Biotechnology and Food Science

Research article

Antioxidant Potential and Phytochemical Content of Selected Fruits and Vegetables Consumed in Cyprus

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Received: 4 December 2017/Available on-line: 15 May 2018

Abstract: *Consuming a diet high in fruits and vegetables can lower the risk of developing numerous chronic diseases, including cancer and cardiovascular disease, due to the presence of multiple antioxidants. Horticultural produce consumed in Cyprus include a large variety of fruits and vegetables, most of which are common components of a Mediterranean-style diet due to the island's localization. The aim of the present study was to evaluate the antioxidant composition and activity of the edible portion of ten fruits and ten vegetables commonly consumed in Cyprus. Total phenolics, total anthocyanins, ascorbic acid and carotenoids contents were determined, while the in vitro antioxidant activity was evaluated by three assays. Antioxidant activity showed great variation, with the highest values found in green olives, capers and red chili peppers and in correlation with total phenolic content. Ascorbic acid was detected in large quantities in parsley, coriander, red guavas and red chili peppers, while red chili peppers, capers and coriander had high levels of carotenoids. Furthermore, sweet cherries, red grapes, and red apples contained significant amounts of anthocyanins. Statistical analysis revealed that phenolic compounds are the most potent antioxidants in fruit and vegetables, while total anthocyanins had a weak contribution to their antioxidant activity. The present study could be a guide for Cypriot as well as Mediterranean diet health-conscious consumers to select specific fruits and vegetables as dietary components. To the best of our knowledge, this is the first study thoroughly describing the antioxidant composition and activity of a wide array of important Cypriot horticultural products.*

Keywords: *phenolic compounds, anthocyanins, ascorbic acid, carotenoids, antioxidant capacity, horticultural products.*

Introduction

Epidemiological studies provide robust evidence that a high dietary intake of fruits and vegetables is clearly associated with the prevention of several chronic

diseases such as coronary heart diseases and different types of cancer [1-2]. The high consumption of fruits and vegetables is also recommended by Mediterranean diet, a food pattern that is linked with reduced risk of mortality [3]. Fruits and vegetables are considered as an excellent reservoir of bioactive compounds that affect various physiological processes related to health benefits. These benefits are mainly contributed to the presence of micronutrients such as polyphenols, carotenoids and vitamins [4-5].

The antioxidant composition in fruits and vegetables displays a great qualitative and quantitative diversity, since they are influenced by many factors including genotype, plant tissues, pedoclimatic conditions, agronomic practices, postharvest treatments and processing [6]. Soil, irrigation and climate play an important role in the bioactive composition of fruits and vegetables resulting in significant differences from region to region.

Cyprus is the third largest island in the Mediterranean Sea laying in the easternmost part of the Mediterranean basin and is found in the crossroad of three continents: Africa, Asia and Europe. It has a subtropical climate – Mediterranean and semi-arid type with very mild winters and warm to hot summers. Rain occurs mainly in winter, with summer being generally dry. These factors may affect the nutraceutical quality of fruits and vegetables grown in Cyprus; however, a few studies have thus far dealt with phytochemical aspects of fruits and vegetables related to the Cypriot market [7-9]. Hence, the evaluation of their bioactive composition will be useful for nutritionists as these horticultural products constitute important Mediterranean food components of Cypriots.

The main objective of the present work was to evaluate the bioactive composition and antioxidant capacity of fruits and vegetables consumed in Cyprus. In particular, major antioxidant groups such as total phenolics, total anthocyanins, ascorbic acid and carotenoids contents were estimated. Furthermore, three *in vitro* assays, namely DPPH radical scavenging activity, FRAP (Ferric Reducing/Antioxidant Power) and phosphomolybdenum assay were applied to determine the antioxidant activity of fruits and vegetables.

Experimental

Materials

Chemicals and reagents

Standard compounds of gallic acid, cyanidin-3-*O*-glucoside, β-carotene and ascorbic acid were purchased from Sigma (St Louis, MO, USA), whereas Folin–Ciocalteu reagent and common solvents were purchased by Scharlau (Barcelona, Spain). Iron (III) chloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-*s*-triazine (TPTZ), 2,6-dichloroindophenol (DCIP) and ammonium molybdate were also obtained from Sigma.

Plant material

In the present study was analyzed the edible part of fresh sweet cherries (skin and flesh), green kiwifruits, green olives (skin and flesh), lemons, mandoras,

oranges, red apples (skin and flesh), red grapes (skin and flesh), red guavas, yellow prickly pears, red chili peppers (skin and flesh), broccoli, capers (fruit and leaves), coriander (aerial part), green beans, parsley (aerial part), potatoes (tuber), red tomatoes (skin, flesh and seeds), shelled black-eyed peas and spring onions (leaves and bulb). Plant tissues were kindly provided by local farmers or markets from the same region (Lemesos) in Cyprus. All fruits and vegetables were washed thoroughly with water, dried carefully with towels, peeled (where applicable), and cut into small pieces, which were flash-frozen using liquid nitrogen. Finally, the edible parts were homogenized using liquid nitrogen and the samples were stored at -80ºC for a short period (3 months) until analyses were carried out. In total, three biological replicates were performed for each fruit or vegetable and each biological replication consisted of five fruits or vegetables.

Methods

Extraction of phenolic compounds

Fruits and vegetables (~1 g) were homogenized in 10 mL methanol using a Polytron (model T 25 D, IKA, Werke GmbH & Co. KG, Staufen, Germany) for 3 min at 10000 rpm. After 5 min incubation in an ultrasonic bath (Ultrasonic Cleaner, Raypa, UCI-150) at 25ºC and centrifugation at 4ºC for 10 min at 16000 xg (Eppendorf Centrifuge 5415 R), crude extract supernatants were decanted and held at -20°C until analyses were carried out.

Determination of total phenolic content

The reaction mixture consisted of 0.5 ml of the diluted methanol extract, 5 ml of distilled water, and 0.5 ml of the Folin-Ciocalteu reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added and the mixture was made up to 10 ml with distilled water. The mixture was thoroughly mixed, allowed to stand for 1 h in the dark at the room temperature, and the absorbance was measured at 765 nm (TECAN, Infinite 200® PRO). Each measurement was repeated three times and the results were expressed as mg gallic acid equivalent (GAE) 100 g^{-1} of fresh weight (FW) [7].

Determination of total anthocyanins

Total anthocyanin content was estimated by the pH-differential assay [10] using two buffer systems: potassium chloride buffer (0.025M) at pH 1.0 and sodium acetate buffer (0.4M) at pH 4.5. Samples were diluted in pH 1.0 and pH 4.5 buffers and their absorbance was subsequently measured at 520 and 700 nm (TECAN, Infinite 200® PRO). Anthocyanin concentration was calculated as cyanidin-3-*O*-glucoside (CY3) equivalents. All measurements were performed in triplicate and expressed as mg 100 g^{-1} FW.

Determination of ascorbic acid

Fruits and vegetables (~1 g) was extracted with 10 mL of 2% w/v meta-phosphoric acid and filtered. One mL of filtrate was added to 9 mL of 50 mmol L^{-1} 2,6-dichloroindophenol and the absorbance was monitored at 515 nm (TECAN,

Infinite 200® PRO). Ascorbic acid (AsA) content was quantified using a standard curve and expressed as mg 100 g^{-1} FW [7].

Determination of carotenoids

Sixteen mL of acetone–hexane mixture (4:6) were added to 1 g of plant material and were thoroughly mixed. When the two phases were separated, an aliquot was taken from the upper phase and the absorbance was measured at 663, 645, 505, and 453 nm (Jenway 6405 UV/Vis. Spectrophotometer). Beta-carotene content was calculated according to the Nagata and Yamashita [11] equation (β-carotene = $0.216A_{663} – 1.22A_{645} – 0.304A_{505} + 0.452A_{453}$) and expressed as μg 100 g^{-1} FW.

Determination of DPPH radical scavenging activity

Two mL of each diluted methanol extract were mixed with 1 mL of 0.3 mmol L^{-1} solution of DPPH in methanol. The absorbance of the mixtures was measured after 30 min incubation time in the dark at 517 nm (TECAN, Infinite 200® PRO). Different concentrations of each sample were tested and the percent (%) of free radical scavenging activity was calculated by the following equation: % scavenging activity = 100 − [(Ab of sample − Ab of blank) 100/Ab of control]. IC_{50} or EC_{50} values are referred to the extract concentration at the 50% of the antioxidant activity [7].

Determination of total antioxidant activity by Ferric Reducing/Antioxidant Power (FRAP) assay

A sample containing 3 mL of freshly prepared FRAP solution (0.3 mol L^{-1} acetate buffer (pH 3.6) containing 10 mmol L^{-1} TPTZ and 40 mmol L^{-1} $FeCl_3$ $10H_2O$) and 100 μL of diluted methanol extract was incubated at 37°C for 4 min in a water bath (NE2-4D 4 Litre Unstirred Digital Water Bath, Clifton Range, Nickel-electro Ltd) and the absorbance was measured at 593 nm (TECAN, Infinite 200® PRO). A standard curve of ascorbic acid was prepared and results were expressed as mmol AsA 100 g^{-1} FW [9].

Determination of antioxidant capacity by the phosphomolybdenum assay

One mL of diluted methanol extract was combined with 1 mL of reagent solution (0.6 mol L^{-1} sulphuric acid, 28 mmol L^{-1} sodium phosphate, and 4 mmol L^{-1} ammonium molybdate). The test tubes were incubated at 95 °C for 90 min in a water bath (Memmert water bath WNB 29). The absorbance of the solution was measured at 695 nm (TECAN, Infinite 200® PRO) against a blank sample, and total antioxidant capacity was expressed as mmol AsA 100 g^{-1} FW [9].

Statistical analysis

Statistical analysis was carried out using the software package SPSS v17.0 (SPSS Inc., Chicago, USA) and the comparison of averages of each treatment was based on the analysis of variance (One-Way ANOVA) according to Duncan's multiple range test at significance level 5% ($P \leq 0.05$). Correlation coefficients (*r*) were also calculated.

Results and Discussion

Antioxidant composition of fruits and vegetables

Polyphenols, anthocyanins, ascorbic acid and carotenoids are the main antioxidants in horticultural products and they are linked with several beneficial health effects. In the present study, the total phenolic content of twenty fruits and vegetables was determined by Folin–Ciocalteu method (Table 1). Results revealed great variation in total phenolic content highlighting that phenolic content is strongly affected by genetic factors. In particular, total phenolic content varied from 7.5 to 714.1 mg GAE 100 g^{-1} FW. Depending on phenolic content, the top five horticultural products were graded as green olives (714.1 mg GAE 100g^{-1}FW), capers (376.3 mg GAE 100g^{-1}FW), red chili peppers (299.0 mg GAE 100g^{-1}FW), coriander (180.4 mg GAE 100g^{-1}FW) and sweet cherries (176.0 mg GAE 100g^{-1}FW). On the other hand, potatoes, red tomatoes and green beans had the lowest phenolic content among examined fruits and vegetables. According to Kaur and Kapoor [12], fruit and vegetables can be divided into three groups, namely high (>200 mg GAE 100g^{-1}FW), medium (100-200 mg GAE 100g^{-1}FW) and low (<100 mg GAE 100g^{-1}FW). Overall, total phenolic contents of Cypriot fruit and vegetables are in line with previous comparative studies for horticultural products of different origin [12-13]. Similar results to present findings were found in oranges (92.4 mg 100 g^{-1} FW), potatoes (43.2 mg 100 g^{-1} FW) and tomatoes (30.8 mg 100 g^{-1} FW) [14] as well as in orange juice (121.5 mg 100 g^{-1} FW) [15], further supported by the study of Kevers et al. [16], regarding phenolic content in tomatoes (35.0 mg 100 g^{-1} FW) and red peppers (296.0 mg 100 g^{-1} FW).

Anthocyanins are water-soluble pigments responsible for blue, purple and red color of many fruits and vegetables. Chemically, anthocyanins belong to a parent class of molecules, the flavonoids, and are glycosides containing a sugar moiety and an aglycone unit [17]. Numerous studies indicate the potential effect that this family of flavonoids may have in reducing the incidence of cardiovascular disease, cancer, hyperlipidemias and other chronic diseases through the intake of anthocyanin-rich foods [18]. Anthocyanin contents were detected only in seven fruits and four vegetables and their concentration ranged between 0.23 to 58.17 mg CY3 100 g^{-1} FW (Table 1). In fruits, sweet cherries (58.17 mg CY3 100 g^{-1} FW), red grapes (16. 39 mg CY3 100 g^{-1} FW), and red apples (2.62 mg CY3 100 g^{-1} FW) had the highest anthocyanin content, while oranges, lemons, mandoras had no anthocyanin. Ferretti and co-workers [19] demonstrated that the total anthocyanin content of seven sweet cherry cultivars varied from 10 to 76 mg CY3 100 g^{-1} FW, whereas the corresponding content of sixteen red grape cultivars was from 4 to 99 mg CY3 100 g^{-1} FW [20]. Similar results regarding the content of anthocyanin in red apples were detected ranging from 1.3-12.3 mg 100g^{-1} FW [21, 22]. Regarding vegetables, their anthocyanin content did not exceed the concentration of 1.25 mg CY3 100 g^{-1} FW, while capers, broccoli, shelled black-eyed peas, green beans, coriander, parsley had no anthocyanin.

In agreement with our study, anthocyanin was not detected in green beans and oranges [14] as well as in lemons [16].

Table 1. Total phenolics, total anthocyanins, ascorbic acid and carotenoids contents in fruits and vegetables grown in Cyprus. * Values within each column followed by the same letter are not statistically significant according to Duncan's multiple range test at significance level P≤0.05. ** nd stands for non-detectable (n = 3, ± SE)

	Total phenolics (mg GAE 100g^{-1} FW)	**Total anthocyanin (mg CY3 100g^{-1} FW)**	**Ascorbic acid (mg AsA 100g^{-1} FW)**	**β-carotene (µg 100g^{-1} FW)**
Fruits				
Sweet cherries	176.0 ± 10.3^{d*}	58.17 ± 3.00^{a}	nd	374.5 ± 29.0^{ij}
Green kiwifruits	49.3 ± 2.1^{ij}	0.36 ± 0.03^{c}	122.4 ± 3.9^{fg}	450.5 ± 15.1^{ij}
Green olives	714.1 ± 8.6^{a}	0.84 ± 0.10^{c}	nd	2235.0 ± 114.9^{f}
Lemons	91.3 ± 8.5^{gh}	nd**	50.0 ± 7.5^{j}	nd
Mandoras	104.9 ± 6.4^{fg}	nd	79.5 ± 0.9^{h}	5305.0 ± 14.3^{e}
Oranges	108.4 ± 2.7^{fg}	nd	113.5 ± 3.6^{g}	2084.1 ± 197.9^{fg}
Red apples	132.4 ± 3.9^{ef}	2.62 ± 0.05^{c}	40.5 ± 1.9^{jk}	510.6 ± 42.1^{ij}
Red grapes	173.9 ± 19.3^{d}	16.39 ± 0.54^{b}	nd	nd
Red guavas	113.5 ± 10.4^{fg}	0.23 ± 0.02^{c}	200.8 ± 5.9^{c}	1511.4 ± 76.0^{h}
Yellow prickly pears	52.9 ± 1.3^{ij}	0.56 ± 0.01^{c}	26.7 ± 4.3^{k}	116.6 ± 13.1^{j}
Vegetables				
Red chili peppers	299.0 ± 4.6^{c}	1.25 ± 0.17^{c}	154.8 ± 4.2^{d}	34529.2 ± 350.7^{a}
Broccoli	51.9 ± 3.8^{ij}	nd	135.2 ± 7.6^{ef}	2028.5 ± 163.4^{fg}
Capers	376.3 ± 29.2^{b}	nd		17369.2 ± 224.0^{b}
Coriander	180.4 ± 12.1^{d}	nd	219.6 ± 4.4^{b}	14144.7 ± 281.6^{c}
Green beans	7.5 ±0.6^{k}	nd	nd	697.0 ± 70.5^{i}
Parsley	155.1 ± 14.0^{de}	nd	226.7 ± 2.6^{a}	8352.7 ± 219.1^{d}
Potatoes	23.4 ± 0.2^{jk}	0.43 ± 0.02^{c}	44.5 ± 4.6^{j}	118.8 ± 11.9 ^{j}
Red tomatoes	21.8 ± 1.9^{jk}	0.25 ± 0.01^{c}	70.4 ± 2.8^{hi}	1673.8 ± 115.9^{gh}
Shelled black-eyed peas	66.4 ± 3.3^{hi}	nd	55.9 ± 4.0^{ij}	1839.9 ± 181.8^{fgh}
Spring onions	50.7 ± 2.5^{ij}	0.59 ± 0.04^{c}	67.0 ± 4.2^{hi}	128.2 ± 16.5^{j}

Ascorbic acid is a water soluble antioxidant known to be important to health due to the presence of a 2,3-enediol moiety. The concentration of ascorbic acid in horticultural products tested ranged between 26.7 to 226.7 mg 100 g^{-1} FW with yellow prickly pears revealing lowest and parsley highest amounts (Table 1). In addition, sweet cherries, red grapes, green olives and green beans had no ascorbic acid. Results were in agreement with previous comparative studies, although a great diversity in ascorbic acid was found within cultivars [21, 23]. Ascorbic acid contents of red peppers and lemons are in agreement with those found by Kevers et al. [16], who reported values of 165.6 mg 100 g^{-1} FW and 61.9 mg 100 g^{-1} FW, respectively. Similarly, ascorbic content in guavas was 243.0 mg 100 g^{-1} FW and in lemons 50.0 mg 100 g^{-1} FW [24].

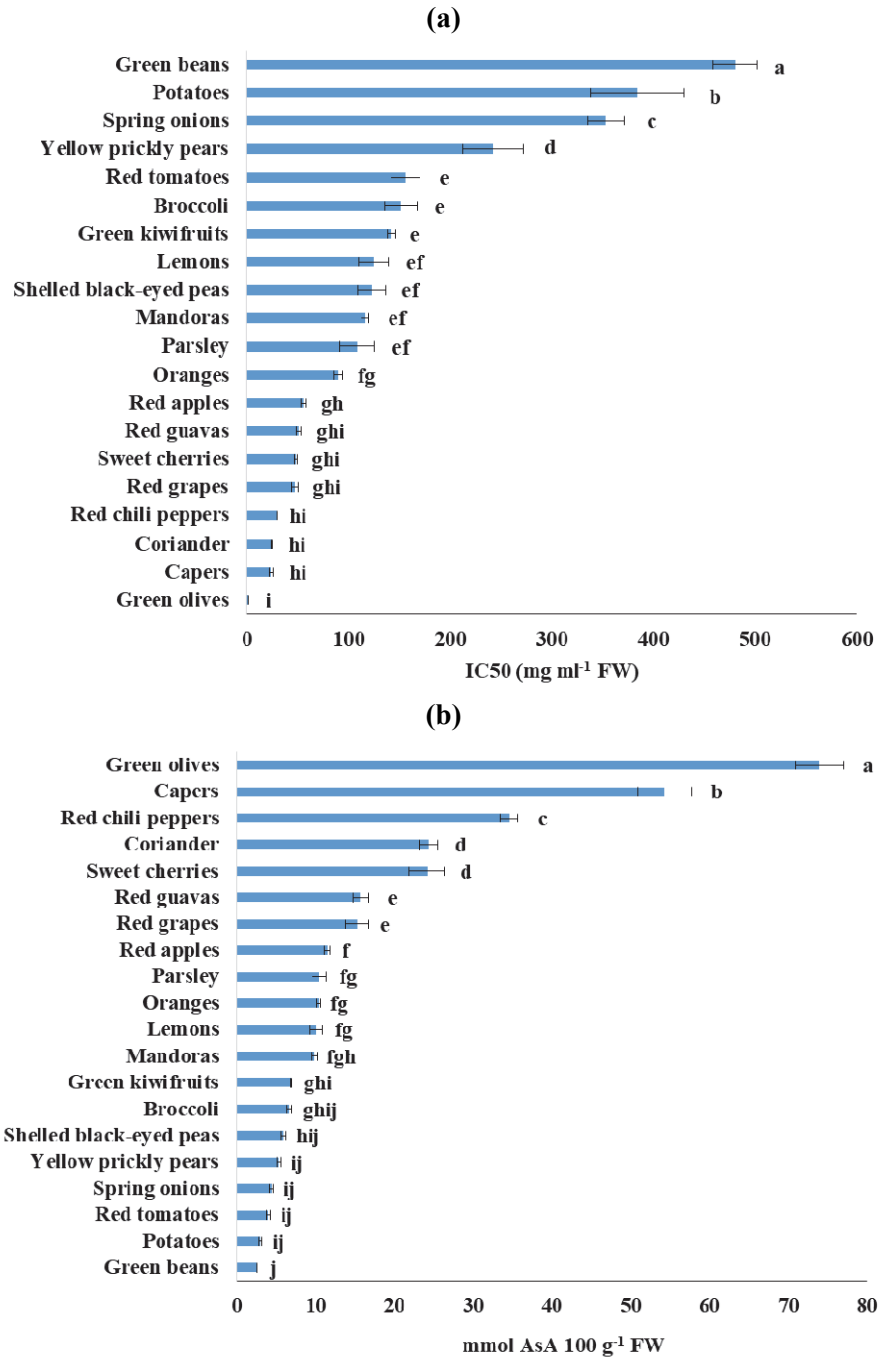
Carotenoids are natural pigments and are generally considered as important contributors to a healthy diet. The most significant aspect of carotenoids in our

diet is the antioxidant and provitamin A activity, and also the color that they impart to our food [25]. In the current work, the total carotenoid content of fruits and vegetables was determined spectrophotometrically and expressed as beta-carotene content. Table 1 shows that they were detected in all vegetables; red chili peppers, capers and coriander were the richest sources of carotenoids. Results also showed that vegetables contain higher amounts of carotenoids than fruits. In particular, carotenoid contents in vegetables ranged between 118.8 and 34529.2 μg β-carotene equivalents 100 g^{-1} FW, whereas fruits have a carotenoid content between 0 and 5305.0 μg β-carotene equivalents 100 g^{-1} FW. With regard to total carotenoids, the highest concentration in fruits and vegetables was detected in mandoras and red chili peppers, respectively. Overall, carotenoid contents of horticultural products are strongly influenced by genetic factors.

Antioxidant activity of fruits and vegetables

The antioxidant activity of fruits and vegetables was evaluated by DPPH, FRAP and phosphomolybdenum assays in order to explore the capacity of antioxidant compounds to scavenge free radicals. The DPPH assay is based on the ability of antioxidants to act as radical scavengers, while FRAP and phosphomolybdate reduction assays to measure the ability of antioxidants to perform as reducing agents [9]. Taking into consideration that the chemical complexity of fruit and vegetables could lead to scattered results, an approach with multiple assays for antioxidant activity was followed. Among a plethora of methods that can be employed for the evaluation of the antioxidant activity, very few are suitable for determining the activity of both hydrophilic and lipophilic compounds, thus ensuring a better comparison of the results and covering a wider range of possible applications [26].

Results generally demonstrated a similar trend between the three assays. The antioxidant activity of fruits and vegetables ranged between 1.2 to 480.3 mg mL^{-1} FW for IC_{50} values in DPPH assay, 2.6 to 73.9 mmol AsA 100 g^{-1} FW for the FRAP assay, 0.8 to 17.7 mmol AsA 100 g^{-1} FW for the phospholybdenum assay (Figure 1a,b,c). All antioxidant activity assays showed that green olives had the highest antioxidant potency, followed by capers and red chili peppers. On the other hand, the antioxidant potency of potatoes and green beans is weak. Another report presented similar results using FRAP, where the antioxidant activity in green beans was low [27]. Furthermore, similar antioxidant activities were determined using FRAP in green olives (65.9 - 190.1 mmol $100g^{-1}$ FW) [28] and orange juice (8.5 mmol $100g^{-1}$ FW) [15].


(a)
Green beans a
Potatoes b
Spring onions c
Yellow prickly pears d
Red tomatoes e
Broccoli e
Green kiwifruits e
Lemons ef
Shelled black-eyed peas ef
Mandoras ef
Parsley ef
Oranges fg
Red apples gh
Red guavas ghi
Sweet cherries ghi
Red grapes ghi
Red chili peppers hi
Coriander hi
Capers hi
Green olives i
0 100 200 300 400 500 600
IC50 (mg ml-1 FW)
(b)
Green olives a
Capers b
Red chili peppers c
Coriander d
Sweet cherries d
Red guavas e
Red grapes e
Red apples f
Parsley fg
Oranges fg
Lemons fg
Mandoras fgh
Green kiwifruits ghi
Broccoli ghij
Shelled black-eyed peas hij
Yellow prickly pears ij
Spring onions ij
Red tomatoes ij
Potatoes ij
Green beans j
0 10 20 30 40 50 60 70 80
mmol AsA 100 g-1 FW

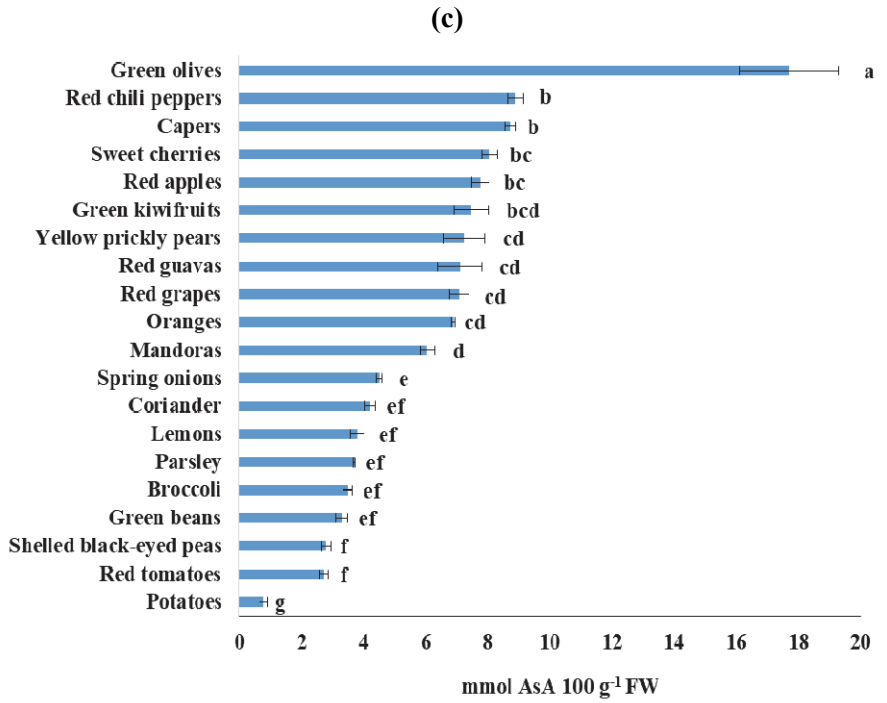


Figure 1. DPPH radical-scavenging activity (a) and antioxidant activity, evaluated by the FRAP (b) and phosphomolybdenum assays (c) of fruits and vegetables grown in Cyprus. Values followed by the same letter are not statistically significant according to Duncan's multiple range test at significance level $P \leq 0.05$. Data are the means of three replications ± SE

With a view to rationalize the antioxidant properties of fruit and vegetables, the correlation coefficients for as total phenolic, total anthocyanin, ascorbic acid and carotenoids contents and antioxidant activity were calculated (Table 2). Data revealed a different response of fruits and vegetables to different antioxidant assays. In particular, a strong correlation was evident between total phenolic content and total antioxidant activity according to all three assays employed (DPPH, FRAP, Phosphomolybdate) for both fruits and vegetables. The same result was also observed with DPPH on another study of several fruits and vegetables in Belgium [16]. In addition, the concentration of total anthocyanins, ascorbic acid, and carotenoids was not linked with the antioxidant activity of examined fruits. In regard with vegetables, all three assays revealed a high correlation of antioxidant activity with carotenoid content, whereas DPPH and FRAP assays demonstrated a moderate contribution of vegetable ascorbic acid to antioxidant activity. Contrarily, the contribution of anthocyanins to antioxidant activity of vegetables was weak.

Table 2. Correlation coefficients (*r*) between groups of antioxidant compounds and antioxidant activity assays

	DPPH	FRAP	Phosphomolybdenum
Fruits			
Total phenolics	-0.614	0.991	0.940
Total anthocyanins	-0.299	0.092	0.003
Ascorbic acid	0.095	-0.376	-0.329
Carotenoids	-0.131	0.170	0.154
Vegetables			
Total Phenolics	-0.727	0.973	0.898
Total anthocyanins	-0.019	0.159	0.412
Ascorbic acid	-0.787	0.520	0.423
Carotenoids	-0.662	0.795	0.849

Summary

In this study, we determined the total phenolic, total anthocyanins, ascorbic acid and carotenoids contents and antioxidant capacity of ten fruits and ten vegetables commonly consumed in Cyprus, an unexplored Mediterranean region. The results showed a wide range of variation with respect to antioxidant composition and activity, with green olives, capers and red chili peppers demonstrating highest bioactive component potential. Interestingly, such produce represent hallmark components of Cypriot and Mediterranean-style diet, and could thus be considered as a guide for health-conscious consumers to choose specific fruits and vegetables as dietary components. Taking into consideration that the antioxidant potency *per se* is a well-established biomarker, which indicates beneficial biological effects of plant derived phenolic substances, further *in vitro* and *in vivo* experiments are essential to confirm the present observations.

Acknowledgements

The support of Cyprus University of Technology internal grant EX032 (VF) is gratefully acknowledged.

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