-01-2759, Quito, Ecuador
[b] Department of Food Science. Swedish University of Agricultural Sciences, P.O. Box 7051, S-750 07 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 30 July 2007
Received in revised form 13 February 2008
Accepted 22 April 2008

Keywords:
Fruits
Ecuador
Phenolic compounds content
Antioxidant capacity
Spectrophotometry

ABSTRACT

Seventeen fruits from Ecuador were analysed for total soluble phenolic compounds content and for antioxidant capacity, using three different methods (DPPH·, FRAP and ABTS·+). For the total phenolic content measured by the Folin–Ciocalteu method, three groups, having <100, 200–500 and >1000 mg GAE/100 g FW, were clearly distinguishable. Andean blackberry, capulí cherry peel and banana passion fruit were classified in the third group, with concentrations of 2167, 1494 and 1010 mg of GAE/100 g FW, respectively. Antioxidant capacity analyses revealed the same classes. FRAP and ABTS·+ gave comparable results and were highly correlated ($y = 0.691x + 6.78$; $r^2 = 0.908$). Spectrophotometric measurements showed that the Andean blackberry and capulí peel but not banana passion fruit contained high levels of anthocyanins ($\lambda_{max} = 520$ nm).

1. Introduction

In addition to their delicious taste and refreshing flavour and aroma, fruits add important vitamins, minerals and other bioactive compounds to the human diet. It has been shown in epidemiological studies that a correlation exists between the consumption of fruits and reduced risk of chronic diseases (Block, Patterson, & Subar, 1992; Chun & Kim, 2004; He, Nowson, Lucas, & Macgregor, 2007; Kuskoski, Asuero, & Troncoso, 2005; Van't Veer, Janson, Klert, & Kok, 2000; Wu et al., 2004). The combination of vitamins, minerals, phenolic antioxidants and fibre seem to be responsible for these effects (Ruxton, Gardner, & Walker, 2006). Parallel with this recognition, the consumption of tropical or "exotic" fruits has increased all over the world.

Different fruits differ markedly in the quantity and types of phenolic antioxidants and their conjugates (Macheix, Fleuriet, & Billot, 1990). The use of simple "total antioxidant capacity" methods differing in their way of generating free radicals, the strategy to measure the end point of the inhibition reaction, and the sensitivity towards the different reducing molecules in the sample (Pellegrini et al., 2003; Roginsky & Lissi, 2005). Therefore, more than one method should be used to gain useful information about the total antioxidant capacity of phenolic compounds.

Similar to other tropical countries, Ecuador is rich in a wide range of delicious fruits that are reaching international markets. This work is a first step to measure the total phenolic content in fruits from Ecuador using the Folin–Ciocalteu reagent and three total antioxidant activity methods.

2. Materials and methods

2.1. Fruit samples

Seventeen fruits, belonging to seven botanical families, which are commonly cultivated and consumed in Ecuador, were chosen for this study. The fruits, which are grown in different parts of Ecuador (Fig. 1) are described in Table 1 with more details below. Samples of the different fruits (1–4 kg, depending on the fruit), were purchased at eating ripeness, at three occasions from three different popular markets in the capital Quito during 2005. Only fruits without blemishes or damage were selected, cleaned and whenever necessary, the peels and seeds were removed, to leave the samples in the same way in which they are consumed. Capulí cherry's peel and pulp were analysed separately because people consume the whole as well as peeled fruits. The edible parts were chopped and their water content was determined in a vacuum oven at 80 °C during 6 h. Ascorbic acid content and °Brix were measured. The fresh samples were stored at −20 °C until freeze–dried. After freeze–drying, the samples were grinded, stored at −20 °C and analysed within three months.

2.2. Chemicals and Reagents

Folin–Ciocalteu reagent (2.0 N), DPPH· (1,1-diphenyl-2-picrylhydrazyl), ABTS·+ (2,2′-azinobis(3-ethylbenzothiaoline-6-sulfonic

* Corresponding author. Address: Department of Food Science and Biotechnology, Escuela Politécnica Nacional, P.O. Box 17-01-2759, Quito, Ecuador. Tel.: +593 2 2 507 138; fax: +593 2 2 507 142.
E-mail addresses: catalina.vasco@lmv.slu.se, iit8@server.epn.edu.ec (C. Vasco).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.04.054

Fig. 1. Distribution of the production areas of fruits in Ecuador by province. For fruit numbers, see Table 1.

acid)), TPTZ (2,4,6-tripyridyl-*s*-triazine), gallic acid, caffeic acid, ferulic acid, quercetin, rutin, tannic acid, DL-α-tocopherol, resveratrol, ascorbic acid, BHA (3-*tert*-butyl-4-hydroxyanisole), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), $FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$ and potassium persulfate were purchased from Sigma-Aldrich (St. Louis, MO), and sodium carbonate was from JT Baker (Phillipsburg, NJ). Methanol and ethanol were of HPLC grade and other reagents were of analytical grade.

2.3. Extraction of fruit samples

The extraction method used is a modification of the procedure described by Larrauri, Rupérez, and Saura-Calixto (1997). Freeze–dried samples (0.5 g) were weighed and twice extracted at room temperature under continuous stirring for 1 h, first with 20 ml of a mixture of methanol: water (50:50 v/v) and then with 20 ml of acetone: water (70:30 v/v) with intermittent centrifugation (4000 rpm, 15 min). The supernatants were pooled in volumetric flasks and the volumes were made up to 50 ml with distilled water. Extractions were performed in triplicate and all analyses were performed on these extracts.

2.4. Total soluble phenolic compound content

The content of soluble phenols was measured using a modified Folin and Ciocalteu (1927) method, employing the reduction of a phosphowolframate–phosphomolybdate complex to blue products by phenolic compounds. Briefly, an aliquot (0.5 ml) of the extract, blank or standard was placed in a 25 ml flask, where the Folin–Ciocalteu reagent (0.5 ml) was added and the mixture was allowed to react for 3 min under continuous stirring before a solution of sodium carbonate (75 g/l, 10 ml) was added and mixed well. The volume was then made up to 25 ml with distilled water and left standing at room temperature for 1 h. The absorbance was then measured at 750 nm using a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The results were expressed as gallic acid equivalents (GAE), using a calibration curve over the range of 50–200 ppm.

2.5. 1,1-Diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$) assay

The antiradical efficiency was assessed, using the $DPPH^{\cdot}$ method, as described by Sánchez-Moreno, Larrauri, and Saura-Calixto (1998). An aliquot (0.1 ml) of the sample extract was added to $DPPH^{\cdot}$ in methanol (3.9 ml) in a 4 ml cuvette. The absorbance was measured using the kinetic mode of the spectrophotometer at time zero and every 10 s until the reaction reached the steady state plateau. For samples with strong and medium antiradical properties dilutions were made, in order to work as close as possible to the EC_{50}. The EC_{50} and t_{EC50} were calculated only for those extracts that reduced $DPPH^{\cdot}$ in the cuvette by >50% when the absorbance reached the plateau. For the other samples, which were considered of low antiradical activity, we reported the concentration of the remaining $DPPH^{\cdot}$, the antioxidant capacity in μmol Trolox/g sample FW and the time to reach the steady state.

Table 1
Description of the 17 studied Ecuadorian fruit samples

Number	English name	Ecuadorian name	Scientific name	Family	Fruit description[a,b]
1	Mango	Mango	*Mangifera indica* L.	*Anacardiaceae*	Drupe 200–570 g/fruit, 7–11 cm diameter, 8.5–12 cm long, 70–80% edible part, 75–84% moisture content, 13.9–17.8 °Brix
2	Red mombin	Hobo	*Spondias mombin* L.	*Anacardiaceae*	Aromatic drupe, 13–33 g/fruit, 2.5–4 cm diameter, 3–4.5 cm long, juicy flesh attached to a white fibrous stone, 70–82% edible part, 77–88% moisture content, 11.2–18.6 °Brix
3	Cherimoya	Chirimoya	*Annona cherimolia* Mill.	*Annonaceae*	Irregular shape, 180–590 g/fruit, 7–11 cm diameter, 5–11 cm height, many seeds, each black seed surrounded by white flesh, 40–65% edible part, 69–80% moisture content, 15.8–21.0 °Brix
4	Zapote	Zapote	*Calocarpum zapota*	*Bombacaceae*	Brown leathery thick skinned fruit, 440–760 g/fruit, 9–12 cm diameter, 8–11 cm height, 4 or 5 seeds surrounded by orange flesh, 40–70% edible part, 81–87% moisture content, 10.0–14.1 °Brix
5	Guava	Guayaba	*Psidium guajava* L.	*Myrtaceae*	Pink fleshed, strong sweet aroma, 35–230 g/fruit, 4–7 cm diameter, 4–10 cm long, many seeds, 100% edible part, 78–84% moisture content, 10.4–11.4 °Brix
6	Passion fruit	Maracuyá	*Passiflora edulis* var. flavicarpa	*Passifloraceae*	Yellow passion fruit, sour taste, strong exotic aroma, 85–165 g/fruit, 6–8.5 cm diameter, 7–11 cm long, several black seeds surrounded by a yellow jelly, 50–60% edible part, 73–79% moisture content, 17.6–21.4 °Brix
7	Granadilla	Granadilla	*Passiflora ligularis* L.	*Passifloraceae*	Hard glossy rind, 56–160 g/fruit, round shaped fruit, 5–7 cm diameter, 6–9 cm height, several seeds surrounded by a transparent white very sweet jelly, 40–55% edible part, 72–80% moisture content, 7.0–8.0 °Brix
8	Banana passion fruit	Taxo	*Passiflora mollissima* L.	*Passifloraceae*	Banana shaped fruit, 42–112 g/fruit, 3–4.5 cm diameter, 7–13 cm long, many seeds surrounded by jelly, 60–70% edible part, 80–83% moisture content, 15.2–17.8 °Brix
9	Strawberry	Frutilla	*Fragaria ananasa* Duch	*Rosaceae*	Berries from 6–16 g/fruit, 2–5 cm diameter, 3–6 cm height, red or red-white flesh, sweet taste, 100% edible part, 86–91% moisture content, 8.5–10.1°Brix
10	Capulí cherry	Capulí	*Prunus serotina var.* Capulí	*Rosaceae*	Black cherries, 2–8 g/fruit, 1–3 cm diameter, single stone, green flesh, 60–85% edible part, 71–78% moisture content, 16.3–22.2 °Brix
11	Plum	Reina-claudia	*Prunus salicina* Lindl	*Rosaceae*	Red or yellowish flesh, dark red skin, 12–43 g/fruit, 3–4 cm diameter, 2–4 cm long, single stone, sweet and sour taste, 80–96% edible part, 83–91% moisture content, 8.1–17.4 °Brix
12	Andean blackberry	Mora de castilla	*Rubus glaucus* Berth	*Rosaceae*	Black berries, 4–11 g/fruit, 1–2 cm diameter, several seeds, 100% edible part, 83–87% moisture content, 10.0–11.0 °Brix
13	Naranjilla	Naranjilla	*Solanum quitoense* Lam	*Solanaceae*	Round aromatic fruit, 40–120 g/fruit, 4–6.5 cm diameter, 3–5.5 cm height, similar to tomato inside, yellow-green flesh with seeds separate by a membrane, sour taste, 70–90% edible part, 82–92% moisture content, 7.3–10.2 °Brix
14	Sweet pepino	Pepino dulce	*Solanum muricatum* Ait	*Solanaceae*	Egg-shaped, watery fruit, 120–290 g/fruit, 5–8.5 cm diameter, 7–10.5 cm long, hollow in the middle with several small seed attached, 70–85% edible part, 89–93% moisture content, 7.3–11.9 °Brix
15	Physalis	Uvilla	*Physalis peruviana* L.	*Solanaceae*	Golden spheres with a papery husk, 2–8 g/fruit, size of marbles, 1–2.5 cm diameter, 90–95% edible part, 76–81% moisture content, 17.5–18.2 °Brix
16	Purple/red and golden/yellow tree tomato	Tomate de árbol (morado y amarillo)	*Cyphomandra betacea* Sendt	*Solanaceae*	Egg-shaped fruit, 60–170 g/fruit, 4–7 cm diameter, 6–10 cm long, in the centre of the fruit the jelly with the seeds can be golden yellow or purple-red, sweet and sour taste, thin peel with a strong bitter taste, 65–85% edible part, 84–88% moisture content, 9.0–11.0 °Brix
17	Tomato	Tomato	*Lycopersicon esculentum* Mill.	*Solanaceae*	Smooth peel fruits, 40–185 g/fruit, 4–8 cm diameter, 4–6 cm height, 96–99% edible part, 91–95% moisture content, 5.2–7.3 °Brix

[a] Number of sets of samples analysed: mango, zapote, guava, passion fruit, granadilla, banana passion fruit, strawberry, capulí cherry, Andean blackberry, naranjilla, sweet pepino, physalis, yellow tree tomato, and tomato ($n = 9$), red mombin ($n = 8$), cherimoya ($n = 7$), plum ($n = 5$), and purple tree tomato ($n = 2$).
[b] Values measured in the laboratory.

2.6. 2,2′-Azinobis-3-ethylbenzotiazoline-6-sulfonic acid ($ABTS^{\cdot+}$)

The $ABTS^{\cdot+}$ assay was performed according to Re et al. (1999). The ABTS radical cation ($ABTS^{\cdot+}$) was produced by mixing ABTS (7 mM final concentration in 25 ml) with potassium persulfate (2.45 mM final concentration in 25 ml) and keeping it in the dark at room temperature for 12–16 h (the reagent was stable for 2 days). For analysis, the reagent was diluted in ethanol until the absorbance at 734 nm was 0.7 ± 0.02. The solution was equilibrated at 30 °C and, after the addition of 1 ml of this solution to 10 μl of the extracts or Trolox standard solution (2.5 mM standard stock solution), the absorbance at 734 nm was measured every 10 s for 6 min.

2.7. Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed, as previously described by Benzie and Strain (1996) and Ahmad and Mukhtar (1999). The fresh FRAP reagent was prepared daily by mixing 25 ml acetate buffer (300 mM, pH 3.6), 2.5 ml TPTZ solution (10 mM in 40 mM HCl) and 2.5 ml of $FeCl_3 \cdot 6H_2O$ solution (20 mM). The reagent was warmed to 37 °C, then 900 μl were placed in a cuvette and the initial absorbance was read. A 100 μl volume of diluted sample (1:4 v/v in water) was added to the cuvette and the absorbance was measured every 10 s. In this study, the reaction was followed until it reached the plateau. Values were calculated according to the calibration curve with aqueous solutions of $FeSO_4 \cdot 7H_2O$ in the range of 100–1000 μM. Data were then converted to Trolox equivalents, using the equivalence determined by Stratil, Klejdus, and Kuban (2006).

3. Results

In this study, 17 different fruits from Ecuador (Table 1) were investigated for their total soluble phenolic compounds and their antioxidant capacity, using three different methods. Some of the fruits selected for the present study are unknown outside their natural range, some are barely known and some are well known and marketed internationally. However, there are no data available on the antioxidant activity of these fruits from Ecuador (Kuskoski et al., 2005).

Sample extraction was performed using 50% methanol, followed by 70% acetone, according to Larrauri et al. (1997). This method enables extraction of a wide range of phenols, i.e., compounds like simple phenols, flavonoid glycosides, procyanidins, and some oligomeric and polymeric proanthocyanidins from diverse sample types (Keinanen, 1993; Robards, 2003). The total soluble phenolic content of the extracts was measured by the Folin–Ciocalteu method and expressed in mg of gallic acid equivalents per 100 g of edible part of the fruit. The antioxidant capacity was analysed with three other methods, i.e., DPPH·, $ABTS^{\cdot+}$, and FRAP (Benzie & Strain, 1996; Re et al., 1999; Sánchez-Moreno et al., 1998).

3.1. Total soluble phenolic compound content

The Folin–Ciocalteu method measures the reduction of the reagent by phenolic compounds with the formation of a blue complex that can be measured at 750 nm against gallic acid as a standard (Imeh & Khokhar, 2002). The samples (Table 2) are arranged from the highest to the lowest total soluble phenols content, which ranged from 26 to 2100 mg GAE/100 g sample FW. Three groups were clearly distinguishable; the first group including Andean blackberry (2167 mg GAE/100 g FW), capulí cherry peel (1494 mg GAE/100 g FW) and banana passion fruit (1010 mg GAE/100 g FW); the second group including samples having 250–400 mg GAE/100 g FW, i.e., plum, guava, capulí cherry pulp, zapote, strawberry, cherimoya and red mombin; and the third group including samples with "low" content < 100 mg GAE/100 g FW, granadilla, naranjilla, physalis, tree tomato, passion fruit, mango, sweet pepino and tomato.

Comparing these results with literature, similar values were reported for strawberry (264 and 368 mg GAE/100 g FW), passion fruit (72 mg GAE/100 g FW), mango (68 mg GAE/100 g FW), tomato (10–26 mg GAE/100 g FW), guava (170–345 mg GAE/100 g FW), and plum (366–478 mg GAE/100 g FW; Brat et al., 2006; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins, 2006; Wu et al., 2004). In another study of plum genotypes, contents ranging from 150–300 mg GAE/100 g FW were obtained after conversion from chlorogenic acid equivalents (Cevallos-Casals, Byrne, Okie, & Cisneros-Zevallos, 2006) to gallic acid equivalents, using the factor determined by Chun and Kim (2004). However, values that differed from our results were found, e.g., 660 mg GAE/100 g FW for common blackberry, 266 mg GAE/100 g FW for mango, and 80–100 mg GAE/100 g FW for tomato (Wu et al., 2004). The variation may be due to differences in varieties, climate, ripeness, extraction method, etc. For the rest of the samples, there is no available literature information about the content of phenolic compounds or the antioxidant capacity but the results are generally in the order of magnitudes reported for fruits, i.e., apple (211–347 mg GAE/100 g), apricot (133–178 mg GAE/100 g), banana (52–231 mg GAE/100 g), blueberry (531–795) mg GAE/100 g, cranberry (709 mg GAE/100 g), grape (145–196 mg GAE/100 g), orange (31–337 mg GAE/100 g), pear (69–220 mg GAE/100 g), and pineapple (47–174 mg GAE/100 g; Brat et al., 2006; Wu et al., 2004).

3.2. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH·) assay

The DPPH· radical absorbs at 515 nm and this absorption is inhibited in the presence of antioxidants (Brand-Williams, Cuvelier, & Berset, 1995). This reduction in absorbance is related to the antiradical efficiency of the sample, extract or standard. The DPPH· antiradical efficiency values of the fruit extracts analysed in this study are presented in Table 2. Here, two groups of samples can be distinguished. The first group contained the samples with high DPPH· antiradical efficiency, coinciding with samples with high and medium total soluble phenolic content, measured by the Folin–Ciocalteu method. In this group, the EC_{50} and t_{EC50} were calculated because the percentage of remaining DPPH· at the steady state was <50%. Among the first group of fruits, the three samples with the highest EC_{50} were capulí cherry peel, banana passion fruit, and Andean blackberry, which are also the three samples with the highest phenolic compound content. The extracts of plum, strawberry, Andean blackberry and capulí peel have a high absorbance at 520 nm, which interferes with the absorbance of DPPH· at 515 nm leading to an underestimation of the antioxidant capacity of these fruits. The second group of fruits, which was considered to have low antiradical efficiency, included granadilla, naranjilla, physalis, tree tomato (purple-red and golden-yellow), passion fruit, mango, sweet pepino, and tomato; fruits with low total soluble phenolic content. Other reasons for the low antiradical efficiency of group 3 fruits might be that the phenolic compounds in these fruits are bound to other molecules, such as carbohydrates, which considerably reduce the activity, or are weak antioxidants *per se*.

The group of fruits with low antiradical efficiency showed kinetic curves that have a decrease in absorbance between 4% and 17% and that reached the steady state in a very short time (0.5–7 min). For the second group of fruits the absorbance decreases gradually to reach the steady state in longer times (16–33 min). The first group formed by blackberry, banana passion fruit and capulí peel showed a very fast decrease to very low absorbances and reached the plateau after 16–22 min. Examples of the curves can be seen in Fig. 2. Plum and cherimoya phenolic compounds reacted slower than the compounds in banana passion fruit, where a marked slope is seen in the first minutes and the plateau is reached after 10 min, when the DPPH· radical is almost completely spent. The curves give an idea of the variety of composition and concentration of the phenolic compounds in every sample, as well as the quality of the compounds with regard to their antiradical efficiency. The fruit extracts have different colour intensities, due to different levels of anthocyanins ($\lambda = 520$ nm). The absorbance curves of the selected fruits extracts are shown in Fig. 3. This indicates that the active antioxidant phenols in banana passion fruit are not anthocyanins.

3.3. $ABTS^{\cdot+}$ and FRAP

Fig. 4 shows the antioxidant capacity of the fruit extracts determined as Trolox equivalents (μmol Trolox/g sample FW) using the $ABTS^{\cdot+}$ and FRAP assays. The analysed fruits showed a wide range of antioxidant capacities, 1.5–124 μmol Trolox/g sample FW. Again the fruits can be divided into three groups that are separated with dotted lines. The highest activities were found in Andean blackberry, capulí cherry peel and banana passion fruit, the rank being the same for $ABTS^{\cdot+}$ and FRAP. Interestingly, some fruits (e.g., guava) have Trolox equivalents comparable to that of Andean blackberry, even if their total phenolic content, measured as GAE, is low. This indicates that these fruits contain some very potent phenolic antioxidants.

The results of the two assays were highly correlated ($r^2 = 0.908$; Fig. 4), a correlation of 0.97 has also been reported by Thaipong et al. (2006). The $ABTS^{\cdot+}$/FRAP ratio of 0.6–2.5 is in the same range reported by Nilsson et al. (2005). The $ABTS^{\cdot+}$ assay measures the scavenging of free radicals as the discoloration of the $ABTS^{\cdot}$ blue reactant, while FRAP measures the potential to reduce the yellow ferric-TPTZ complex to a blue ferrous-TPTZ complex by electrodonating substances under acidic conditions (Nilsson et al., 2005). FRAP assay detects compounds with redox potentials of <0.7 V, the redox potential of ferric-TPTZ, and is comparable with the $ABTS^{\cdot+}$ redox potential (0.68 V; Prior, Wu, & Schaich, 2005).

Table 2
Total soluble phenolic compound content, ascorbic acid content and DPPH· antioxidant capacity[a]

Phenolic compounds	English name	Ascorbic acid (mg/100 g FW)	Total soluble phenolic compound content (mg GAE/100 g sample FW)	DPPH· assay[b]				
				Experimental parameters			Calculated parameters[c]	
				Antioxidant capacity (μmol Trolox/g sample FW)	DPPH· remaining at the steady state (%)	Time to reach the steady state (min)	EC_{50} (g sample FW/g DPPH·)	t_{EC50} (min)
High level	Andean blackberry	10–11	2167 ± 835	41 ± 16	43 ± 7	14 ± 1	701 ± 181	13 ± 1
	Capulí cherry peel	1–2	1494 ± 385	76 ± 21	42 ± 8	22 ± 3	352 ± 86	20 ± 2
	Banana passion fruit	63–93	1010 ± 198	70 ± 4	46 ± 3	16 ± 1	407 ± 125	15 ± 2
Intermediate	Guava	74–84	462 ± 128	30 ± 7	40 ± 9	16 ± 1	900 ± 297	14 ± 1
	Plum		440 ± 272	16 ± 8	54 ± 9	33 ± 8	1583 ± 371	33 ± 4
	Capulí cherry pulp	1–2	331 ± 56	13 ± 5	54 ± 14	19 ± 5	2055 ± 42	19 ± 1
	Cherimoya	4–6	323 ± 83	23 ± 6	37 ± 12	25 ± 5	1087 ± 236	20 ± 2
	Zapote	2–3	258 ± 124	17 ± 7	30 ± 14	18 ± 3	1718 ± 633	17 ± 2
	Red mombin	27–36	249 ± 67	17 ± 8	37 ± 17	16 ± 6	1368 ± 395	14 ± 1
	Strawberry	50–66	238 ± 86	11 ± 3	34 ± 6	24 ± 3	2395 ± 663	22 ± 2
Low	Granadilla	16–25	91 ± 43	0.5 ± 0.2	95 ± 2	0.5 ± 0.2	–	–
	Naranjilla	11–13	91 ± 17	3.2 ± 0.9	77 ± 4	10 ± 3	–	–
	Physalis	58–68	87 ± 19	0.7 ± 0.4	86 ± 4	1.2 ± 0.6	–	–
	Tree tomato (purple-red)	16–24	81 ± 29	4.2 ± 1.0	72 ± 27	6.5 ± 2.8	–	–
	Tree tomato (golden-yellow)	16–24	81 ± 13	2.6 ± 0.4	83 ± 2	6.0 ± 1.4	–	–
	Passion fruit	30–40	61 ± 32	0.5 ± 0.3	94 ± 1	0.5 ± 0.3	–	–
	Mango	19–20	60 ± 26	3.1 ± 0.6	84 ± 3	2.9 ± 0.8	–	–
	Sweet pepino	20–38	56 ± 15	0.3 ± 0.2	93 ± 2	1.3 ± 0.4	–	–
	Tomato	18–21	26 ± 12	0.8 ± 0.3	88 ± 2	2.2 ± 0.7	–	–

[a] Results presented as mean ± SD (except for ascorbic acid where the range is shown); all analyses were performed in triplicate; the number of samples was nine except for red mombin, capulí cherry pulp, plum, cherimoya and tree tomato purple-red.
[b] Concentration of the initial DPPH solution was 0.025 g/l.
[c] Parameters that were not calculated when the concentration of the remaining DPPH· did not reach less than 50% at the steady state.

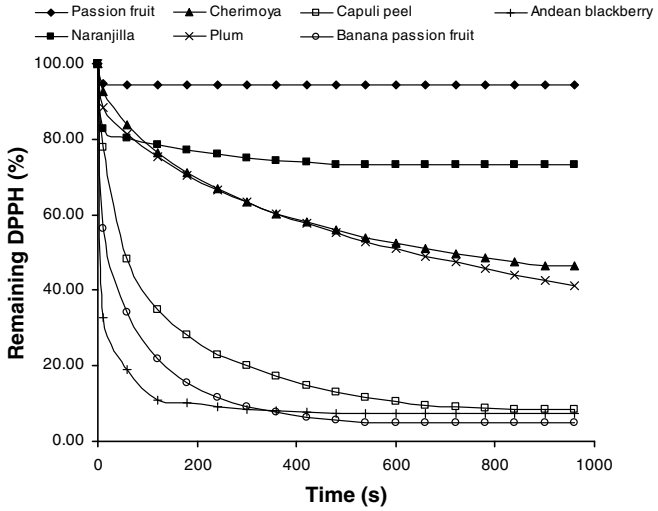

Fig. 2. Examples of DPPH· kinetic curves for samples with low, medium and high antiradical efficiency.

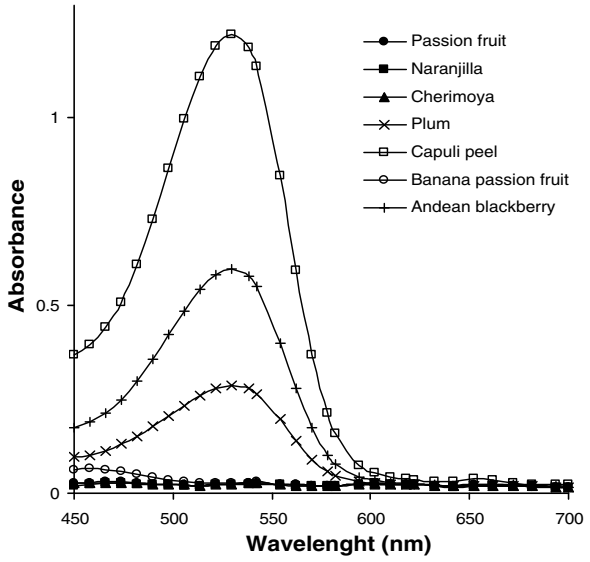

Fig. 3. Absorbance spectra for the samples shown in Fig. 2.

Correlations between total phenolic compounds and antioxidant capacity methods FRAP and ABTS·+ were 0.62 and 0.56, respectively, (Fig. 5a and b). Other studies report correlations between −0.58 and 0.97, depending on the extraction solvent, the hydrophilicity of the compounds, the sample and the type of phenolic compound, which means that different phenolic compounds react in different ways in these assays (Thaipong et al., 2006). Correlations were recalculated without the three strongest samples, showing a very high linear correlation this time where the new values were 0.90 and 0.96, respectively. The three removed samples were correlated separately and they presented a negative correlation (0.91 for FRAP and 0.89 for ABTS·+) suggesting that although banana passion fruit has a lower amount of phenolic compounds, these compounds are very strong antioxidants, compared to the compounds in capulí peel and blackberry.

To compare the results obtained with the DPPH· method with those obtained with ABTS·+ and FRAP, a calibration curve with Trolox as standard was prepared and the values recalculated (see Table 2). Among the 17 fruits, capulí peel has the highest antioxidant capacity, whereas sweet pepino has the lowest capacity. Also, the samples are divided into three groups: the first three fruits showed high antioxidant capacity, fruits from guava to strawberry showed medium antioxidant capacity and the rest showed low antioxidant capacity. Even though the correlation with ABTS·+ and FRAP was very high (0.97 with FRAP and 0.90 with ABTS·+), values are lower than for the other methods. This may be due to drawbacks with the method itself, i.e., some test compounds have spectra that overlap DPPH· at 515 nm and many antioxidants, which are highly reactive with peroxyl radicals, may react slowly or do not react at all with DPPH· (Prior et al., 2005).

4. Discussion

Using total soluble polyphenols content and the antioxidant capacity measured by three methods (DPPH·, FRAP and ABTS·+), 17 fruits from Ecuador were divided into three groups of high, medium and low phenolic content and antioxidant capacity. Positive correlations were obtained between phenolic content and antioxidant capacity, measured as DPPH·, FRAP and TEAC, of 0.66, 0.62 and 0.56, respectively. The same comparisons but without the group of the three fruits with high antioxidant activity (Andean blackberry, banana passion fruit and capulí peel) showed higher correlations (0.86, 0.90 and 0.96). In fact, negative correlations were obtained for the three samples (0.67, 0.92 and 0.89), showing that banana passion fruit has low levels of phenolic compounds but very strong antioxidant capacity. This fruit has a high ascorbic acid content, comparable to that in guava, a fruit with relatively high antioxidant capacity (group 2) despite a relatively low polyphenol content.

The correlations FRAP–ABTS·+, FRAP–DPPH·, and ABTS·+–DPPH· were very high (0.9, 0.97 and 0.9, respectively). Each method only provides an estimate of antioxidant capacity that is subjective to its conditions and reagents. Therefore, the use of different methods helps to identify variations in the response of the compounds extracted from the fruit samples and the response of pure compounds. The three methods gave information about the estimated antioxidant capacity but FRAP and TEAC were easier to handle in terms of kinetics and calculations. The DPPH· method used in this study has a different interpretation and the comparison was very difficult since it is also dependent on the initial concentration of the DPPH· radical and a recalculation of data with Trolox as standard was necessary. Antioxidant capacity may be underestimated comparing fruit samples and individual compounds because there are interactions with other phenolic compounds or other compounds that can happen in complex mixtures such as fruit extracts and do not occur in pure compounds. These methods indeed only measure phenolic compounds in which the phenolic groups are free, i.e., not glycosylated or ester linked.

In this study, we determined the total phenolic content and antioxidant capacity of 17 fruits from Ecuador including 13 fruits that have not been studied before. The results showed a wide range of variation with respect to total phenolic content and antioxidant capacity in the different assays but three clear groups of fruits could be identified as having high, medium and low phenolic compounds content and antioxidant capacity. Besides Andean blackberry, the banana passion fruit is very high in antioxidant activity and deserves further investigations. In our future research, we are in the process of analysing the phenolic compounds in different fruits by liquid chromatography with ultra-violet and mass spectrometric detectors.

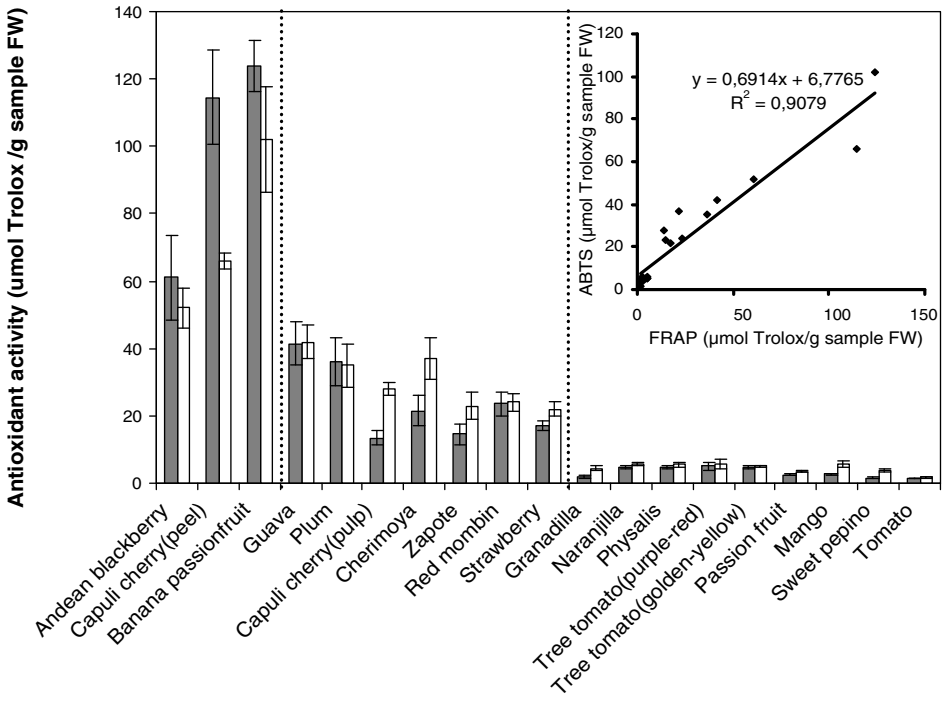

Fig. 4. The antioxidant capacity of Ecuadorian fruit extracts measured as Trolox equivalents (µmol Trolox/g sample FW) by FRAP (■) and ABTS· (□) assays. Correlation between the two methods FRAP and ABTS·+. Number of samples $n = 9$, except for red mombin $n = 8$, plum $n = 5$, cherimoya $n = 7$ and tree tomato purple-red $n = 2$.

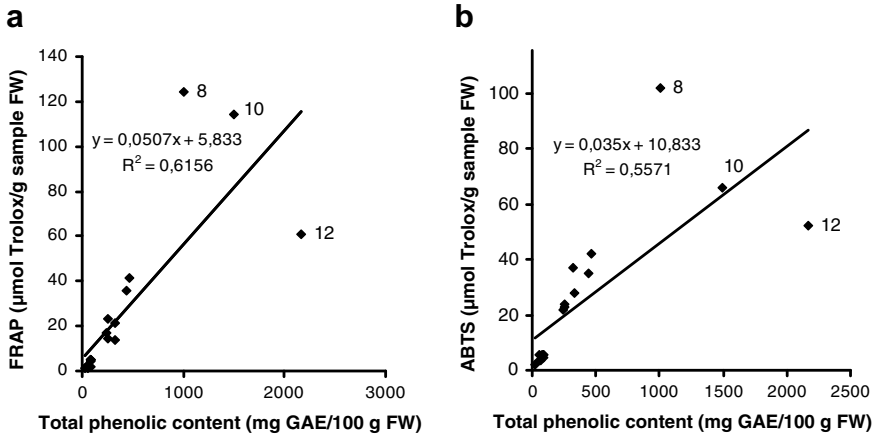

Fig. 5. Correlation between total phenolic content and antioxidant capacity measured by (a) FRAP and (b) ABTS·+. Sample numbers are explained in Table 1.

Acknowledgements

This research work (Project EC: 01) was supported by Grants from The International Programme in the Chemical Sciences (IPICS, Uppsala University, Uppsala, Sweden).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2008.04.054.

References

Ahmad, N., & Mukhtar, H. (1999). Green tea polyphenols and cancer: Biologic mechanisms and practical implications. *Nutrition Reviews, 57*(3), 78–83.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The frap assay. *Analytical Biochemistry, 239*, 70–76.

Block, G., Patterson, B., & Subar, A. (1992). Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. *Nutrition and Cancer, 18*, 1–29.

Brand-Williams, W., Cuvelier, M., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie, 28*, 25–30.

Brat, P., George, S., Bellamy, A., Chaffaut, L. D., Scalbert, A., Mennen, L., et al. (2006). Daily polyphenol intake in France from fruit and vegetables. *The Journal of Nutrition*, 2368–2373.

Cevallos-Casals, B., Byrne, D., Okie, W., & Cisneros-Zevallos, L. (2006). Selecting new peach and plum genotypes rich in phenolic compounds and enhanced functional properties. *Food Chemistry, 96*, 273–280.

Chun, O. K., & Kim, D.-O. (2004). Consideration on equivalent chemicals in total phenolic assay of chlorogenic acid-rich plums. *Food Research International, 37*, 337–342.

Folin, O., & Ciocalteu, V. (1927). On tyrosine and tryptophane determinations in proteins. *Journal of Biological Chemistry, 73*, 627–650.

He, F., Nowson, C., Lucas, M., & Macgregor, G. (2007). Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: Meta-analysis of cohort studies. *Journal of Human Hypertension*. doi: 10.1038/sj.jhh.1002212.

Imeh, U., & Khokhar, S. (2002). Distribution of conjugated and free phenols in fruits: Antioxidant activity and cultivar variations. *Journal of Agricultural and Food Chemistry, 50*, 6301–6306.

Keinanen, M. (1993). Comparison of methods for the extraction of flavonoids from birch leaves (*Betula pendula* Roth.) carried out using high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 41*, 1988–1990.

Kuskoski, M., Asuero, A., & Troncoso, A. (2005). Aplicación de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. *Ciência e Tecnologia de Alimentos, 25*(4), 726–732.

Larrauri, J. A., Rupérez, P., & Saura-Calixto, F. (1997). Mango's peel fibers with antioxidant activity. *Zeitschrift für Lebensmittel-Untersuchung und Forschung A, 205*, 39–42.

Macheix, J.-J., Fleuriet, A., & Billot, J. (1990). *Fruit phenolics*. Boca Raton: CRC Press Inc.

Nilsson, J., Pillai, D., Onning, G., Persson, C., Nilsson, A., & Akesson, B. (2005). Comparison of the 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) methods to asses the total antioxidant capacity in extracts of fruit and vegetables. *Molecular Nutrition and Food Research, 49*, 239–246.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., et al. (2003). Total antioxidant capacity of plant foods, beverages, and oils consumed in Italy assessed by three different in vitro assays. *The Journal of Nutrition, 133*, 2812–2819.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9/10), 1231–1237.

Robards, K. (2003). Strategies for the determination of bioactive phenols in plants, fruits and vegetables. *Journal of Chromatography A, 1000*(1–2), 657–691.

Roginsky, V., & Lissi, E. (2005). Review of methods to determine chain-breaking antioxidant activity in food. *Food Chemistry, 92*, 235–254.

Ruxton, C., Gardner, E., & Walker, D. (2006). Can pure fruit and vegetable juices protect against cancer and cardiovascular disease too? A review of the evidence. *International Journal of Food Science and Nutrition, 57*, 249–272.

Sánchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture, 76*, 270–276.

Stratil, P., Klejdus, B., & Kuban, V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables – Evaluation of spectrophotometric methods. *Journal of Agricultural and Food Chemistry, 54*, 607–616.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins, D. (2006). Comparison of ABTS, DPHH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis, 19*, 669–675.

Van't Veer, P., Janson, M., Klert, M., & Kok, F. (2000). Fruits and vegetables in the prevention of cancer and cardiovascular disease. *Public Health Nutrition, 3*, 103–107.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry, 52*, 4026–4037.