JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Evolution of Antioxidant Capacity during Storage of Selected Fruits and Vegetables

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Interest in the consumption of fresh fruits and vegetables is, to a large extent, due to its content of bioactive nutrients and their importance as dietary antioxidants. Among all of the selected fruits and vegetables, strawberries and black grapes have relatively high antioxidant capacities associated with high contents of total phenolic compounds, ascorbic acid, and flavonols. More interesting, the results of this study indicated that in most fruits and vegetables storage did not affect negatively the antioxidant capacity. Better, in some cases, an increase of the antioxidant capacity was observed in the days following their purchase, accompanied by an increase in phenolic compounds. In general, fruits and vegetables visually spoil before any significant antioxidant capacity loss occurs except in banana and broccoli. When ascorbic acid or flavonoids (aglycons of flavonols and anthocyanins) were concerned, the conclusions were similar. Their content was generally stable during storage.

KEYWORDS: Antioxidant; ascorbic acid; flavonoids; flavonols; fruits; phenolics; storage; vegetables

INTRODUCTION

There has been increasing interest for the inclusion of fresh fruits and vegetables in the human diet, mainly for the health benefits associated with their consumption (*1*, *2*). A major benefit from a higher intake of fruits and vegetables may be the increased consumption of vitamins (vitamin C, vitamin A, vitamin B6, thiamin, and niacin), minerals, and dietary fiber. Other constituents that may lower the risk of cancer and heart disease as well as prevent degenerative diseases include antioxidant compounds such as carotenoids, flavonoids, and other phenolics (*3*). These compounds are found ubiquitously in edible plants and are important constituents of the human diet. Epidemiologic studies that analyze the health implications of dietary components must estimate the intake in sample populations. Therefore, the availability of appropriate and complete food composition data is crucial. Due to the chemical diversity of antioxidant compounds present in foods, complete databases on food antioxidant content are not yet available.

Polyphenol concentrations in foods vary according to numerous genetic, environmental, and technological factors, some of which may be controlled to optimize the polyphenol content of foods (*4*). The postharvest life of fruits and vegetables has been traditionally defined in terms of visual appearance (freshness, color, and absence of decay or physiological disorders) and texture (firmness, juiciness, and crispness). Although this concept involves aesthetic appeal and mechanical properties associated with quality, it disregards flavor and nutritional quality (*5*). Flavor plays an important role in consumer satisfaction and influences further consumption of fruits and foods in general (*6*). Fruits form an important part of our diet mainly as a source of energy, vitamins, minerals, and antioxidants. Postharvest losses in nutritional quality, particularly vitamin C content, can be substantial and are enhanced by physical damage, extended storage duration, high temperatures, low relative humidity, and chilling injury of chilling-sensitive commodities (*7*–*9*).

The first objective of the present work was to determine the antioxidant activity and contents in commercially available fruits and vegetables in the Belgian market. The second and main objective was to evaluate the antioxidant activity and contents in fresh fruits and vegetables during storage after their purchase. Antioxidant capacity, total phenolic compounds, ascorbic acid, total flavonoids, total anthocyanins, and flavonols contents were evaluated during storage of selected fresh fruits and vegetables.

MATERIALS AND METHODS

Plant Material. All fruits and vegetables were obtained from the wholesale distribution center Delhaize in Boncelles (Liège, Belgium). The materials were immediately taken out after their arrival on the date indicated on **Table 1** and used directly for analyses at time zero. Some fruits and vegetables were stored at room temperature and others at 4 °C as indicated in **Table 1** and analyzed after several storage times. Storage was stopped when fruits or vegetables presented visual spoilage. The edible part analyzed was determined according to usual Belgian consumer habits (as indicated in **Table 1**). Seeds and stone were discarded for all of the fruits before analysis.

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10.1021/jf071736j CCC: $37.00
Published on Web 09/20/2007

Table 1. Fruits and Vegetables, Their Storage Conditions, and Material Used for Analysis

fruit or vegetable	storage condition	storage duration (days)	material used	purchase date
apple var. 'Jonagold'	RT[a]	19	with skin and without core	March 22, 2006
apricot var. 'Galta Roja'	RT	7	without hard core	May 31, 2006
asparagus	4 °C	22	all material	May 10, 2006
banana	RT	8	without skin	March 15, 2006
broccoli	4 °C [b]	27	all material	April 26, 2006
carrot	4 °C	51	without peel	April 19, 2006
celery	4 °C	8	without peel	June 14, 2006
cherry var. 'Brooks'	RT	7	without hard core	May 31, 2006
cucumber	4 °C	8	with skin	June 8, 2006
French bean	4 °C	8	all material	May 10, 2006
garlic	RT	30	without peels	June 14, 2006
grape (black) var. 'Ribier'	RT	14	all fruit	June 21, 2006
grape (green) var. 'Dauphine'	RT	14	all fruit	June 21, 2006
green pepper	4 °C	14	without pips	March 29, 2006
kiwifruit	RT	19	without skin	March 22, 2006
leek	4 °C	23	without green leaves	April 26, 2006
lemon	RT	21	without skin	March 15, 2006
lettuce	4 °C[c]	8	all material	June 14, 2006
melon var. 'Charentais'	RT	7	without skin and pips	May 31, 2006
nectarine	RT	8	without skin	April 19, 2006
onion	RT	23	without peel	April 26, 2006
orange	RT	15	without skin	March 15, 2006
pear var. 'Conference'	RT	12	with skin and without core	March 22, 2006
plum var. 'Black Plum'	RT	30	without hard core	April 19, 2006
red pepper	4 °C	14	without pips	March 29, 2006
spinach	4 °C[c]	19	all material	May 4, 2006
strawberry	4 °C	22	all fruit	May 20, 2006
tomato	4 °C	36	all fruit	May 4, 2006
yellow pepper	4 °C	34	without pips	March 29, 2006

[a] Room temperature. [b] Packaged with polypropylene films. [c] Packaged in sealed polypropylene bags.

Sample Preparation. For each fruit or vegetable, on the same day, three samples of 75 g of fresh material (as generally consumed as indicated in **Table 1**) were used. To collect 75 g a set or a part of fruits or vegetables was needed. In this last case, three different fruits or vegetables were used. The samples were ground in a blender with 150 mL of extraction solvent: acetone (70%), water (28%), acetic acid (2%) (*10*). The mixture was shaken for 1 h at 4 °C and centrifuged at 17000*g* for 15 min. The supernatant was removed, and the pellet was extracted again with 150 mL of the same solvent, incubated for 15 min, and centrifuged using the same procedure. The supernatants were pooled, and 70% of the volume was evaporated at 30 °C. The volume was then adjusted to 300 mL with water. Each sample was independently extracted in triplicate or more, and analyses were performed the same day except for ORAC assay and flavonol and anthocyanin analyses. In these cases, 1 mL samples were lyophilized for use later.

Total Phenolics. Total phenolic contents were determined according to the Folin–Ciocalteu method (*11*). Appropriately diluted extracts (3.6 mL) were mixed with 0.2 mL of Folin–Ciocalteu reagent, and 3 min later, 0.8 mL of sodium carbonate (20% w/v) was added. The mixture was heated at 100 °C for 1 min. After cooling, the absorbance at 750 nm was measured. Chlorogenic acid (Sigma) was used as standard, and results were expressed as milligrams of chlorogenic acid equivalents (CAE) per 100 g of fresh weight (FW). Analyses were performed in duplicate on each sample.

Antioxidant Capacity (DPPH). Antioxidant capacity was determined by scavenging of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Tadolini et al. (*12*). Stock solution was prepared by stirring 75 mg of DPPH in 1 L of methanol overnight. Trolox [(±) 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; Fluka Chemie GmbH, Buchs, Switzerland] was used as a standard and methanol as a blank. In the assay, 0.75 mL of extract, standard (0–0.1 mM Trolox) or blank (methanol), and 1.5 mL of DPPH solution were mixed. The absorbance at 517 nm using an Uvikon 931 spectrophotometer (BIOTEK Instruments) of samples, standards, and blanks was determined after 5 min. The percentage of the remaining DPPH was proportional to the antioxidant concentration, calculated relative to the antioxidant capacity of Trolox, and expressed as micromolar Trolox equivalents (TE) per 100 g of FW. Analyses were performed in duplicate.

Hydrophilic Antioxidant Capacity. ORAC assays were carried out on a fluoroskan Ascent FL Thermolabsystems (Finland) plate reader. The temperature of the incubator was set to 37 °C. Procedures were based on the method of Wu et al. (*13*). Briefly, AAPH was used as peroxyl radical generator, Trolox as standard, and fluorescein as fluorescent probe. Fluorescence filters were used for an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Twenty-five microliters of diluted sample, blank, or Trolox calibration solutions were mixed with 150 μL of 4 μM fluorescein and incubated for 15 min at 37 °C before injection of 25 μL of AAPH solution (173 mM). The fluorescence was measured every 2 min for 4 h. All samples were analyzed in duplicate at three different dilutions. The final ORAC values were calculated using the net area under the decay curves and were expressed as micromolar Trolox equivalents (TE) per 100 g of FW.

Ascorbic Acid. The 2,6-dichloroindophenol (DCIP) method of the Association of Vitamin Chemists (*14*) was used to measure only reduced ascorbic acid. Briefly, each molecule of vitamin C converts a molecule of DCIP into a molecule of $DCIPH_2$, and that conversion can be monitored as a decrease in the absorbance at 520 nm. A standard curve was prepared using a series of known ascorbic acid concentrations. Diluted samples in 5% metaphosphoric acid or ascorbic acid calibration solutions (600 μL) were mixed with 500 μL of 10% metaphosphoric acid, 300 μL of citrate buffer (pH 4.15), and 300 μL of DCIP (0.1 mg mL^{-1}). The optical density blanching was used; for each sample, the blank value was determined after the addition of 60 μL of ascorbic acid (1 mg mL^{-1}) with the aim to measure the interference due to the sample color. The results were expressed as milligrams of AA per 100 g of FW.

Total Flavonoids. Total flavonoid content was measured following the method of Lamaison and Carnat (*15*). Appropriately diluted extracts (1 mL) were mixed with 1 mL of reagent ($AlCl_3 \cdot 6H_2O$ 2% in methanol). The absorbance at 430 nm was measured 10 min later. Quercetin (Sigma) was used as standard, and results were expressed as milligrams of quercetin equivalents (QE) per 100 g of FW. Analyses were performed in duplicate on each sample.

Total Anthocyanins. Anthocyanin quantification was performed by the pH-differential method (*16*). The extract was diluted in a pH 1.0 solution (0.1 M HCl, 25 mM KCl) and in a pH 4.5 solution (0.4 M

Table 2. Antioxidant Capacity (DPPH and ORAC) and Fruit Content in Total Phenolics, Ascorbic Acid, Total Flavonoids, Flavonol Aglycons (Myricetin, Quercetin, and Kampferol), and Anthocyanins[a]

						flavonols			
	total phenolics (mg of CAE 100 g^{-1} of FW)	DPPH (μM TE 100 g^{-1} of FW)	ORAC (μM TE 100 g^{-1} of FW)	ascorbic acid (mg of AA 100 g^{-1} of FW)	total flavonoids (mg of QE 100 g^{-1} of FW)	myricetin (μg 100 g^{-1} of FW)	quercetin (μg 100 g^{-1} of FW)	kampferol (μg 100 g^{-1} of FW)	anthocyanins (mg of CE 100 g^{-1} of FW)
black grape	582 ± 26	443 ± 16	1746 ± 96	9.5 ± 1.5	11.8 ± 1.0	0	239 ± 43	176 ± 64	142 ± 11
banana	475 ± 108	523 ± 84	783 ± 256	49.6 ± 6.3	0.7 ± 0.1	143 ± 33	292 ± 36	12 ± 2	0
green grape	407 ± 15	328 ± 8	719 ± 32	13.4 ± 2.1	0.3 ± 0.1	0	48 ± 7	18 ± 3	0
lemon	324 ± 16	304 ± 40	843 ± 196	61.9 ± 4.5	3.2 ± 0.3	998 ± 166	0	61 ± 17	0
strawberry	313 ± 4	683 ± 40	2118 ± 96	53.6 ± 1.2	6.7 ± 0.5	979 ± 369	123 ± 6	99 ± 20	8 ± 1
plum	311 ± 15	224 ± 4	1978 ± 52	1.8 ± 0.2	3.0 ± 0.3	0	115 ± 21	5 ± 01	102 ± 8
appel	272 ± 13	92 ± 24	1139 ± 104	4.1 ± 0.9	3.3 ± 0.3	0	321 ± 81	53 ± 15	0
orange	243 ± 20	224 ± 8	1318 ± 292	57.5 ± 10.5	6.1 ± 0.3	2193 ± 632	686 ± 229	176 ± 64	0
cherry	226 ± 10	180 ± 4	2026 ± 308	5.3 ± 2.7	4.2 ± 0.4	0	102 ± 10	242 ± 2	27 ± 2
apricot	117 ± 13	88 ± 4	1027 ± 136	2.8 ± 0.3	0.9 ± 0.2	0	376 ± 60	8 ± 1	0
kiwifruit	112 ± 17	80 ± 40	360 ± 44	41.2 ± 1.5	0.4 ± 0.1	0	0	22 ± 6	0
melon	70 ± 1	56 ± 8	384 ± 36	4.0 ± 1.7	4.2 ± 0.2	0	71 ± 8	210 ± 24	0
pear	63 ± 13	140 ± 4	519 ± 132	3.4 ± 0.3	0.7 ± 0.1	0	214 ± 38	138 ± 39	0
nectarine	45 ± 30	56 ± 4	643 ± 104	1.5 ± 0.4	0.7 ± 0.1	0	77 ± 14	42 ± 1	0

[a] Assays were run immediately after fruits were obtained from distribution center.

Table 3. Antioxidant Capacity (DPPH and ORAC) and Vegetable Content in Total Phenolics, Ascorbic Acid, Total Flavonoids, and Flavonol Aglycons (Myricetin, Quercetin, and Kampferol)[a]

						flavonols		
	total phenolics (mg of CAE 100 g^{-1} of FW)	DPPH (μM TE 100 g^{-1} of FW)	ORAC (μM TE 100 g^{-1} of FW)	ascorbic acid (mg of AA 100 g^{-1} of FW)	total flavonoids (mg of QE 100 g^{-1} of FW)	myricetin (μg 100 g^{-1} of FW)	quercetin (μg 100 g^{-1} of FW)	kampferol (μg 100 g^{-1} of FW)
red pepper	296 ± 13	1207 ± 48	875 ± 152	165.6 ± 15.8	4.8 ± 0.5	0	98 ± 29	164 ± 22
yellow pepper	284 ± 10	1207 ± 124	1011 ± 120	171.3 ± 18.9	2.3 ± 0.1	218 ± 29	78 ± 13	20 ± 3
green pepper	215 ± 31	1163 ± 104	907 ± 196	135.3 ± 12.9	2.1 ± 0.4	0	56 ± 6	23 ± 6
spinach	177 ± 2	184 ± 4	1558 ± 64	12.4 ± 0.5	6.6 ± 0.5	0	6476 ± 506	241 ± 32
broccoli	127 ± 32	188 ± 8	1586 ± 328	15.2 ± 4.2	0.3 ± 0	0	127 ± 17	51 ± 13
garlic	113 ± 4	60 ± 4	1370 ± 392	4.9 ± 0.7	0.2 ± 0.1	1612 ± 59	1739 ± 219	255 ± 80
leek	77 ± 3	180 ± 4	675 ± 44	21.9 ± 1.2	0.4 ± 0	1320 ± 189	28 ± 3	232 ± 70
celery	75 ± 4	60 ± 4	679 ± 68	0.5 ± 0.1	0.2 ± 0.1	0	375 ± 85	224 ± 42
onion	53 ± 7	60 ± 4	739 ± 144	7.0 ± 0.7	3.3 ± 0.4	0	32 ± 11	29 ± 9
asparagus	44 ± 4	72 ± 4	296 ± 68	9.2 ± 0.5	0.2 ± 0.1	0	51 ± 19	0
tomato	35 ± 2	84 ± 4	216 ± 4	8.2 ± 1.2	2.3 ± 0.2	0	0	0
French bean	34 ± 1	68 ± 4	511 ± 88	0.5 ± 0	5.0 ± 0.2	0	28 ± 3	65 ± 5
lettuce	32 ± 1	56 ± 4	184 ± 12	0 ± 0	2.6 ± 0.3	0	121 ± 28	9 ± 5
cucumber	20 ± 2	0 ± 0	160 ± 32	0.4 ± 0.3	0.2 ± 0.0	0	102 ± 7	55 ± 4
carrot	0 ± 0	0 ± 0	276 ± 100	1.6 ± 0.2	0.7 ± 0.1	0	21 ± 2	72 ± 3

[a] Assays were run immediately after vegetables were obtained from distribution center.

CH_3COONa). The absorbance of the mixtures was then measured at 534 and 700 nm against distilled water. The value $(Abs_{535} - Abs_{700})_{pH1.0} - (Abs_{535} - Abs_{700})_{pH4.5}$ corresponds to the absorbance due to the anthocyanins. Calculation of the anthocyanins concentrations was based on a cyanidin 3-glucoside molar extinction coefficient of 25.965 and a molecular mass of 449.2 g mol^{-1}. Results were expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g of FW.

Flavonol Analysis. For hydrolysis of the flavonols, lyophilized material was mixed in 1 mL of hydrolysis solution: 1.2 M HCl, 50% methanol, and 3 mg mL^{-1} ascorbic acid. The mixture was heated at 80 °C during 60 min; 334 μL of 4 M NaOH were then added to stop the hydrolysis.

Analyses of the aglycons were performed in a liquid Elite Lachrom Merck Hitachi chromatograph equipped with an L2450 photodiode array detector. Separation was carried out using a LiChroCART steel cartridge, 240 mm × 4 mm, filled with 5 μm particles RP (reversed phase) 18, and thermostated at 30 °C.

The mobile phase was a linear gradient of water/acetonitrile (50:50, v/v) adjusted to pH 1.8 with perchloric acid (solvent B) in water/acetonitrile (95:5, v/v) adjusted to pH 1.8 with perchloric acid (solvent A), at a flow rate of 1.2 mL min^{-1} as previously described (*17*). Spectra were recorded between 250 and 400 nm (sampling period, 400 ms; spectral bandwidth, 4 nm). Standards of flavonols were purchased from Extrasynthese (Genay, France).

All results presented are the means (± SE) of the three independent extractions. Statistical analysis (linear regression or ANOVA with statistical significance level fixed at $p < 0.05$) was carried out using Microsoft Excel (Microsoft, Roselle, IL).

RESULTS

Comparison of Antioxidant Contents in Different Fresh Fruits and Vegetables. *Total Phenolic Compounds.* Among fresh fruits, black grape had the highest phenolic content (582 mg of CAE per 100 g) and was followed by bananas, green grape, lemon, strawberry, and plum (**Table 2**), whereas melon, pear, and nectarine had the lowest phenolics content.

Red and yellow peppers were the vegetables with the highest phenolic content (respectively, 296 and 284 mg of CAE per 100 g), followed by green peppers, spinach, and broccoli (**Table 3**). The phenolic content of the other vegetables tested was lower, especially in cucumber and carrots.

Antioxidant Capacity. As for phenolics, the antioxidant activity measured with the DPPH method was higher in grapes, bananas, and lemon (**Table 2**). The exception was strawberry, with a very high antioxidant capacity.

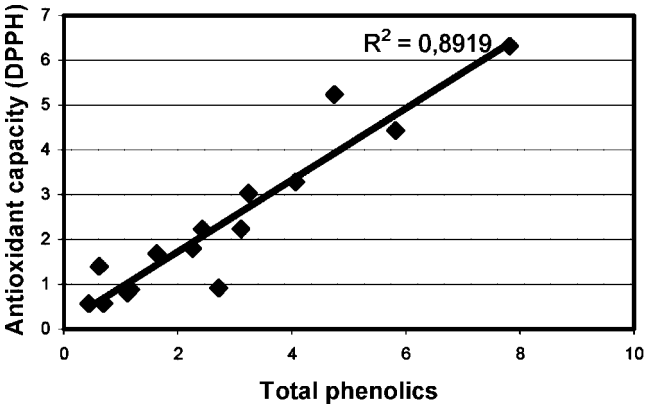

Figure 1. Regression analysis between total phenolics and antioxidant capacity (DPPH) of fruits.

Among the vegetables, peppers (red, yellow, or green) had a very high antioxidant capacity measured by the DPPH method, 6 times more than the other vegetables (**Table 3**).

The ORAC assay confirmed that strawberry fruits have a very high antioxidant capacity. For other fruits the results differed from those obtained by the DPPH assay. Strawberry, cherry, plum, and black grape had a higher antioxidant capacity, whereas kiwifruit and melon had the lowest antioxidant capacity (**Table 2**).

The antioxidant capacity of peppers measured with the ORAC assay was also high, but some other vegetables also had a higher capacity (spinach, broccoli, and garlic), whereas lettuce, cucumber, and carrots were always the vegetables with the lowest antioxidant capacity with tomato (**Table 3**).

Ascorbic Acid. Concerning the fruits, the higher content in ascorbic acid (~50 mg 100 g^{-1} of FW) was found in lemon, orange, strawberry, banana, and kiwifruit (**Table 2**).

The content of ascorbic acid in pepper was very high (150 mg 100 g^{-1} of FW) (**Table 3**). Leek, broccoli, and spinach had ascorbic contents above 10 mg 100 g^{-1} of FW, whereas in the other vegetables this content was lower.

Flavonoids. Black grape had the highest flavonoid content, followed by strawberry and orange, whereas flavonoid content was very low in banana, green grape, apricot, kiwifruit, pear, and nectarine (**Table 2**). Among the three main flavonol aglycons, myricetin was found only in banana, lemon, strawberry, and orange, in which this aglycon level was very high. Quercetin was found in almost all fruits and was generally the most important flavonol aglycon. Kampferol was present in all fruits, but its level was often lower than that of quercetin. Anthocyanins were found in only intensely colored fruits such as black grape, plum, cherry, and strawberry.

Among vegetables, spinach, French bean, and red pepper had the highest level of flavonoids (**Table 3**). In broccoli, garlic, leek, celery, asparagus, cucumber, and carrots the flavonoid content was very low. A high level of the aglycon myricetin was observed in garlic and leek. Quercetin was present in all vegetables except tomato, and a particularly high level was found in spinach. Kampferol was detected at a low level in all of the vegetables except asparagus and tomato. No anthocyanin was found in the vegetables tested.

Relationship between Antioxidant Capacity and Phenolic Contents. A high correlation (**Figure 1**) between total phenolics and DPPH measurements within the fruits tested (except strawberry) can be observed ($R^2 = 0.892$), whereas no correlations were found between phenolics and ORAC measurements ($R^2 = 0.463$) or between the two techniques (DPPH and ORAC) used to determine the antioxidant capacity ($R^2 = 0.377$). For the vegetables (except spinach), similar correlations were found: high for phenolics versus DPPH ($R^2 = 0.891$), not significant for phenolics versus ORAC ($R^2 = 0.375$) and ORAC versus DPPH ($R^2 = 0.084$). Neither were correlations found between flavonoid or anthocyanin content and antioxidant capacity.

Evolution of Antioxidant Content and Capacity during Storage of Fresh Fruits and Vegetables. *Total Phenolic Compounds.* In most cases, total phenolic compounds were stable during storage (**Figure 2**). There were some exceptions where there was a transient increase of phenolic compounds, during a few days, as in plum (**Figure 2D**), tomato (**Figure 2F**), broccoli, or black grape (data not shown). In contrast, a transient decrease of these compounds was observed in citrus (−76% on day 2), lettuce (−31% on day 2), and celery (−50% on day 5) (data not shown).

In leek and asparagus, the phenolic content increased during the first days and was stable afterward. In banana (**Figure 2B**), in contrast, the phenolic contents decreased rapidly. Only 20% was still present after 2 days.

Antioxidant Capacity. As for phenolics, the antioxidant capacity measured with DPPH was generally stable during storage (**Figure 2E**). In some cases, a transient increase of the antioxidant capacity was measured as in yellow pepper (**Figure 2C**), asparagus, and plum (**Figure 2D**). The antioxidant capacity decreased during storage in apricot (25%) and decreased by >50% in spinach, banana (**Figure 2B**), broccoli, and leek. In contrast, in orange and apple, the antioxidant capacity doubled rapidly and afterward was stable. In onion, the antioxidant capacity continuously increased during storage (>10 times after 23 days).

When the measurements were done with the ORAC assay, a transient increase of the antioxidant capacity in yellow pepper (**Figure 2C**), broccoli, and plum (**Figure 2D**), a transient decrease in leek and lettuce, decreases of around 20% in spinach and around 40% in melon, celery, and apricot (**Figure 2A**), and an increase, superior to 50%, in citrus and garlic were observed. Antioxidant capacity was stable in other fruits and vegetables (as in green grape, **Figure 2E**).

Ascorbic Acid. In most fruits and vegetables, the ascorbic acid content was relatively stable during storage (**Figure 3C–F**). In some others, the ascorbic acid content decreased rapidly, during the first day of storage. This was the case of apricot (**Figure 3A**), banana (**Figure 3B**), spinach, melon, cherry, citrus, and leek.

Flavonoids. Generally, the flavonoid content of fruits and vegetables increased during storage (**Figure 3A**,**D**,**E**) or was stable (**Figure 3C**) except in banana (**Figure 3B**), where it decreased rapidly, notably the flavonol aglycons. In the other materials, the flavonol aglycone level was stable during storage (**Figure 3A**,**E**) or had a low decrease (**Figure 3C**). A transient increase in flavonol aglycon and anthocyanin levels was observed in plum (**Figure 3D**).

DISCUSSION

Various methods have been developed in recent years to evaluate in a simple way the total antioxidant capacity of biological samples and food. Despite all of the methods developed, there is still missing a method that can measure accurately the total antioxidant capacity of samples. Several publications have focused the differences between these methods (*18–20*).

To evaluate the antioxidant capacities of foods, three methods have emerged as the most popular: the determination of total phenolic content (bioactive antioxidant compounds); the scav-

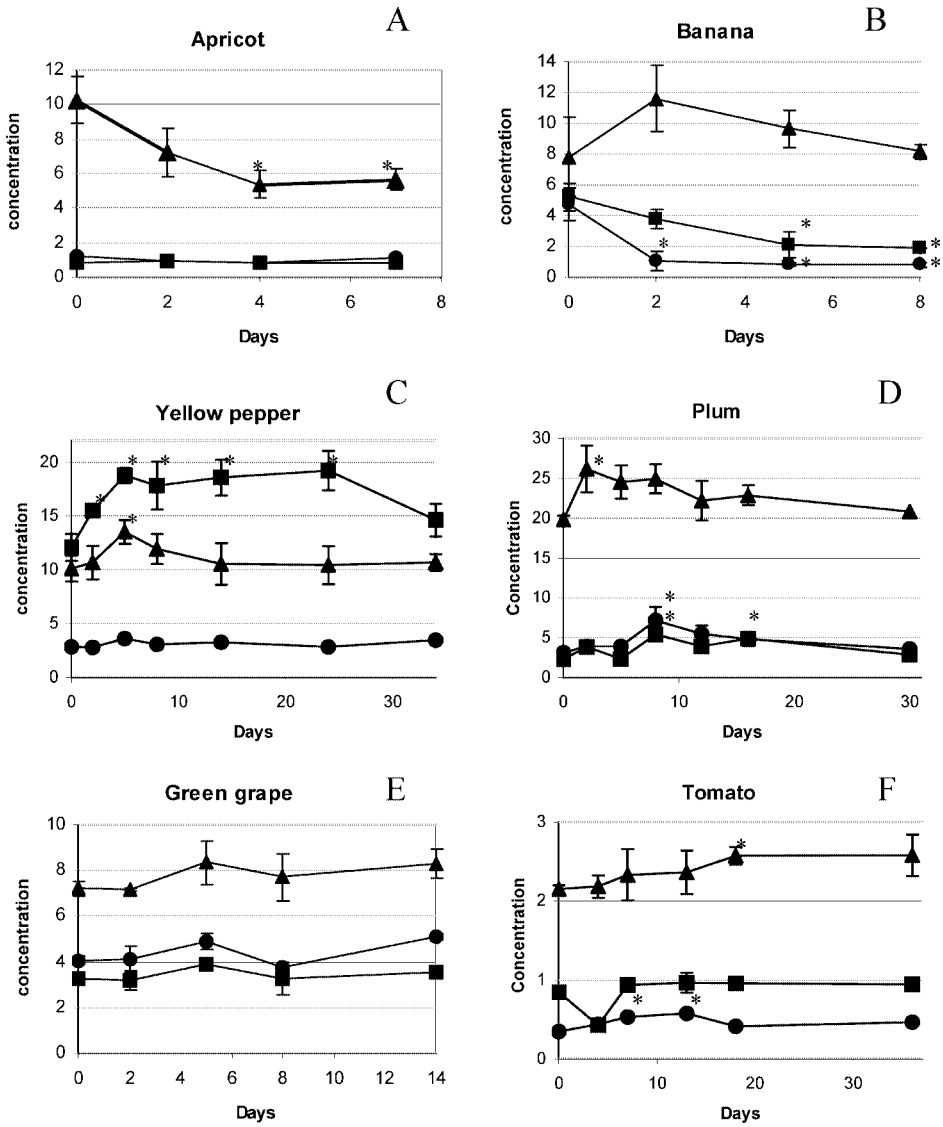

Figure 2. Evolution of antioxidant capacity (μM TE g^{-1}; ■, DPPH; ▲, ORAC) and total phenolic content (mg of CAE g^{-1}, ●) during storage in various fresh fruits and vegetables. An asterisk indicates significant difference from average value at time 0 by ANOVA ($p < 0.05$).

enging effect on the radical DPPH, simple and rapid; and, overall, the oxygen radical absorbance capacity (ORAC), where the measurement of fluorescence increases sensitivity and permits a much lower molar ratio of antioxidant sample. The merits and disadvantages of these methods have been fully discussed in several reviews (*20–22*).

The scatterplots done with results obtained in this study on fruits (**Figure 1**) and vegetables had a significant correlation between total phenolic content and antioxidant activity measured with DPPH radical, whereas no significant correlation can be found between phenolic compounds and ORAC or between the DPPH method and ORAC assay. This linear correlation between phenolic and DPPH measurements (**Figure 1A**) can suggest that the presence of the phenolic compounds largely accounted for the antioxidant capacity measured with DPPH as already observed (*17, 23–25*). Most of the bioactive antioxidant compounds were phenolics. On the other hand, correlations between phenolic compounds and ORAC measurements were found in potatoes (*26*), in different common foods (*13*), and in wines (*27*). Thus, the radical source used in the assay can have dramatic effects on the results because of the differential response of different types of antioxidant compounds to the radical source (*28*).

Recently, some fairly large-scale analyses were done to evaluate the antioxidant capacity of foods from different countries such as Italy (*29*), the United States (*13*), the Czech Republic (*24*), and France (*30*). Unfortunately, the methods used in most of these studies were different, and it was not easy to make a comparison with our results. The range of phenolic contents and antioxidant capacity was large among the different fruits (**Table 2**). Of all our fruit samples, strawberries and black grapes have a relatively high total phenolic content and antioxidant capacities determined with DPPH or by ORAC test, whereas those of melons were relatively low. Some divergences exist between the results obtained with the ORAC assay and the DPPH method: bananas, green grapes, and lemons, which have the highest total phenolic contents, had high antioxidant capacity as assayed by the DPPH method, whereas plums, oranges, and cherries had higher antioxidant capacity when assayed by the ORAC assay. The high antioxidant capacity [1540 ORAC units per 100 g (*31*)] of strawberry was, however, recognized (*32*) behind those of grapes and blackberries. Grapes are usually known for their high polyphenol content, due to their high proanthocyanidin (*32*) and flavonoid contents. Most of the fruits with high antioxidant capacity had also a high level of ascorbic acid (strawberries, bananas, lemons, and oranges).

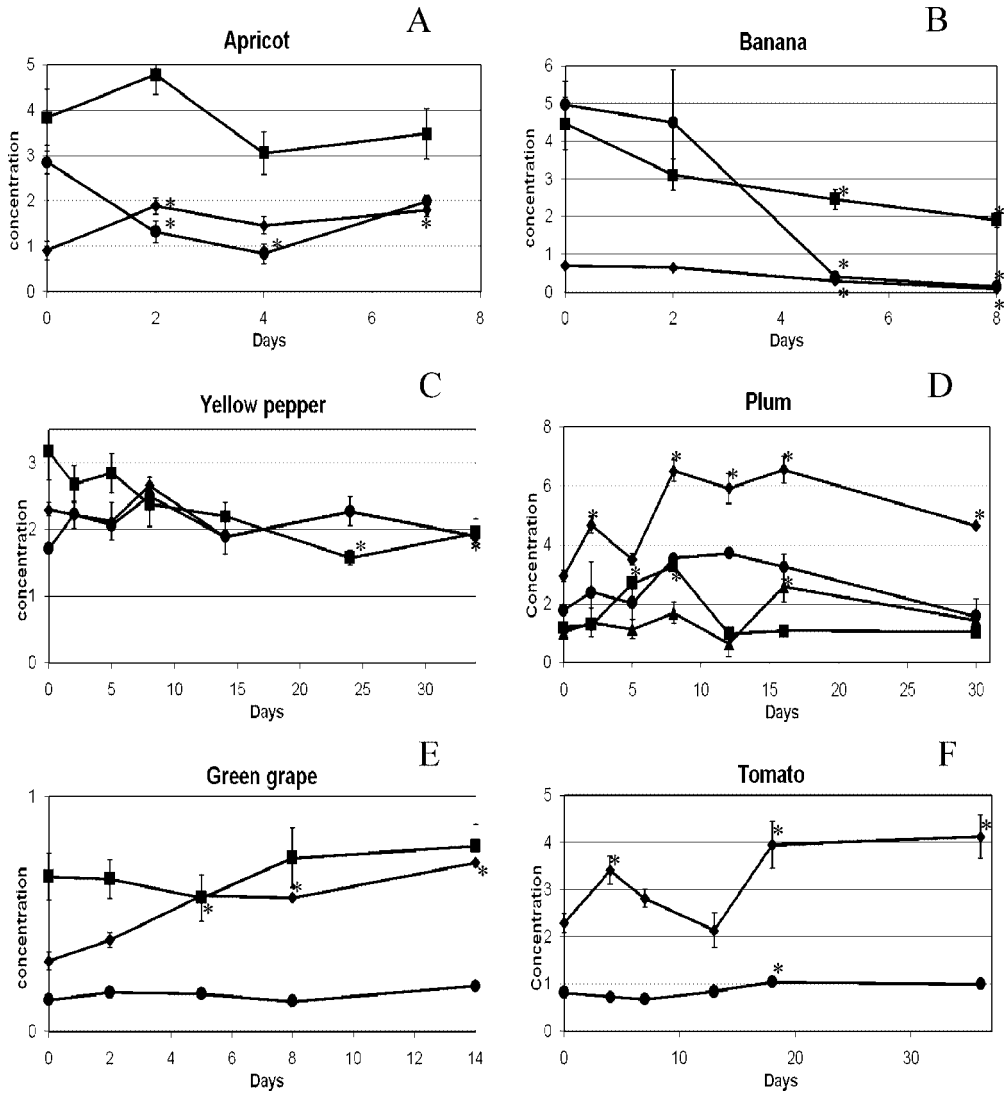

Figure 3. Evolution of ascorbic acid (yellow pepper and green grape, 10^{-3} g g^{-1}; tomato and banana, 10^{-4} g g^{-1}; apricot and plum, 10^{-5} g g^{-1}, ●), total flavonoids (10^{-5} g of QE g^{-1}, ♦), flavonol aglycons (myricetin + quercetin + kampferol, μg g^{-1}, ■), and total anthocyanins (mg g^{-1}, ▲) contents during storage in various fresh fruits and vegetables. An asterisk indicates significant difference from average value at time 0 by ANOVA ($p < 0.05$).

The range of phenolic and ascorbic acid contents and antioxidant capacities determined with the DPPH method in fresh vegetables was not as great as that in fruits (**Table 3**) except for peppers. Peppers had extremely high values compared to all other vegetables as already attested (*9*) and cucumber the lowest value as already observed (*33*). Far behind peppers were spinach, broccoli, and garlic. These three vegetables had also the highest antioxidant capacity measured by the ORAC assay, as previously indicated (*31*–*33*). The highest total flavonoid content was determined in spinach as previously observed (*34*).

Quercetin is a typical flavonoid ubiquitously present in vegetables and fruits (**Tables 2** and **3**). It is generally the most important flavonol aglycon. In contrast, anthocyanins were detected in only some fruits, as previously observed by Wu et al. (*35*). Quercetin (*36*) and anthocyanins (*37*) have been shown to be strong antioxidants.

A validation of the antioxidant capacity approach is essential for investigating the role of food antioxidants in human health. From published data of various areas in the world, some fruits (strawberry, grape, banana, berries) and vegetables (peppers, broccoli, and spinach) always appeared to be rich in antioxidants. In addition to the difference due to the methods used, the variance observed between reported data can be explained by various factors such as extraction procedure (*38*), cultivar (*39*), ripening state (*9*), and weather conditions of the production season (*40*).

Among the many sensory characteristics of fresh fruits and vegetables, appearance, texture, and flavor are of prime importance. In addition to these general sensory characteristics, consumers are nowadays more and more concerned with nutritional qualities. Although fairly large-scale analyses were recently done to evaluate the antioxidant capacity of foods, no important studies were done to evaluate the influence of storage on the antioxidant capacity. Data on only some fruits or vegetables were available.

The preservation of fruit phenolic content has a great impact on the quality of fruits because of the contribution of phenols not only in enzymatic browning reactions but also on nutritional value of the product, as antioxidant capacity. The results of this study (**Figure 2**) indicated that in most fruits and vegetables the storage did not affect negatively the antioxidant capacity. In some cases an increase of the antioxidant capacity was observed in the days following their purchase. Similar observations were previously done on some fruits (*5*, *41*) or vegetables (*42*) stored at room temperature or in the refrigerator. In only some cases did the antioxidant capacity decrease during storage,

as in broccoli. Serrano et al. (*43*) have also shown a decrease of antioxidant capacity in broccoli and indicated the importance of the type of film package on the maintenance of antioxidant capacity and phenol content during storage. In banana, phenolic content and antioxidant capacity (DPPH) decreased drastically. Kondo et al. (*44*) have, in contrast, shown that the antioxidant capacity (DPPH) on the banana skin increased during storage. In apricot, Bartolini et al. (*45*) also observed a decrease of the antioxidant capacity during storage at low temperature.

When ascorbic acid or flavonoids were concerned (**Figure 3**), the conclusions were similar. Ascorbate content was generally stable except in some cases when its level decreased. Jimenez et al. (*42*) have observed an increase of its content in peppers during storage at 20 °C, whereas in our study, at 4 °C, no significant modification was found. The total flavonoid content was relatively stable or increased during storage as already observed in spinach (*46*) or apple (*47*) and olives (*48*), respectively. In the same way, no significant changes of flavanone content were observed after chilled (4 °C) storage of orange juice (*49*). Loss of flavonoids during storage was observed only in some fruits or vegetables such as banana and lettuce (*50*). The monitoring of specific classes of polyphenolic constituents, including total flavonoids made by Dourtoglou et al. (*48*), has indicated that not all phenolics were affected in the same manner, as they presented a different evolution pattern throughout the storage period. The content of the aglycons of flavonols (quercetin, myricetin, and kampferol) and anthocyanins was relatively stable during storage. Only a transitory increase of quercetin or anthocyanins was observed in our conditions.

In conclusion, the new and interesting result of this study was the relative stability of the antioxidant capacity in most fruits and vegetables during storage. In general, fruits and vegetables visually spoil before any significant antioxidant capacity loss occurs. Nevertheless, it could be stressed that, in general, polyphenolic content increased. Increased levels of antioxidant capacity generally accompanied this increase, which should be considered as an important assurance for the impact of storage evolution of phenolics on the nutritional value of fruits and vegetables.

ACKNOWLEDGMENT

We thank the distribution center Delhaize for providing the plant material and HEPL-Rennequin Sualem for technical assistance. The skillful assistance of the APE personnel (provided to CEDEVIT by the regional government of Wallonia) was greatly appreciated.

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Received for review June 13, 2007. Revised manuscript received August 16, 2007. Accepted August 22, 2007. J.T. gratefully acknowledges the Luxemboug Ministry of National Education.

JF071736J