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Agronomic Characteristics and Physicochemical Properties of Selected *Citrus* Cultivars Grown in Tunisia

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ABSTRACT

Citrus is a popular horticultural crop. Interest in assessing agronomic and bioactive compounds with antioxidant capacity and potential health benefits in *Citrus* is increasing. Besides some agronomic characteristics, the variability in total carotenoids and lycopene contents of ten *Citrus* cultivars (five oranges including the pigmented cultivars 'Moro', 'Tarocco', 'Sakasli', and 'Maltaise', 'Demi Sanguine' and the blond cultivar 'Maltaise Blonde'; two mandarins 'Fortune' and 'Minneola'; one citron 'Marsh'; one pomelo 'Star Ruby'; and one Clementine 'Hernandina' were investigated. The results showed significant differences in total carotenoids and lycopene contents between *Citrus* cultivars. Total carotenoid content ranged from 5.33 mg/kg FW in 'Hernandina' to 23.66 mg/kg FW in 'Star Ruby'. Lycopene content ranged from 0.27 mg/kg FW in 'Maltaise Blonde' to 17.93 mg/kg FW in 'Star Ruby'. Therefore, the highest total carotenoids and lycopene values were shown by the pomelo 'Star Ruby'. This study demonstrates that the amount of total carotenoid and lycopene was influenced by genotype, emphasizing the need to evaluate *Citrus* biodiversity in order to improve its nutritional value and to contribute towards increasing the intake of antioxidants.

Keywords: carotenoid, *Citrus*, lycopene, pigmented *Citrus* cultivars, quality

INTRODUCTION

Citrus is one of the main horticultural crops grown and consumed in Tunisia and is therefore of economic importance. In fact, in 2010, 20,723 ha were dedicated to this crop and its production amounted to 261,000 t (DGPA 2010).

Lycopene and anthocyanin are the main types of pigments responsible for the red colour in *Citrus* fruits. These pigments, particularly lycopene, not only add color attraction but also nutritional benefits (Colditz *et al*. 1985; Olson, 1986; Brady *et al*. 1996; Arena *et al*. 2001; Wang *et al*. 2008). β-Carotene is the major dietary precursor of Vitamin A. However, lycopene has a strong dietary antioxidant; it plays a role in the prevention of cancer and chronic disease, and exhibits significant tumor suppression activity which has attracted interest in its pharmaceutical potential (Edge *et al*. 1997). The typical lycopene-pigmented *Citrus* is deep coloured grapefruit, e.g. 'Ruby Red' (Dhillon 1982; Bowman and Gmitter 1990). Several epidemiological studies have inversely correlated the consumption of *Citrus* fruits with the risks of colorectal, esophageal, gastric and stomach physiological disorders (Peterson *et al*. 2006; Tripoli *et al*. 2007).

Recently, attention has been given to antioxidant compounds in fruits and vegetables (Ilahy *et al*. 2009; Tlili *et al*. 2009; Ilahy *et al*. 2010; Tlili *et al*. 2010; Ilahy *et al*. 2011). In fact, their estimation is becoming an important evaluation parameter for the nutritional quality of food and its quantification allows a real evaluation of this nutritional value. In addition, it is known that the amount of each antioxidant in *Citrus* fruits is strongly influenced by type of cultivar and a large number of external factors such as agrotechnical process, climatic conditions and ripeness during harvest and post harvest manipulation (Patil *et al*. 2004; Huang *et al*. 2007; Goulas and Manganaris 2012).

Despite the great health benefit of *Citrus* fruit showed recently by Wong (2009) who studied the pharmacological actions of grapefruit extracts (naringin and naringinin) and as source of dietary polyphenols (Fernàndez-López *et al*. 2009), few studies have focused on total carotenoid and lycopene contents of *Citrus* cultivars. Goulas and Manganaris (2012) focused on the phytochemical content and the antioxidant potential of different *Citrus* fruits grown in Cyprus and found a clear phytochemical diversity particularly in carotenoid content among the studied *Citrus* fruits.

Therefore, and based on these facts, the aim of this study was to evaluate some agronomic and physicochemical properties of 10 *Citrus* cultivars ('Fortune', 'Hernandina', Maltaise Blonde', 'Maltaise Demi sanguine', 'Marsh', 'Minneola', 'Moro, 'Sakasli, 'Star Ruby', and 'Tarocco') grown in Tunisia.

MATERIALS AND METHODS

Fruit sampling

Fruits of five orange cultivars differing in their intensity of flesh pigmentation including the pigmented 'Moro', 'Tarocco', 'Sakasli', and 'Maltaise Demi Sanguine' and the blond cultivar 'Maltaise Blonde', two mandarin cultivars 'Fortune' and 'Minneola', one citron cultivar 'Marsh', one pomelo cultivar 'Star Ruby' and the Clementine cultivar 'Hernandina' were evaluated. Ninety fruits similar in fruit quality, from 90 orange trees were randomly divided into 3 groups as three replications. *Citrus* trees were grown in the experimental station of the National Research Institute of Tunisia located in the north east of Tunisia (El Gobba). All fruit were harvested at a commercial stage, based on size uniformity and external colour. Pulps from equatorial part of the fruits were collected, and stored at -80°C until physicochemical analysis.

Chemicals

Sodium hydroxide and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich, Chemical Co., Milan (Italy). Hexane, acetone and ethanol were of analytical grade and also obtained from Sigma-Aldrich.

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Determination of physicochemical properties

Soluble solids content in orange juice (°Brix) was measured by taking a small sample of the homogeneous juice and squeezing the juice into a digital refractometer (Atago PR-100, NSG Precision Cells Inc., Farmingdale, NY) calibrated with a 10% sucrose solution. The pH was carried out directly on the juice using an electronic pH-meter (WTW, Microprocessor pH Meter, PH 539, Weilheim, Germany). Titratable acidity was estimated after titration at a pH of 8.1 with a sodium hydroxide solution (0.1 M) and results were expressed in percentage of citric acid (National Canners Association 1968). The density was measured by accurately weighing 25 ml of centrifuged juice into a 25 ml pycnometer (Brand GmBH, Wertheim, Germany).

Determination of bioactive compounds

Total carotenoids and lycopene extraction and determination were conducted as described by Lee (2001) and Fish *et al.* (2002), respectively. The method uses a mixture of hexane: ethanol: acetone (2: 1: 1, v/v) containing 0.05% BHT. During the extraction process, some precautions were taken, like working in a reduced luminosity room and wrapping glass materials in aluminium foil to avoid lycopene loss by photo-oxidation. For total carotenoid and lycopene quantification, the absorbance of the hexane extract was read at 450 and 503 nm, respectively using a Beckman DU 650 spectrophotometer (Beckman Coulter, Fullerton, CA, US). Total carotenoids were expressed in milligrams β-carotene equivalents per kilogram of fresh weight (mg β-CaE kg^{-1} FW). Lycopene molar extinction $\varepsilon = 17.2\ 10^4\ M^{-1} cm^{-1}$ in *n*-hexane was used for lycopene content determination and results were expressed in milligrams per kilogram of fresh weight (mg kg^{-1} FW).

Statistical analysis

The analysis of variance was carried out according to the General Linear Models (GLM) procedure developed by the Statistical Analysis Systems Institute (SAS, V6.0, Cary, NC). Means and standard errors were calculated. The LSD test was used for testing significant differences between means with a 95% confidence level.

RESULTS AND DISCUSSION

Physicochemical properties

The soluble solids content, titratable acidity and density of the studied fruit *Citrus* cultivars are shown in **Table 1**. All studied parameters varied significantly between the studied *Citrus* cultivars ($P < 0.01$). Soluble solids varied from 7.93 °Brix in the citron variety 'Marsh' to 16.63 °Brix in the Clementine variety 'Hernandina'. The two pigmented variety 'Tarocco' and 'Sakasli' had similar soluble solids content. Regarding titratable acidity, the lowest and the highest values were obtained by the pigmented cultivars 'Tarocco' and 'Moro' with 0.21 and 1.14% citric acid, respectively. Regarding fruit juice density, the lowest value was obtained by 'Maltaise Blonde' (1.00 g cm^{-3}) and the highest was recorded for the pigmented variety 'Tarocco' (1.04 g cm^{-3}).

Bioactive compounds

The total carotenoid and lycopene contents of the different *Citrus* cultivars are shown in **Table 2**. The results showed significant differences among *Citrus* cultivars in total carotenoid and lycopene ($P < 0.01$). Total carotenoid values varied from 5.33 mg kg^{-1} FW in 'Hernandina' to 23.66 mg kg^{-1} FW in 'Star Ruby'. 'Star Ruby', 'Moro' and 'Sakasli' were determined to be richest cultivars in carotenoids.

Concerning lycopene, values ranged from 0.27 mg kg^{-1} FW in 'Maltaise Blonde' to 17.93 mg kg^{-1} FW in 'Star Ruby'. 'Star Ruby', 'Tarocco' and 'Marsh' were determined to be richest cultivars in lycopene.

The obtained results confirmed that both carotenoids and lycopene were detected in *Citrus* cultivars and are in agreement with those reported by Xu *et al.* (2006) who identified the major couloured pigments and found that lycopene and β-carotene were detected in Citrus. They also found that carotenoids and lycopene contents vary among different studied *Citrus* cultivars and that 'Star Ruby' was found to be the richest content of β-carotene and lycopene attaining 93.03 µg g^{-1} DW and 283.57 µg g^{-1} DW, respectively compared to the other cultivars.

The obtained results are also in agreement with those of Goulas and Manganaris (2012) who concluded recently that the studied orange fruit particularly the cultivar 'Valencia' represents an excellent source of bioactive compounds. In fact, *Citrus* fruit extracts showed in recent studies anti-cancer, anti-inflammatory, anti-tumour and blood clot inhibition activities (Du and Chen 2010; Huang and Ho 2010).

Table 1 Some agronomic characteristics of the different studied *Citrus* cultivars. n (sample size) = 30.

Cultivars	Soluble solids (°Brix)	Titratable acidity (citric acid %)	Density (g m^{-3})
Tarocco	13.26 b	1.14 a	1.04 a
Moro	11.46 c	0..21 g	1.01 dc
Sakasli	14.26 b	1..57 f	1.03 ab
Maltaise demi sanguine	9.26 de	0..90 cde	1.00 de
Maltaise blonde	10.15 dc	0.80 e	1.00 e
Minneola	10.22 dc	0..59 f	1.02 bc
Fortune	10.36 dc	0.82 de	1.01 dc
Hernandina	16.63 a	0..98 bc	1.02 bc
Marsh	7.93 e	1.03 b	1.00 de
Star Ruby	10.8 dc	0..92 bcd	1.00 de
Significance	**	**	**

Significance: ** Probability level of 1%. Values in the same column followed by the same letters do not differ significantly (LSD test, $P < 0.05$).

Table 2 Lycopene and total carotenoids contents of the different studied *Citrus* cultivars. n (sample size) = 30.

Cultivars	Lycopene (mg kg^{-1} FW)	Total carotenoids (mg kg^{-1} FW)
Tarocco	10.40 b	11.55 de
Moro	0.84 e	20.86 b
Sakasli	0.94 e	21.33 b
Maltaise demi sanguine	0.46 e	18.60 c
Maltaise blonde	0.27 e	13.40 d
Minneola	4.10 d	8..33 f
Fortune	7.03 c	11.00 e
Hernandina	3.19 d	5.33 g
Marsh	10.97 b	17.56 c
Star Ruby	17.93 a	23.66 a
Significance	**	**

Significance: ** Probability level of 1%. Values in the same column followed by the same letters do not differ significantly (LSD test, $P < 0.05$).

CONCLUSIONS

This study confirmed the presence of carotenoids and lycopene in different *Citrus* cultivars grown and consumed in Tunisia. The pomelo 'Star Ruby' had the highest content of these antioxidant compounds. The pigmented cultivars 'Moro', 'Tarocco' and 'Sakasli' have higher total carotenoid and lycopene contents. The variability detected among the *Citrus* cultivars emphasized the need to evaluate more *Citrus* genotypes to improve their nutritional value.

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