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Full Length Research Paper

Pulp antioxidant activities, mineral contents and juice nutritional properties of Algerian Clementine Cultivars and Mandarin

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Six cultivars of Clementine fruits including 'Cadoux', 'Monreal', 'St Martin', 'Merme', 'Cheylard', 'Rocamora' and one mandarin fruit cultivar were chemically analysed for their juice characteristics (water content, pH, acidity, total soluble solids and ascorbic acid). Ascorbic acid content varied from 35.98 to 69.41 mg/100 ml, Mandarin variety showed the highest value, whereas Clementine St Martin cultivar was the lowest. Lyophilized citrus pulps were analysed for mineral contents and bioactive compounds. Mineral analysis showed that all the fruit pulps were good sources of K, Fe, and Na. Statistical differences among the citrus pulps were established for the contents of phenols, flavonoids and carotenoids, the quantification of bioactive compounds revealed distinct contents amongst the analysed materials. Phenolic and flavonoid contents presented a value between 3108.78 and 4046.20 mg gallic acid/100 g dw and between 946.42 and 1281.98 mg Catechin/100 g dw respectively and in both cases the highest amount was found in Merme cv. and the lowest in St Martin cv. whereas for carotenoid contents, the amount varied from 38.31 to 75.14 mg β-carotene/100g dw and Mandarin was the richest fruit. All citrus fruit pulps exhibited 1,1-di-phenyl-2-picryl-hydrazil (DDPH) scavenging capacity, and reducing power in a concentration-dependent manner, the Merme cv. being the most active one and the St Martin cv. was the weakest. Our results indicate that the Merme cv. was outranking with regard to the contents of phenolic and flavonoid, DPPH and reducing power activity while St Martin exhibited the lowest amounts and the weakest antioxidant activity.

Key words: Clementine fruit, Mandarin, antioxidant activity, bioactive compounds, ascorbic acid, minerals.

INTRODUCTION

The human diet contains important micronutrients, such as vitamin C, vitamin E, carotenoids and flavonoids, essential for human health maintenance. Several epidemiological and clinical evidences demonstrate a significant decrease in mortality from cardiovascular and other diseases among fruit and vegetable consumers (Gey et al., 1993; Hertog et al., 1995; Liu et al., 2000; Bazzano et al., 2002; Kris-Etherton et al., 2002). These beneficial effects have been attributed to the various antioxidants in fruits and vegetables (Knekt et al., 2002; Huxley and Neil, 2003).

Growing knowledge about the health-promoting impact of antioxidants in everyday foods, combined with the assumption that a number of common synthetic preservatives may have hazardous effects, has led to increased investigations in the field of natural antioxidants (Moure et al., 2001; Bursal and Gülçin, 2011; Gülçin et al., 2011), the reason why In recent years, evaluation of antioxidative activity of naturally occurring substances has been the focus of many studies (Anagnostopoulou et al., 2006; Peschel et al., 2006; Oliveira et al., 2009).

Fruits are attractive and nutritional food, due to their

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colour, shape, specific taste and smell, enriched minerals, vitamins and other beneficial components. Citrus (Citrus L.), which is one of the most important world fruit crops, is consumed mostly as fresh produce or juice because of its nutritional value and special flavour. It is among a major products of Algerian agriculture and many species are cultivated, such as sweet orange (*C. sinensis*), mandarin (*C. reticulata*), grapefruit (*C. paradisi*), lemon (*C. limon*) and clementine (*C. clementina*). Consumption of citrus fruit or juice is found to be inversely associated with several diseases (Joshipura et al., 2001).

According to Agriculture Ministry Statistics, in 2005, about 62126 hectares are dedicated to citrus cultivation. Algeria produces more than 627 thousand tonnes of citrus fruits, which commercially represents an important crop.

Clementine (*C. Clementina*) is a hybrid between orange and mandarin, discovered by Father Clement Rodier in Misserghin, near Oran, in Algeria (Khan, 2007) and various Clementine clones are commercially very important in Mediterranean and North African countries (Ladaniya, 2008). In Algeria, Clementine fruit production comes in the second position after oranges. In 2005, Clementine production represented 17.52% of total citrus fruit (Agriculture Ministry Statistics, 2005), which reflects the preference of Algerian population for this specie.

Several studies have already been realised on the bio-active compounds and antioxidant activity of citrus fruits (Gorinstein et al., 2004; Wang et al., 2006; Vanamala et al., 2006), however, there is no information about Algerian citrus fruits. So far, to our knowledge, the antioxidant compounds of these different Clementine cultivars were not investigated yet. So the current study aims at highlighting some nutritional properties of juices and to determine the mineral content, the levels of bioactive compounds and their antioxidant activities in the edible portions (pulp), of six cultivars of Clementine fruits and Mandarin, cultivated in Algeria.

MATERIALS AND METHODS

Fruit and sample preparation

Six cultivars of Clementine fruits, belonging to the mandarin group and can be distinguished by a rather deep colour of the rind and its sweet taste were harvested at maturity stage from local farm of ITAF institute (Technical Institute of Fruit Arboriculture and Vine) at December 10th, 2008 in Blida region and one cultivar of Mandarin (*C. reticulata*) was collected from Bejaia region, both regions are located in north Algeria with Mediterranean climate. Immediately after harvesting, the fruits were cleaned with tap water and dried, then separated into edible and inedible portions. The freeze dried pulp were ground into a fine powder using a homogenizer. To achieve a standard size of particles, all the ground material was sieved through a 0.5mm metal sieve then stored at -20°C until analysed.

Clementine fruits and Mandarin were used also for juice extraction which was achieved by means of household juice extractor. The juices were placed in sealed amber vials and refrigerated at 4°C and then used for the following analyses.

Nutritional properties of Clementine and Mandarin juices

After filtering the juices through filter paper, the Clementine and Mandarin juice samples were studied to determine the following parameters: pH, titratable acidity (% of citric acid), water content (H%), total soluble solids (TSS) using refractometer, and ascorbic acid (mg/100 ml of juice.) using a dye (2, 6-diclorophenol indophenol) according to the official method (AOAC, 1990).

Mineral contents of Clementine and Mandarin pulps

For the content determination of the following minerals: Ca, Mg, k, Na, Zn, Fe, Cu and Mn, the lyophilized pulp samples (2 g) placed in platinum crucibles were calcined in fumace at 450 °C for 6 h, the ash was then weighed and put in solution in 5 ml HNO3/ HClO4 (nitric acid and perchloric acid solution) (2/1), the solution was filtered, recovered in 100 ml flask, added with pure HNO_3 heated on the hot plate, at 200°C and diluted to volume with deionized water. The minerals were then analysed using Flame atomic absorption spectrometry AAS (AA-6501F. Ver 1.10 Shimadzu Corporation). The amounts of minerals were calculated with a standard curve of each element (Leterme et al., 2006).

Bioactive contents of Clementine and Mandarin pulps

Extraction of phenolic compounds

Approximately 0.4 g aliquot of lyophilized samples and 10 ml of 1% HCl in 80% aqueous methanol solvent was added and left under stirring at room temperature for 3 h. The mixture was then centrifuged at 4000 rpm for 20 min at 4°C. The pellet was re-extracted three times more by repeating the same steps under the same conditions. Following centrifugation the supernatants were combined and filtered through Whatman filter paper using a funnel (Wang et al., 2006).

Total phenolic contents

The concentration of total phenols was measured by the method using the Folin-Ciocalteu reagent (Gutfinger, 1981; Koksal et al., 2011). Shortly, 0.5 ml of appropriately diluted samples or standard solutions of gallic acid was pipetted into test tube, along with 5 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent, and the mixture was allowed to react for 3 min. 1 ml of 20% Na_2CO_3 solution was added and mixed well then left to stand for 1 h at room temperature for colour development. Absorbance was measured at 725 nm and the total phenolic content was derived by comparison with a gallic acid standard curve. The estimation of total phenolics in all the extracts was carried out in triplicate in Shimadzu 1240 MINI UV-VIS spectrophotometer (the same equipment was used in other analyses) and the result means were presented.

Total flavonoid contents

The total flavonoid content of the samples was measured using colorimetric method of Marinova et al. (2005). Properly diluted fruit extract (1 ml), was added to 4 ml of distilled water; followed by 0.3 ml of 5% $NaNO_2$ and the mixture was kept for 6 min at room temperature. 0.3 ml of 10% $AlCl_3$ methanol solution was added and

Table1. Means of nutritional values of Clementine and Mandarin juices.

Fruit	H (%)	pH	Acidity (g/l)	TSS (%)	Ascorbic acid (mg/100 ml)
Mandarin	86.18 ± 0.26^{a}	3.68±0.02^{e}	7.47 ± 0.37^{a}	13.62 ± 0.07^{c}	66.35 ± 0.88^{a}
Rocamora	81.25 ± 0.35^{e}	3.72 ± 0.00 ^{d}	6.67 ± 0.46^{bc}	14.45 ± 0.13^{b}	63.80 ± 0.88^{b}
Merme	84.50 ± 1.20^{bc}	3.91 ± 0.01^{a}	4.61 ± 0.00^{f}	12.45 ± 0.05^{d}	65.84 ± 0.00^{b}
Cheylard	85.53 ± 0.41^{ab}	3.78 ± 0.03^{b}	5.93 ± 0.15^{d}	11.03 ± 0.23^{f}	60.43 ± 2.98^{c}
St Martin	82.26 ± 0.28^{de}	3.75 ± 0.01^{c}	6.36 ± 0.07^{cd}	14.27 ± 0.03^{b}	47.97 ± 0.44^{e}
Cadoux	82.58 ± 0.90^{d}	3.69 ± 0.00^{de}	7.40 ± 0.39^{ab}	14.70 ± 0.17^{a}	55.12 ± 0.00^{d}
Monreal	84.33 ± 0.72^{c}	3.80 ± 0.01^{b}	5.29 ± 0.20^{e}	11.83 ± 0.06^{e}	65.33 ± 0.88^{b}

Mean values of the same column, followed by the same letter, are not significantly different ($p<0.05$). TSS, Total soluble solid.

the mixture was incubated for 6 min again. After 5 min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with distilled water, the absorbance was measured at 510 nm after incubation for 10 min at room temperature against prepared reagent blank. The total flavonoid content was expressed as Catechin Equivalents (CE) per 100 g of lyophilized pulp using a calibration curve.

Determination of carotenoids

150 mg of lyophilized pulp was extracted with 50 ml of (hexane, acetone, ethanol, 50:25:25. v/v/v) containing 0.01% BHT with pre-child mortar and pestle until the extract were colorless, the top layer of hexane containing the color was recovered and transferred to a 25ml volumetric flask. The combined hexane phases are centrifuged for 5 min at 6500 rpm at 5°C. The content of carotenoids is determined by the measurement of hexane extract absorbance at 450 nm. The results are expressed in mg of β-carotene equivalent per 100 g of lyophilized pulp (Sass-Kiss et al., 2005).

Total antioxidative potential determination

DPPH radical scavenging assay

The free radical scavenging activity using the 1,1-di-phenyl-2-picryl-hydrazil (DPPH) reagent was determined by the reduction of the reaction colour between DPPH solution and sample extracts (Molyneux, 2004; Gülçin et al., 2010).

Hundred microliter of various concentrations of the extracts was added to 2.9 ml of DPPH solution (6×10^{-5}). The mixture was shaken immediately after adding DPPH solution and allowed to stand at room temperature in the dark and the decrease in absorbance at 515 nm was measured after 30 min. All tests were performed in triplicate (Milardović et al., 2006).

Blank was made from 2.9 ml of DPPH and 100 µl of methanol and measured absorbance at t = 0. The scavenging of DPPH was calculated according to the following equation: % DPPH scavenging = [(Abs (t = 0) - Abs (t = 30) / Abs (t = 0)] *100; Where Abs (t = 0) = absorbance of DPPH radical + methanol at t = 0 min; Abs (t=30) = absorbance of DPPH radical + phenolic extracts at t = 30 min.

IC_{50}, the necessary concentration of sample to decrease by 50% the initial DPPH concentration, was derived from the percentage of DPPH scavenging vs. sample concentration plot.

Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each extract in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6, and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min in water bath. After incubation, 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 6000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm against a blank in spectrophotometer.

Statistical analysis

All analyses were done in triplicate and the statistical comparison of data was performed by a one way analysis of variance (ANOVA) and the LSD (Least Significant Difference) test to reveal significant differences for each parameter among cultivars.

RESULTS AND DISCUSSION

Nutritional properties of Clementine and Mandarin juices

Acidity, pH, water content, total soluble solids, and ascorbic acid of different juices are illustrated in Table 1. The acidity of different juices ranged from 4.61 ± 0 g of citric acid /l (for Merme cv.) and 7.47 ± 0.37 (for Mandarin), with a pH varied between 3.91 ± 0.01 and 3.68 ± 0.02 for the same cultivars respectively which explained the good taste with less acidity compared to orange juices (6.78-13.75) studied by Topuz et al. (2005). This result shows a high negative correlation between pH and acidity (r = -0.94).

Citrus fruits are classified as acid fruits, since their soluble solids are composed mainly of organic acids and sugars (Kale and Adsule, 1995), which are used as the main index of maturity and one of the major analytical measures of flavour quality. These organic acids contribute to the particular flavour and palatability of citrus juice, and the high acidity protects against the development of pathogens (Esteve et al., 2005). The main acids of citrus fruits are citric and malic acids. In addition, traces of tartaric, benzoic, oxalic and succinic acids have also been reported (Kale and Adsule, 1995; Karadeniz, 2004).

The samples were found to be a good source of sugars. Cadoux cv. contained the highest total soluble

Table 2. Mineral contents of Clementine and Mandarin pulps **(mg/g of dw)**.

Fruit	Na	K	Mg	Ca	Fe	Mn	Cu	Zn
Mandarin	1.80	4.27	1.07	0.39	2.07	0.05	0.04	0.34
Rocamora	1.38	5.16	0.60	0.26	1.92	0.05	0.01	0.37
Merme	3.43	5.22	0.66	0.42	2.43	0.34	0.01	0.32
Cheylard	2.46	5.08	1.69	0.30	2.01	0.34	0.00	0.22
St Martin	3.43	5.31	0.78	1.46	2.77	0.53	0.01	0.63
Cadoux	3.21	6.00	0.52	0.28	2.64	0.45	0.03	0.19
Monreal	3.05	5.43	1.63	0.29	2.60	0.47	0.03	0.31

solids (14. 7% ± 0. 17) whereas Cheylard cv. showed the lowest value (11.03% ± 0. 23), which gave relatively a sweet taste in comparison with other orange juices total soluble solids that range between 10.9 and 12.4% (Topuz et al., 2005). The differences in chemical composition of juices can be attributed to the genetic influence occurring among different cultivars and physiological factors (Del Caro et al., 2004; Sharma et al., 2006). According to Sturm et al. (2003) the chemical composition (TSS, sugar, organic acids) of strawberry fruits significantly varies among the genotype and the stage of maturity of fruits.

Citrus are well-known to be a nutrient source of vitamin C in dietary intake. Ascorbic acid content ranged from 47.97 ± 0.44 to 66.35 ± 0.88 mg ascorbic acid /100ml, Mandarin variety showed the highest value whereas St Martin cv. was the lowest.

The ascorbic acid content varies between 41.7 and 78.14 mg/100ml among orange variety juices (Rapisarda et al., 1999), whereas according to Dhuique-Mayer et al. (2005), the range for ascorbic acid content is from 40 to 62 mg /100ml for all of the studied citrus juices. The Mandarin/Clementine group has lower values (40 and 52.8 mg/100 ml, respectively), which is slightly lower than our results.

Several factors influence the ascorbic acid content. Lee and Kader (2000) reported that preharvest factors include climatic conditions (sunlight exposure and weather), cultural practices (fertilizers), maturity at harvest, harvesting method, postharvest handling conditions (storage), species, cultivars, tissues, as well as the genotype and treatment (Huang et al., 2006). All these factors are responsible for the wide variation in vitamin C content of fruits and vegetables.

Mineral contents of Clementine and Mandarin pulps

The aim of this analysis was to evaluate and compare the mineral content of several Clementine and Mandarin cultivars that are widely consumed by Algerian population and find out their pattern of occurrence in the pulp. To the best of the authors' knowledge, no such study on Clementine cultivar pulps has been reported before. The composition of minerals is detailed in Table 2.

Plant ash varied depending on the fruit cultivars. K, Na and Fe content presented the highest values in all samples, ranged between (1.92 - 6.00 mg/g dried weight (dw)). The abundance of these elements showed the mineral rich nature of Clementine and Mandarin cultivars, they constituted the major elements. On the contrary Zn, Mn and Cu presented the lowest concentrations (0.00 - 0.63 mg/g dw), these remaining elements occurred only at trace level.

K content was relatively variable. Cadoux cv. had the highest value (6 mg/g dw) whereas Mandarin had the lowest (4.27 mg/g dw), Topuz et al. (2005) found that K is the major present element in all samples, with a range of 1011–1364 mg/kg in the investigated citrus cultivars.

Iron concentration ranged between 1.92 and 2.77 mg/g dw in all studied citrus fruits, while the highest value of Mg content was found in Cheylard cv. (1.69 mg/g dw) and Calcium was most abundant in St Martin cv. (1.46 mg/g dw).

Globally, the mineral profile content obtained for the Clementine and Mandarin fruits are comparable to that obtained for other citrus fruit by Gorinstein et al. (2001), who found that the Fe content is higher than Zn, Mn and Cu in some citrus fruits.

Leterme et al. (2006) reported that the variability in the mineral composition depends on the cultivation conditions, such as soil fertility and pH, water supply, climate and seasonal variations.

Bioactive contents of Clementine and Mandarin pulps

All the studied Clementine samples in this paper were harvested in the same field and year and, therefore, were produced under the same conditions of climate to reduce additional sources of variance except for the Mandarin specie which was collected from Oued Ghir field located in Bejaia region, used for the comparison. Table 3 summarizes the quantitative determination of phenols, flavonoids and carotenoids in various samples.

Total phenolic contents

Of all the selected cultivars, the total polyphenols

Table 3. Means of total phenol, flavonoid and carotenoid contents per 100g dw of Clementine and Mandarin pulps.

Fruit	Phenol (mg gallic acid)	Flavonoids (mg catechin)	Carotenoids (mg β –carotene)
Mandarin	3888 ± 43.71[b]	1078.92 ± 23.74[b]	75.14 ± 0.79[a]
Rocamora	4006.45 ± 25.86[ab]	1088.33 ± 17.25[b]	38.31 ± 0.82[e]
Merme	4046.2 ± 123.2[a]	1281.98 ± 23.04[a]	57.39 ± 1.64[b]
Cheylard	3355.09 ± 133.25[d]	1030.77 ± 17.14[c]	49.69 ± 1.56[c]
St Martin	3108.78 ± 66.5[e]	946.42 ± 16.31[e]	42.89 ± 1.83[d]
Cadoux	3144.24 ± 92.93[e]	983.3 ± 6.14[d]	44.54 ± 1.69[d]
Monreal	3504.7 ± 31.85[c]	1068.53 ± 30.34[b]	56.2 ± 1.21[b]

Mean values of the same column, followed by the same letter, are not statistically different ($p<0.05$).

contents ranged from 3108.78 ± 66.50, to 4046.20 ± 123.20 mg gallic acid/ 100g lyophilized pulp. The contents of total phenolics were significantly higher in C.Clementina Merme cv. whereas C.Clementina St Martin cv. had the lowest amount of total phenols.

According to Wang et al. (2006), the total polyphenols content ranges between 36.9 and 75.9 mg GAE/ g dw) of eight different citrus fruit, while in white grapefruit and his hybrid, the phenolic content are 63.0 and 69.6 (mg gallic acid equivalent /g dw) (Gorinstein et al., 2004).

Our result of phenolic content in Clementine cultivars and Mandarin was much higher than that of Algerian date varieties which ranges between 2.49 to 8.36 mg gallic acid equivalent per 100 g fresh fruit (Mansouri et al., 2005), and was in the lower limit than that of citrus fruit range in the references mentioned above (Gorinstein et al., 2004; Wang et al. 2006).

As far as we know, there is no such investigation of these Clementine cultivars. According to Sharma et al. (2001) and Abeysinghe et al. (2007) several factors such as the genetic differences amongst different cultivars and species, the tissue analysed, as well as the geographical origin and the clones (Stefanovits-Bányai et al., 2003), plant height (Lisiewska et al., 2006), extraction time, temperature and solvent (Li et al., 2006; Spigno et al., 2007), maturation stages (Yoo et al., 2004) and vegetative rootstocks (Jakobek et al., 2009) could influence the phenolic content.

Total flavonoid contents

Total flavonoids varied from 946.42 ± 16.31 to 1281.98 ± 23.04 mg/100g dw (Catechin equivalents). The cultivar Merme and Rocamora had the highest levels (1281.98 ± 23.04 and 1088.33 ± 17.25 mg/100g dw Catechin equivalents respectively), while C. Clementina St Martin cv. and Cadoux cv. had the lowest (946.42 ± 16.31 and 983.3 ± 6.14 mg/100 g dw) respectively.

Wang et al. (2006) found that flavonoid content in different studied citrus fruits ranges from 8.41 to 21.6 mg Rutin Equivalent/ g dw, whereas the content of total flavonoids (mg Catechin equivalent/100 g fresh weight) in peeled hybrids and white grapefruits are 47.12 ± 4.1 and 37.7 ± 3.2 (Gorinstein et al., 2004).

Flavanone is the major flavonoid in oranges (Wang et al., 2006). Naringin and hesperidin, so-called citrus flavonoids, are two major flavanone glycosides present in citrus fruits (Kawaii et al., 1999).

According to Kawaii et al. (1999), the abundance order of flavonoid compounds in Clementine is hesperidin, narirutin, neoponcirin, neoeriocitrin, diosmin, eriocitrin with the absence of naringin, whereas according to Nogata et al. (2006) the order is hesperidin, narirutin, followed by diosmin, isorhoifolin, neoponcirin and rutin.

In our study, the total flavonoid levels were within the range of Wang et al. (2006) study, citrus flavonoid composition appears to vary greatly among the tested fruits depending on their genetic origin, the time of fruit collection, and the different parts of the used fruit (peel, and edible parts) (Lu et al., 2006). The presence and/or concentrations of flavonoids can be affected by the fruit development stage (Castillo et al., 1993; Ortuno et al., 1995). Furthermore, Vanamala et al. (2006) reported that naringin content of grapefruit juice from the same grove and trees fluctuates during a season and varies considerably between crop years.

Determination of carotenoids

Total carotenoid content was much lower than those of total polyphenol and flavonoid, and ranged from 38.31 ± 0.82 to 75.14 ± 0.79 mg/100 g dw (β-carotene equivalents). Significant differences in the contents of carotenoids also observed in selected citrus species (Table 3). Carotenoid content was significantly higher in Mandarin than that in the Clementine cultivars, the carotenoid level exhibited the following order: Mandarin> Merme > Monreal > Cheylard> Cadoux> St Martin> Rocamora.

According to Wang et al. (2006), the carotenoid level of eight citrus fruit varies between 0.019 and 0.336 mg β-carotene/g dw which is lower than our results, at the same time the Clementine carotenoid content is much higher than Algerian date varieties which varies between

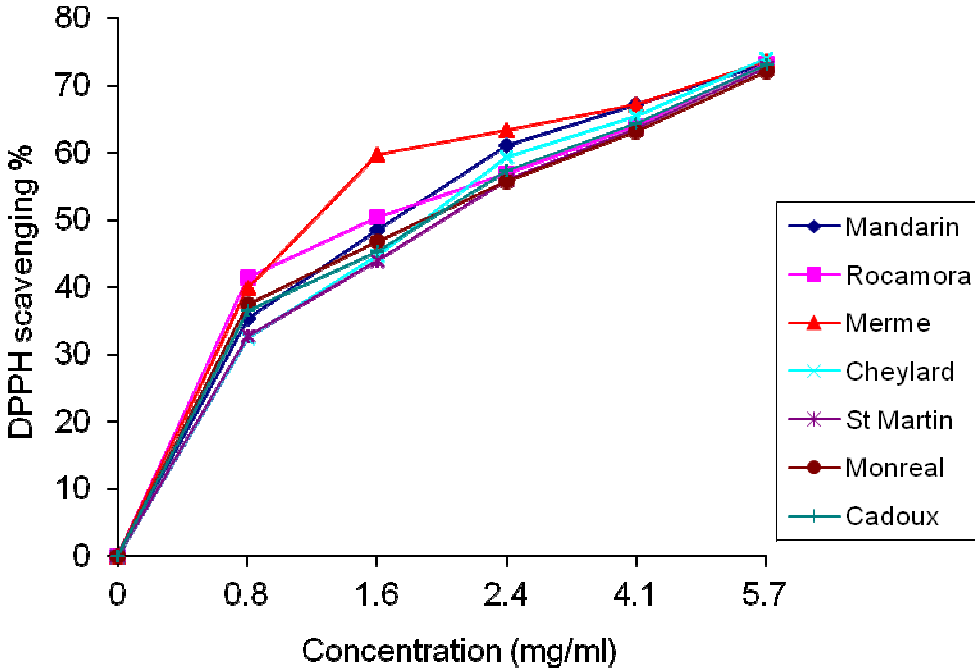

Figure 1. Effect of extract concentrations of Clementine and Mandarin pulps on DPPH scavenging percentage.

32.6 to 773 µg β-carotene equivalent/ 100 g fresh weight (Boudries et al., 2007). This difference could be explained by several factors such as variety, maturation stages, sunshine period, rainfall, temperature, geographical origin (Mercadante et al., 1998; Markus et al., 1999; Boudries et al., 2007).

Total antioxidative potential determination

To screen the antioxidant properties of the Clementine and Mandarin fruits, two different *in vitro* assays were performed: DPPH free radical scavenging capacity and reducing power.

DPPH free radical scavenging capacity

The DPPH method is rapid, sensitive, reproducible, and require simple conventional laboratory equipment for assessing the antioxidant activity of fruit and vegetable extracts (Du et al., 2009). All the citrus fruit extracts exhibited a concentration dependent DPPH radical scavenging activity (Figure 1).

The antioxidant capacities of different samples using DPPH assay gave relatively high IC_{50} values which varied from 1.14 ± 0.05 to 1.91 ± 0.06 (mg/ml) in different cultivars (Table 4).

The Merme cv. was shown to be the most active material, followed by the Rocamora cv. (IC_{50} of 1.48 ± 0.05 (mg/ml)), while St Martin cv. clearly showed a lower antioxidant capacity.

A lower absorbance indicates a higher scavenging effect. The antioxidant react with the stable organic nitrogen free radical DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and convert it to 1,1 diphenyl -2- picryl hydrazine, due to its hydrogen donating ability at a very rapid rate with decolouration (Soares et al., 2009), which is monitored spectrophotometrically at 515 nm. Therefore, compounds with high antioxidant activity result in a rapid decline in the absorbance of the DPPH (Guimarães et al., 2010).

Reducing power

Figure 2 shows the reducing power of the different cultivar methanolic extracts as a function of their concentrations. The reducing power of the extracts increased with their concentrations in the medium.

The values of absorbance at 700 nm for the pulp extracts of all Clementine and Mandarin cultivars revealed that all samples had a capacity to reduce iron (III) and had electron donor properties for neutralizing free radicals by forming stable products. The outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging (Tohma and Gülçin, 2010), and the Reducing Power values of Clementine cultivars and Mandarin were significantly different, and decreased in the order of Merme, Mandarin, Rocamora, Cheylard, Monreal, St Martin and Cadoux.

Table 4. Means of IC_{50} of Clementine and Mandarin pulps using DPPH test.

Fruit	IC_{50} (mg/ml)
Mandarin	1.65 ± 0.01^{c}
Rocamora	1.48 ± 0.05^{d}
Merme	1.14 ± 0.05^{e}
Cheylard	1.80 ± 0.09^{b}
St Martin	1.91 ± 0.06^{a}
Cadoux	1.83 ± 0.11^{ab}
Monreal	1.77 ± 0.00^{b}

Mean values of the same column, followed by the same letter, are not significantly different ($p<0.05$).

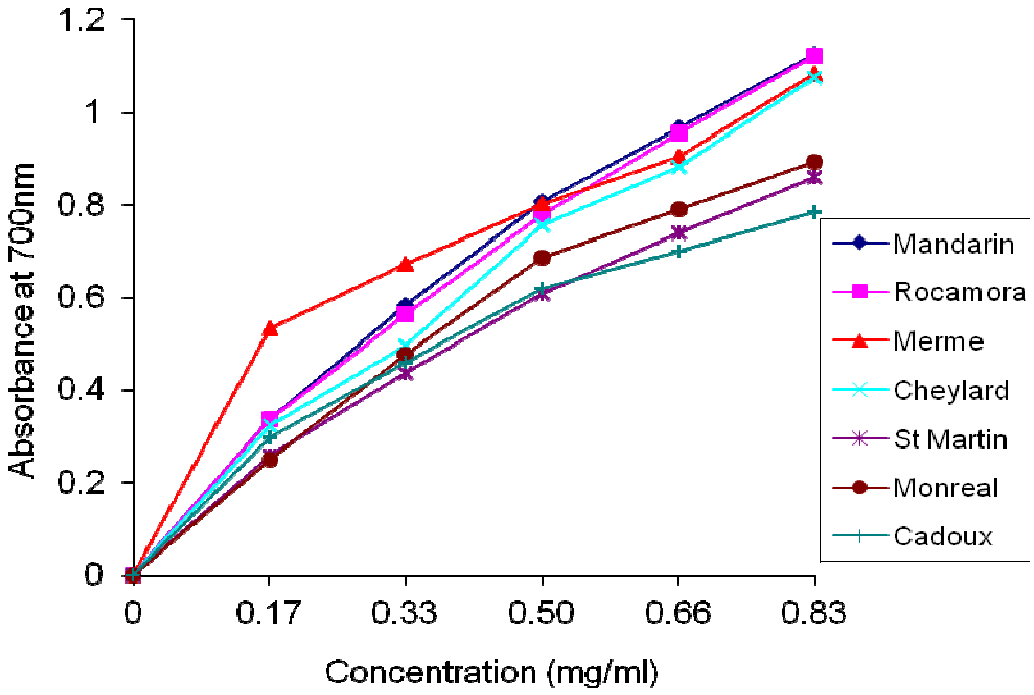

Figure 2. Effect of extract concentrations of Clementine and Mandarin pulps on reducing power.

In this assay, the reducing power of the Clementine pulp extracts was measured by direct electron donation in the reduction of $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$. The product was visualised by addition of free $Fe3^{+}$ ions after the reduction reaction, by forming the intense Prussian blue colour complex, Fe4[Fe(CN^{-})6]3, and quantified by absorbance measurement. An increase in absorbance of the reaction mixture would indicate an increase in reducing capacity due to an increase in the formation of the complex (Ribeiro et al., 2008; Gülçin, 2010). The yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound (Soares et al., 2009).

The antioxidant potential exhibited by Clementine and Mandarin fruits, determined by their composition, is obviously influenced by species and cultivars which is accordance with Du et al. (2009).

The antioxidant activity of citrus fruit extracts may be due to the presence of phenols, flavonoids, carotenoids and ascorbic acid (Gardner et al., 2000; Gorinstein et al., 2001, 2004; Yoo et al., 2004; Jayaprakasha and Patil, 2007; Abeysinghe et al., 2007).

However, not only the qualitative characterisation of different constituents was important but also the knowledge of their amounts and ratios was so relevant. Thus, the action of the different compounds, which might comprise synergic and antagonist effects, present in Clementine and Mandarin fruits result in the total antioxidant activity.

Several studies focused on the relationship between

the antioxidant activity of the phenolics compounds, as hydrogen donating free radical scavengers and their chemical structure. The scavenging properties of antioxidant compounds are often associated with their ability to form stable radicals (Majo et al., 2005).

It has been shown that the presence of the -CH=CH-COOH group in the hydroxylated cinnamates ensures greater H-donating ability and subsequent radical stabilization than the carboxylate group in the hydroxy benzoates (Rice-Evans et al., 1996). The anti-radical, and the ability to scavenge free radicals by phenolic acids is positively correlated with (a) the number of hydroxyl groups bonded to the aromatic ring; (b) the ortho and para system of hydroxyl substitution in relation to each other, and (c) the character of substituents (carboxyl or acetyl group) and their position in relation to the hydroxyl groups seem to influence also the antioxidant or anti-radical features of the phenolic compounds (Sroka and Cisowski, 2003). The same observation was reported by Heim et al. (2002) concerning flavonoids compounds, multiple hydroxyl groups confer to the molecule substantial antioxidant and chelating activity. Methoxy groups introduce unfavorable steric effects and increase lipophilicity. A double bond and carbonyl function in the heterocycle or polymerization of the nuclear structure increases activity by affording a more stable flavonoid radical through conjugation and electron delocalization. A clear relationship between the planar character of flavanone compounds and antioxidant power which is influenced by: the number and position of hydroxyl groups, the position of the methylation and the kind of sugar (Majo et al., 2005).

Conclusion

In summary, Clementine cultivars and Mandarin appeared as outstanding mineral sources especially in K, Fe and Na, which constituted the major minerals followed by Ca and Mg which were present in appreciable quantity.

The studied fruits contained a group of natural antioxidants that had a high antioxidant activity. Polyphenols and flavonoids contributed significantly to the total antioxidant activity of all Clementine and Mandarin cultivars, while vitamin C, the hydrosoluble antioxidant which was largely present in the juice, certainly improved greatly the antioxidant activity. This study also confirmed the high contents of carotenoids in citrus varieties cultivated under the Mediterranean climate.

Given that the growing evidence of the versatile health benefits of antioxidant compounds through epidemiological surveys, further studies are needed on the isolation and characterisation of individual compounds to elucidate their different antioxidant mechanisms and the existence of possible synergism, if any, among the compounds present in Clementine fruits.

Therefore, the results of this study strongly showed that the Clementine fruit can be used as easily accessible source of natural antioxidant to protect the body against various oxidative stresses, and made it the preferable fruit for dietary prevention of cardiovascular and other diseases. Thus, it is recommended to consume Clementine for all these nutritional values.

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REFERENCES

Abeysinghe DC, Li X, Sun CD, Zhang W S, Zhou CH, Chen KS (2007). Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species. Food Chem. 104: 1338-1344.

Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimopoulou AN, Boskou D (2006). Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). Food Chem. 94: 19-25.

AOAC (1990). Official methods of analysis (17th Ed.). Washington, DC: Association of Official Analytical Chemists.

Bazzano LA, He J, Ogden LG, Loria CM, Vupputuri S, Myers L, Whelton PK (2002). Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first national health and nutrition examination survey epidemiologic follow-up study. Am. J. Clin. Nutr. 76: 93-99.

Boudries H, Kefalas P, Hornero-Mendez D (2007). Carotenoid composition of Algerian date varieties (P*hoenix dactylifera*) at different edible maturation stages. Food Chem. 101: 1372-1377.

Bursal E, Gülçin I (2011). Polyphenol contents and in vitro antioxidant activities of lyophilised aqueous extract of kiwifruit (*Actinidia deliciosa*). Food Res. Int. 44: 1482-1489.

Castillo J, Benavente O, Del Rio JA (1993). Hesperetin 7-Oglucoside and prunin in Citrus species (*C. aurantium* and *C. paradisi*). A study of their quantitative distribution in immature fruits and as immediate precursors of neohesperidin and naringin in *Citrus aurantium*. J. Agric. Food Chem. 41: 1920-1924.

Del Caro A, Piga A, Vacca V, Agabbio M (2004). Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. Food Chem. 84: 99-105.

Dhuique-Mayer C, Caris-Veyrat C, Ollitrault P, Curk F, Amiot MJ (2005). Varietal and Interspecific Influence on Micronutrient Contents in Citrus from the Mediterranean Area. J. Agric. Food Chem. 53: 2140-2145.

Du G, Li M, Ma F, Liang D (2009). Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. Food Chem. 113: 557-562.

Esteve MJ, Frigola A, Rodrigo C, Rodrigo D (2005). Effect of storage period under variable conditions on the chemical and physical composition and colour of Spanish refrigerated orange juices. Food Chem. Toxicol. 43: 1413-1422.

Gardner PT, White TAC, McPhail DB, Duthie GG (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chem. 68: 471-474.

Gey KF, Stahelin HB, Eichholzer M (1993). Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke. Basel prospective study. Clin. Investigator. 71: 3-6.

Gorinstein S, Martin-Belloso O, Park YS, Haruenkit R, Lojek A, Ciz M, Caspi A, Libman I, Trakhtenberg S (2001). Comparison of some biochemical characteristics of different citrus fruits. Food Chem. 74: 309–315.

Gorinstein S, Zachwieja Z, Katrich E, Pawelzik E, Haruenkit R,
Trakhtenberg S, Martin-Belloso O (2004). Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and his new hybrid. Lebensm.-Wiss. u.-Technol. 37: 337-343.
Guimarães R, Barros L, Barreira JCM, Sousa MJ, Carvalho AM, Ferreira ICFR (2010). Targeting excessive free radicals with peels and juices of citrus fruits: Grapefruit, lemon, lime and orange. Food Chem. Toxicol. 48: 99-106.
Gülçin I (2010). Antioxidant properties of resveratrol: A structure-activity insight. Innov. Food Sci. Emerg. Technol. 11: 210-218.
Gülçin I, Huyut Z, Elmastas M, Aboul-Enein HY (2010). Radical scavenging and antioxidant activity of tannic acid. Arab J. Chem. 3: 43-53.
Gülçin I, Topal F, Çakmakçı R, Bilsel M, Gören AC, Erdogan U (2011). Pomological features, nutritional quality, polyphenol content analysis and antioxidant properties of domesticated and three wild ecotype forms of raspberries (*Rubus idaeus* L.). J. Food Sci. 76: C585-C593.
Gutfinger T (1981). Polyphenols in olive oils. J. Amer. Oil Chem. Soc. 966-968.
Heim KE, Tagliaferro AR, Bobilya DJ (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13: 572-584.
Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, Simic BS, Toshima H, Feskens EJ, Hollman PC, Katan MB (1995). Flavonoid intake and long term risk of coronary heart disease and cancer in the seven countries study. Arch. Int. Med. 155: 381-386.
Huang YC, Chang YH, Shao YY (2006). Effects of genotype and treatment on the antioxidant activity of sweet potato in Taiwan. Food Chem. 98: 529-538.
Huxley RR, Neil HAW (2003). The relationship between dietary flavonol intake and coronary heart disease mortality: A meta-analysis of prospective cohort studies. Eur. J. Clin. Nutr. 57: 904-908.
Jakobek L, Seruga M, Voca S, Sindrak Z, Dobricevic N (2009). Flavonol and phenolic acid composition of sweet cherries (cv. Lapins) produced on six different vegetative rootstocks. Sci. Hortic. 123: 23-28.
Jayaprakasha GK, Patil BS (2007). In vitro evaluation of the antioxidant activities in fruit extracts from citron and blood orange. Food Chem. 101: 410-418.
Joshipura KJ, Hu FB, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, Colditz G, Ascherio A, Rosner B, Spiegelman D, Willett WC (2001). The effect of fruit and vegetable intake on risk for coronary heart disease. Ann. Int. Med. 134: 1106-1114.
Kale PN, Adsule PG (1995). Citrus, Handbook of fruit science and technology. Production, composition, storage and processing. Van Nostrand Reinhod, New York, USA. pp. 39-65.
Karadeniz F (2004). Main Organic Acid Distribution of Authentic Citrus Juices in Turkey. Turk. J. Agric. For. 28: 267-271.
Kawaii S, Tomono Y, Katase E, Ogawa K, Yano M (1999). Quantitation of Flavonoid Constituents in *Citrus* Fruits. J. Agric. Food Chem. 47: 3565-3571.
Khan IA (2007). Citrus Genetics, Breeding and Biotechnology. CAB international, Cambridge, USA. pp: 19-45
Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Heliovaara M, Reunanen A, Maatela J. (2002). Flavonoid intake and the risk of chronic diseases. Am. J. Clin. Nutr. 76: 560-568.
Koksal E, Bursal E, Dikici E, Tozoglu F, Gülçin I (2011). Antioxidant Activity of Melissa officinalis leaves. J. Med. Plant Res. 5: 217-222.
Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am. J. Med. 113: 71-88.
Ladaniya MS (2008). Citrus fruit, biology, technology and evaluation. Academic Press, Burlington, USA. pp: 13-63.
Lee SK, Kader AA (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol. 20: 207-220.
Leterme P, Buldgen A, Estrada F, Londono AM (2006). Mineral content of tropical fruits and unconventional foods of the Andes and the rain forest of Colombia. Food Chem. 95: 644-652.
Li BB, Smith B, Hossain MM (2006). Extraction of phenolics from citrus peels. I. Solvent extraction method. Sep. Purif. Technol. 48: 182-188.
Lisiewska Z, Kmiecik W, Korus A (2006). Content of vitamin c, carotenoids, chlorophylls and polyphenols in green parts of drill (*Anthum Graveolens* L) depending on plant height. J. Food Compost. Anal. 19: 134-140.
Liu S, Manson JE, Lee IM, Stephen RC, Hennekens CH, Wilett WC, Buring JE (2000). Fruit and vegetable intake and risk of cardiovascular disease: the women's health study. Am. J. Clin. Nutr. 72: 922-928.
Lu Y, Zhang C, Bucheli P, Wei D (2006). *Citrus* Flavonoids in Fruit and Traditional Chinese Medicinal Food Ingredients in China. Plant Food Hum. Nutr. 61: 57-65.
Majo DD, Giammanco M, Guardia ML, Tripoli E, Giammanco S, Finotti E (2005). Flavanones in Citrus fruit: Structure–antioxidant activity relationships. Food Res. Int. 38: 1161-1166.
Mansouri A, Embarek G, Kokkalou E, Kefalas P (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (Phoenix Dactylifera). Food Chem. 89: 411-420.
Marinova D, Ribarova F, Atanassova M (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J. Univ. Chem. Technol. Metall. 40: 255-260.
Markus F, Daood HG, Kapitany J, Biacs PA (1999). Change in the carotenoid and antioxidant content of spicy red pepper (paprika) as a function of ripening and some technological factors. Am. Chem. Soc. 47: 100-106.
Mercadante AZ, Rodriguez-amaya DB (1998). Effects of ripening, cultivar differences, and processing on the carotenoid composition of Mango. J. Agric. Food Chem. 46: 128-130.
Milardović S, Ivekovic D, Grabarić BS (2006). A novel ampirometric method for antioxidant activity determination using DPPH free radical. Bioelectrochemistry, 68: 175-180.
Molyneux P (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxdant activity. J. Sci. Technol. 26: 211-219.
Moure A, Cruz J, Franco D, Dominguez JM, Sineiro J, Dominguez H, Núñez MJ, Parajó JC (2001). Natural antioxidants from residual sources. Food Chem. 72: 145-171.
Nogata Y, Sakamoto K, Shiratsuchi H, Ishii T, Yano M, Ohta H (2006). Flavonoid Composition of Fruit Tissues of Citrus Species. Biosci. Biotechnol. Biochem. 70: 178-192.
Oliveira AC, Valentim IB, Silva CA, Bechara EJH, Barros MP, Mano CM, Goulart MOF (2009). Total phenolic content and free radical scavenging activities of Methanolic extract powders of tropical fruit residues. Food Chem. 115: 469-475.
Ortuno A, Garcıa-Puig D, Fuster MD, Perez ML, Sabater F, Porras I, Garcia-Lidon A, Del Rio JA (1995). Flavanone and nootkatone levels in different varieties of grapefruit and pummelo. J. Agric. Food Chem. 43: 1-5.
Oyaizu M (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44: 307-315.
Peschel W, Sanchez-Rabaneda F, Diekmann W, Plescher A, Gartzıa I, Jimenez D, Lamuela-Raventós R, Buxaderas S, Codina C (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem. 97: 137-150.
Rapisarda P, Tomaino A, Casio RL, Bonina F, Pasquale AD, Saija A (1999). Antioxidant Effectiveness As Influenced by Phenolic Content of Fresh Orange Juices. J. Agric. Food Chem. 47: 4718-4723.
Ribeiro SMR, Barbosa LCA, Queiroz JH, Knodler M, Schieber A (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. Food Chem. 110: 620-626.
Rice-Evans C, Miller NJ, Paganga G (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Radic. Biol. Med. 20: 933-956.
Sass-Kiss A, Kiss J, Milotay P, Kerek MM, Toth-Markus M (2005). Differences in anthocyanin and carotenoid content of fruits and vegetables. Food Res. Int. 38: 1023-1029.
Sharma RR, Goswami AM, Singh CN, Chhonkar OP, Singh G (2001). Catecholase and cresolase activities and phenolic content in mango (*Mangifera indica* L.) at panicle initiation. Sci. Hortic. 87: 147-151.
Sharma RR, Singh R, Saxena SK (2006). Characteristics of citrus fruits

in relation to granulation. Sci. Hortic. 111: 91-96.
Soares AA, De Souza CGM, Daniel FM, Ferrari GP, Da Costa SMG, Peralta RM (2009). Antioxidant activity and total phenolic content of Agaricus Brasiliensis (agaricus blzei Murril) in two stages of maturity. Food Chem. 112: 775-781.
Spigno G, Tramelli L, Faveri DM (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolic. J. Food Eng. 81: 200-208.
Sroka Z, Cisowski W (2003). Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem. Toxicol. 41: 753-758.
Stefanovits-Bányai É, Tulok MH, Hegedûs A, Renner C, Varga IS (2003). Antioxidant effect of various rosemary (Rosmarinus officinalis L.) clones. Acta Biol. Szegediensis. 47: 111-113.
Sturm K, Korn D, Stampar F (2003). The composition of fruit of different strawberry varieties depending on maturity stage. Food Chem. 83: 417-422.
Tohma HS, Gülçin I (2010). Antioxidant and radical scavenging activity of aerial parts and roots of Turkish liquorice (Glycyrrhiza glabra L.). Int. J. Food Prop. 13: 657-671.
Topuz A, Topakci M, Canakci M, Akinci I, Ozdemir F (2005). Physical and nutritional properties of four orange varieties. J. Food Eng. 66: 519-523.
Vanamala J, Reddivari L, Yoo KS, Pike LM, Patil BS (2006). Variation in the content of bioactive flavonoids in different brands of orange and grapefruit juices. J. Food Compost. Anal. 19: 157-166.
Wang YC, Chuang YC, Ku YH (2006). Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan. Food Chem. 102: 1163-1171.
Yoo KM, Lee KW, Park JB, Lee HJ, Hwang IK (2004). Variation in major antioxidants and total antioxidant activity of Yuzu (Citrus Junos Sieb ex Tanaka) during maturation and between cultivars. J. Agric. Food Chem. 52: 5907-5913.