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Evaluating the hydrophilic antioxidant capacity in different citrus genotypes

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Abstract

Antioxidants are the body's defense system against the damage caused by reactive oxygen species, formed naturally during many physiological activities. In vegetables and fruits, various antioxidant compounds such as vitamin C, polyphenols, flavonoids, and carotenoids have been identified. Because fruits and vegetables are the primary antioxidant sources in our daily diet, it is necessary to determine their antioxidant capacity. Citrus fruit consumption per capita has steadily increased over the world over the last 30 years. Citrus fruits are high in vitamin C as well as other active ingredients like phenols and flavonoids that are beneficial to human health. Using carotenoid complement and pigmentation genetic diversity, the objective of this research was to see how vitamin C and carotenoids contributed to the capacity of hydrophilic antioxidants of the citrus fruits' pulp. Six citrus cultivars were chosen for this purpose: two sweet orange genotypes, Valencia Ruby and Valencia Late; two grapefruit genotypes, Star Ruby and Marsh; and two mandarin genotypes, Nadorcott and Clemenules. In proportion to their color singularity, total carotenoid composition and content in fruit pulp differed dramatically. A good and clear connection was found between hydrophilic antioxidant capacity and vitamin C concentration in the pulp of various fruit species, as measured by DPPH and ABTS tests. The proportion of vitamin C to the total HAC was calculated to be between 15% and 30%.

Keywords: vitamin C; citrus fruit; carotenoids; antioxidant capacity.

Practical Application: In the current research it was aimed to see how vitamin C and carotenoids contributed to the capacity of hydrophilic antioxidants of the citrus fruits' pulp.

1 Introduction

Citrus fruit is the world's most traded horticulture product and one of the most widely cultivated horticultural commodities (Herrera-Barros et al., 2021; Rangel et al., 2011). Around two-thirds of the overall citrus, output comes from the United States, China, the Mediterranean nations, and Brazil (Lacirignola & D'Onghia, 2009). Citrus fruits are among the most valuable cultivars in world commerce, and they're in great demand for food additives in meals and drinks, freeze concentrates, juice processing, and fresh consumption all over the world (Czech et al., 2020; Xu et al., 2008). Colour is a crucial determinant in consumer acceptance and internal and external quality across the many citrus cultivars and species (Hoppu et al., 2021). The genus citrus comes in a wide range of colors both on the outside and inside (Ballistreri et al., 2019; Monselise, 2019): from the new sweet orange cultivars or pink or red lycopene-accumulating grapefruits, and orange of mandarins to the citrons, many grapefruits' cultivars, pummelos, and yellow color of lemons (Alquezar et al., 2013; Rodrigo et al., 2013). Pectin, carotenoids, anthocyanins (blood oranges), limonoids, coumarins, phenolic acids, flavonoids, mineral elements, Vitamins E, C, and A are just a few of the phytochemicals found in citrus fruits (Zou et al., 2016). Citrus fruit intake has been linked to significant health advantages as well as a lower risk of chronic illnesses, according to findings from multiple in vivo and in vitro investigations (Ahmed & Azmat, 2019; Aune, 2019; Durazzo et al., 2019; Ma et al., 2020; Wallace et al., 2020).

The antioxidant activity of such substances has been linked to their positive impacts on health (Rodrigues et al., 2011; Shahidi & Ambigaipalan, 2015; Yashin et al., 2017). Antioxidants

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are organic or artificial substances that quench or scavenge nitrogen species or reactive oxygen, such as free radicals, to postpone or avoid physiological oxidants' oxidative cell damage (Lobo et al., 2010; Sen et al., 2010). Citrus fruit contains carotenoids, which are one of the significant phytochemicals. With a wide range of carotenoids in regards to amounts and types across various cultivars and species, citrus fruits are among the highly sophisticated suppliers of carotenoids (Kato, 2012). A unique mix of hydrophilic molecules is responsible for the antioxidant activity of citrus fruit (Buljeta et al., 2021). Carotenoids, which are effective antioxidants that scavenge peroxyl radicals and singlet molecular oxygen, serve a vital function in plants in shielding biological systems from oxidative damage and photooxidative processes (Kancheva & Kasaikina, 2013; Krause, 2019). Over the years, a considerable number of researchers have investigated the citrus fruit extracts' antioxidant properties concentrating on antioxidant evaluation of particular solvents by the bioactive components of a single extraction, regardless of each component family's preferential solubility in the mixture of extraction (Durazzo, 2017; Gómez-Mejía et al., 2019; Jayaprakasha et al., 2008). The results of multiple studies reveal that overall phenolic compounds, vitamin C content, and antioxidant activity in the juice and pulp of various citrus cultivars and species are all positively correlated (Brito et al., 2014; Dong et al., 2019; Ramful et al., 2011; Elkhatim et al., 2018). Regardless of the fact that certain carotenoids have long been known to have antioxidant properties, their proportional role to total antioxidant capacity (TAC) in foods remains debated (Koszewska & Kuzak, 2021).

The main objective of this work has been to assess the hydrophilic antioxidant capacity (HAC) fractions using the ABTS and DPPH radical scavenging tests, employing the genetic variation in citrus fruits' carotenoid and coloring makeup. For such purpose, we investigated Vit C content, as well as carotenoids composition and content, in fruits from two genotypes of oranges with differing pulp coloring, two mandarins, and two grapefruits.

2 Material and methods

Two mandarin genotypes, Nadorcott and Clemenules, two grapefruit genotypes, Star Ruby and Marsh, and two sweet orange genotypes, Valencia Ruby and Valencia Late, were chosen for their distinctive pulp colors (Figure 1).

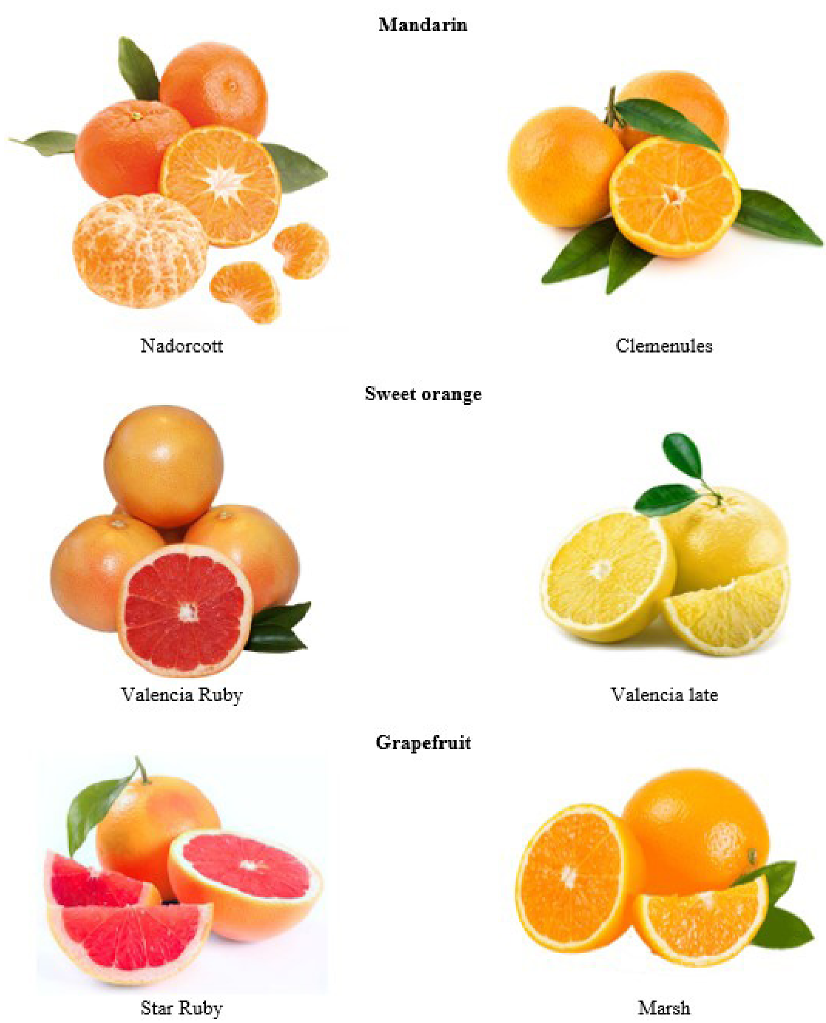

Figure 1. Image of citrus cultivars' mature fruit utilized in this research.

Fruits from mature trees grown within the typical growing and agronomic conditions were gathered for each genotype. Fruits have been chosen based on size consistency and the absence of any exterior defect or injury. After the pulp color was determined, pulp tissue was prepared by excising tiny cube portions of around 1 cm^2 comprising free of segment membranes juice vesicles. Utilizing a Braun MPZ22 Citrus Juicer, the remaining pulp was squeezed for juice and filtered through a 0.8 mm metal sieve. Liquid nitrogen was used to quickly freeze the samples, which were then kept at -80 °C, awaiting examination. A Minolta Chroma Meter CR-400 was used to measure the color of the pulp from three distinct locations.

The color of citrus fruit was determined using a well-known relation, the Hunter *a*/*b* ratio was used to express color, and the Hunter input variables *a* and *b* were calculated (Carmona et al., 2010; Venkatram et al., 2017). Color index data are also the averages of 10 fruits minimum ± SD for each cultivar. A digital refractometer was used to measure the total titratable acidity (TA) and total soluble solids (TSS) of the juices (Teerachaichayut & Ho, 2017; Włodarska et al., 2017). The TSS/TA relation was used to determine the maturity index (MI). Each sample's carotenoid composition was determined by employing high-performance liquid chromatography (HPLC) (Ahamad et al., 2007; Hodisan et al., 1997). According to Alós et al. (2021), total Vit C was evaluated following ascorbic acid extraction. The antioxidant capacity was determined using the ABTS test. TAC was measured using the method published by Curi et al. (2021), with a few tweaks, allowing for the determination of antioxidant capacity due to hydrophilic chemicals. With slight modifications, Girennavar et al. (2007) DPPH free radical test was also used to assess the hydrophilic antioxidant activity. As Trolox equivalent antioxidant capacity (TEAC), a variety of bioactive substances' relative antioxidant capacity (RAC) was determined by Müller et al. (2011). We calculated the correlation of Vit C and carotenoids contained in the samples' hydrophilic extracts to the capacity of antioxidant using these results as a benchmark. Following the assessment of the HAC, as well as the amounts of various chemicals, their contribution to the capacity of antioxidant was estimated. XLSTAT software's one-way analysis of variance was used to calculate the statistical significance, and any substantial variations across cultivars at $p < 0.05$ were determined using Tukey's test.

3 Results and discussion

Figure 2 shows the fruit quality metrics (MI, TA, TSS, and pulp color or a/b Hunter) of the six citrus genotypes used in this investigation.

The key factors for choosing these genotypes were the color of the pulp (Hunter ratio) and variations across crops of the same species. As a result, Nadorcott's orange coloring was brighter than Clemenules' mild orange pigmentation when it came to mandarin fruit. Clemenules mandarin, on the other hand, has less strong internal and exterior color compared to other mandarin cultivars, although having great fruit quality and economic value. Compared to Clementine mandarin, the TSS of Nadorcott was somewhat greater, implying a fairly late-ripening, while in both genotypes, the MI at reaping season

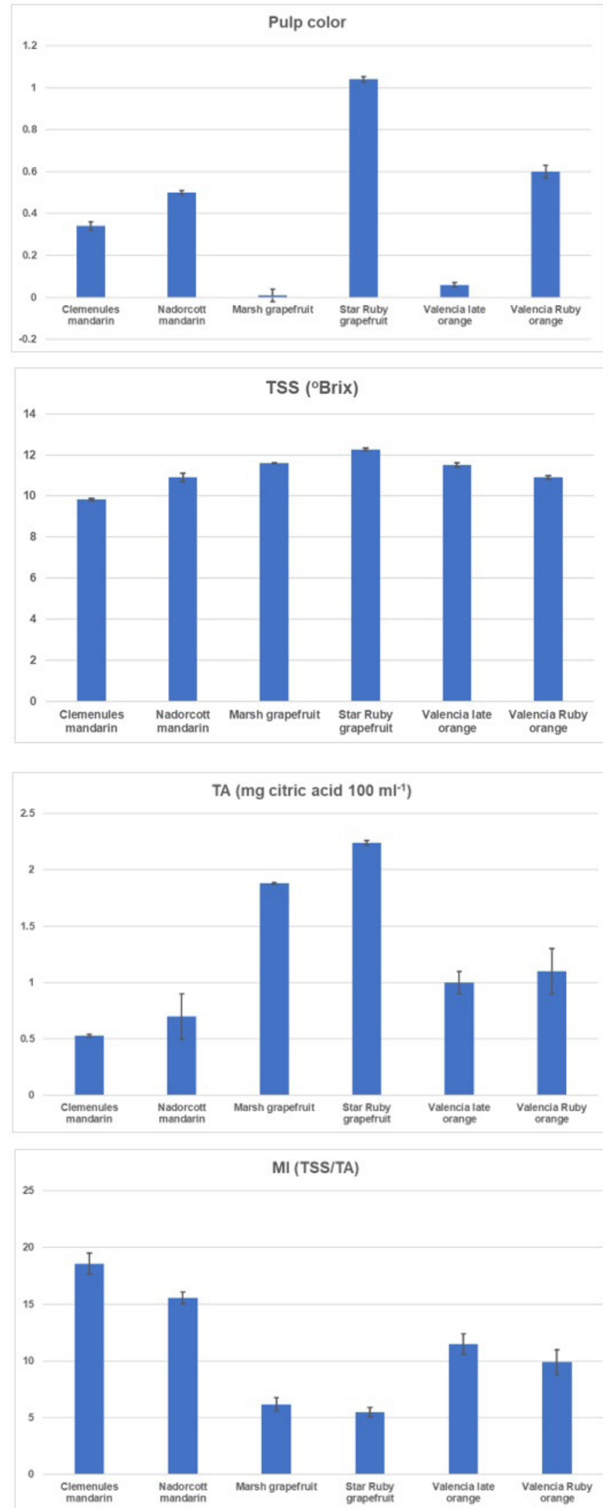

Figure 2. MI (Maturity Index), TA (Total Acidity), TSS (Total Soluble Solids), and Hunter ratio (color index) of the pulp of citrus genotypes studied (mean ± standard deviation, n = 3).

was identical. Contrary to Marsh grapefruit pulp's customary pale yellowish coloring, Star Ruby grapefruit has a distinctive red coloration. Internal MI, on the other hand, was comparable in both grapefruits (Figure 2), showing that, apart from fruit coloring, the aging process under Mediterranean circumstances tends to follow an identical pattern in both cultivars. The pale orange tint of Valencia's late orange fruit contrasts sharply with the reddish coloring of the pulp taken at the same stage of maturity and under the same climatic environments, demonstrating the phenotypic difference. Having moderate to low color and carotenoid concentration, Valencia late is a well-known orange cultivar that is grown all over the world. Internal maturation metrics in Valencia oranges were statistically equal, showing that the mutant's additional ripening mechanisms unaffected (Figure 2). The carotenoid composition and content of the six citrus genotypes fruits studied were compared to the variations in pulp coloring. Amongst the six genotypes, pulp's total carotenoid concentration differed greatly (Table 1).

Nadorcott's pulp has 3.2 times more carotenoid concentration than Clemenules'. In comparison with their corresponding counterpart, carotenoids were found to be much greater in orange and the red-fleshed grapefruit. Relative to the red Star Ruby, the carotenoids in the white Marsh grapefruit were significantly low. In the Valencia Ruby's pulp, the total carotenoids have been over 20 times greater than in Valencia's pulp (Table 1). Despite the fact that other carotenoids were practically similar in Clemenules, the Nadorcott mandarin pulp had 4 and 6 times more violaxanthin and β-cryptoxanthin, respectively. The deeper orange coloration detected in the Nadorcott pulp relative to Clemenules may be explained by these fundamental variations in these xanthophylls (Table 1). The major carotenoid in mandarin juice and pulp is β-cryptoxanthin, which gives the fruit its bright orange color. Vitamin C is probably best known for its antioxidant properties in plant cells, and it's a big part of the citrus fruit's health advantages. The content of vitamin C in the six cultivars pulp used for this investigation is shown in Figure 3.

The antioxidant properties of the citrus varieties were studied to determine the role of carotenoid concentration and Vit C to the citrus fruit's antioxidant capacity. ABTS and DPPH scavenging assays were used to estimate the HAC fraction (Figure 4).

For the six citrus cultivars investigated, the value of R^2 of 0.91 and 0.85 for the ABTS and DPPH tests, correspondingly, show a good correlation between Vit C and HAC. According to these findings,

Table 1. Composition and content of carotenoids in the pulp of the samples under study (mean ± SD).

Carotenoid (mg 100 g^{-1} FW)	Oranges		Grapefruits		Mandarins	
	Valencia late	Valencia Ruby	Marsh	Star Ruby	Clemenules	Nadorcott
Antheraxanthin	0.14 ± 0.01	0.11 ± 0.03	0.01 ± 0.01	0.02 ± 0.02	0.10 ± 0.01	0.32 ± 0.02
β-Carotene	nondetected	0.04 ± 0.01	nondetected	0.57 ± 0.02	traces	0.24 ± 0.12
β-Cryptoxanthin	0.06 ± 0.02	0.03 ± 0.01	nondetected	0.01 ± 0.01	0.31 ± 0.03	1.83 ± 0.50
ξ-Carotene	0.03 ± 0.01	0.06 ± 0.01	nondetected	0.03 ± 0.01	0.07 ± 0.01	0.26 ± 0.13
Luteoxanthin	0.06 ± 0.02	0.02 ± 0.01	nondetected	nondetected	0.02 ± 0.01	0.11 ± 0.03
Lycopene	nondetected	0.86 ± 0.02	nondetected	0.74 ± 0.08	nondetected	nondetected
Phytoene	0.1 ± 0.01	13.31 ± 0.11	0.03 ± 0.01	0.81 ± 0.02	0.26 ± 0.03	0.27 ± 0.14
Phytofluene	0.02 ± 0.01	1.84 ± 0.36	0.01 ± 0.01	0.32 ± 0.01	0.31 ± 0.27	0.28 ± 0.13
Violaxanthin	0.38 ± 0.02	0.23 ± 0.01	0.01 ± 0.01	nondetected	0.25 ± 0.01	1.00 ± 0.04
Zeaxanthin	0.07 ± 0.02	0.06 ± 0.01	nondetected	0.03 ± 0.03	0.04 ± 0.01	0.09 ± 0.02
Total carotenoids	0.86 ± 0.03	16.51 ± 0.28	0.06 ± 0.01	2.60 ± 0.15	1.44 ± 0.26	4.69 ± 1.06

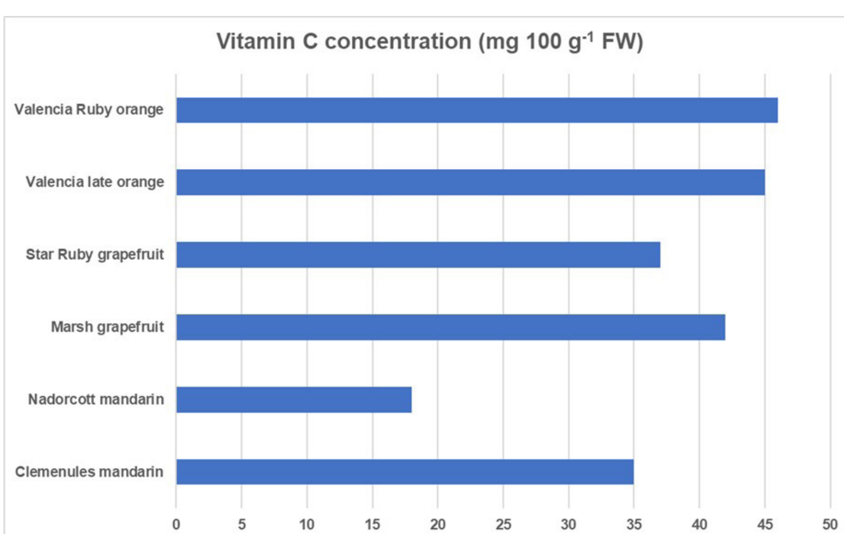

Figure 3. Vitamin C levels of the citrus cultivars' pulp under study.

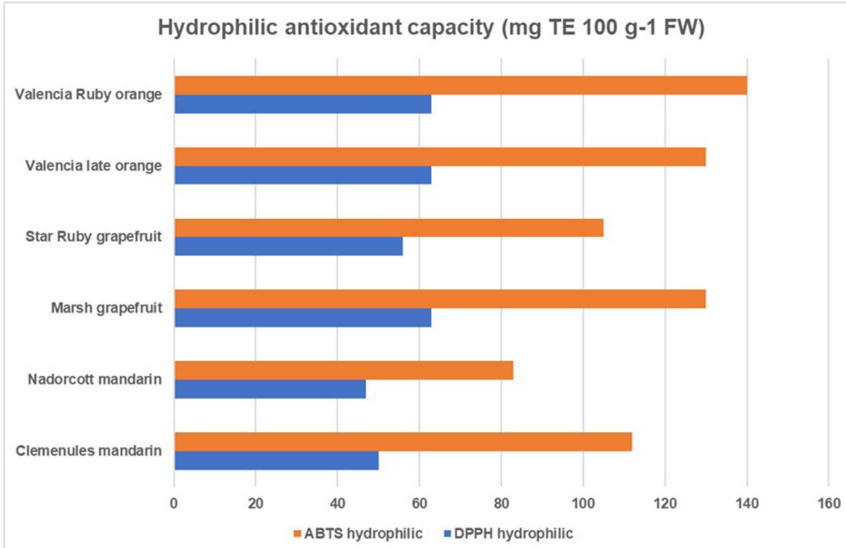

Figure 4. In the citrus cultivars' pulp, the HAC fraction was measured using ABTS and DPPH.

vitamin C content in the pulp of sweet oranges, grapefruits, and mandarins is linked to their HAC. In comparison, both ABTS and DPPH tests revealed a near-complete lack of association between carotenoid concentration and hydrophilic activity.

4 Conclusion

In vitro, antioxidant tests could be valuable markers for estimating the healthful qualities of the consumable section of fruits. Regrettably, due to the absence of standardization, the techniques' differences, and the potential of chemicals found to quench or scavenge various radicals, inconsistencies can arise, leading to an underestimation or overestimation of the particular contribution of the various hydrophilic constituents of a food sample. To assess HAC in the citrus cultivars' pulp throughout this research, we employed two antioxidant assays with different carotenoid composition, content, and coloring. The findings revealed a link between vitamin C concentration and HAC. The proportion of vitamin C to the total HAC was calculated to be between 15% and 30%.

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