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Chemical composition of blood orange varieties from Turkey: A comparative study

Kafkas Ebru[1]*, Ercisli Sezai[2], Kemal Koc Namik[1], Baydar Konce[1], Yilmaz Huseyin[3]

[1]*Subtropical Fruits Research and Application Center, Cukurova University, Balcali, Adana, 01330, Turkey*
[2]*Department of Horticulture, Ataturk University, Erzurum, 25240, Turkey*
[3]*Netkim Private Company, Adana, Turkey*

***Author for Correspondence:** ubru@cu.edu.tr; Mobile no: 90 53*

ABSTRACT

Three blood orange varieties, Moro Blood, Sanguinello and Cara Cara grown together in Mediterranean region of Turkey were characterized for their total lipid, fatty acids, sugars, organic acids, vitamin C, total phenols, total flavonoid contents and aroma compounds. The total phenol content of blood orange varieties was in range of 441.0 to 527.0 mg/L, expressed as gallic acid equivalents (GAE). Total flavonoid concentrations ranged from 121.70 to 239.80 mg/L. The order of vitamin C, expressed as mg per 100 mL among different blood orange varieties was Sanguinello (41.59 mg)>Cara Cara (34.24 mg)>Moro Blood (31.83 mg). Moro Blood variety had the highest total lipid ratio (2.01 %), and followed by cv. Cara Cara (0.65 %) and cv. Sanguinello (0.59 %), respectively. Sixteen fatty acids were detected in blood orange varieties and C18:2 (linoleic acid) was predominant for all varieties ranged from 23.15 to 31.83 %. A total of 46 aroma compounds were identified in juices and among varieties, cv. Cara Cara had the highest number of aroma compounds. The study revealed that dietary intake oranges may supply substantial health components.

KEYWORDS: aroma, citrus, blood oranges, total flavonoid, total phenols, vitamin C, fatty acids

INTRODUCTION

Horticultural plants particularly fruits are beneficial to human health and contribute to the prevention of degenerative processes (1). In the past few years consumer acceptance in the nutraceutical aspects of fruits has increased significantly. There is also an increase in the acceptance of formulated foods containing compounds extracted from fruits (2).

Fruits contain several important phytonutrients such as flavonoids, vitamins, caretonoids, fatty acids, phenolic acids etc and these compounds have been reported to have strong antioxidant capacities (3). Epidemiological studies have revealed that dietary intake of flavonoids and phenolic acids are inversely related to subsequent coronary heart disease (4). Flavonoids are also reported to act as antiulcer, antispasmodic, antisecretory, or antidiarrhoeal agents in the gastrointestinal tract (5), possess antihepatotoxic properties (6).

Oranges *(Citrus sinensis* L.) constitute by far the most important class of commercial citrus grown in the world. A large portion of the world orange crop is used in the production of orange juice, which is probably the most appreciated beverage worldwide with pleasant flavor and color, and rich in nutritional and biological active compounds, such as potassium, vitamin C, and amino acids (7). Additionally, organic acids and sugars are among the major compounds of citrus fruit. Their nature and concentration largely affect the taste characteristics and organoleptic quality (8). Citrus fruit flavor and aroma

are composing of complex combinations of soluble compounds (malic acids, sugars and flavonoids) and volatile compounds. The latter consists mostly of mono and sesquiterpenes (the major components of citrus essential oils). Oranges can be divided into 3 groups including sweet, blood and bitter oranges (9). Among them, blood oranges are characterized by their unique flesh and rind colour due to red pigments belonging to the anthocyanin class (10) and a considerable body of evidence indicates that the anthocyanins present in blood orange can play a key role in the prevention of human pathologies related to oxidative damage of biomolecules, including heart diseases and cancer (11). Furthermore, blood oranges had also been reported higher antioxidant capacity because of their higher biological active substances (12).

Turkey is an important citrus producing country and orange is the third largest fruit crop after grape and apple grown in Turkey with an annual production of around 1,300,000 tons (13). The majority of orange orchards in the country are located in the East Mediterranean region. The main blood orange varieties in this region are Moro, Cara Cara and Sanguinello. These varieties differ from the other orange groups by the presence in the flesh and sometimes in the rind of red pigments belonging to anthocyanin classes (10). The interest in pigmented orange among consumers is due to different factors, including better taste and higher biological properties. Saija et al (14), reported the inclusion of Moro on blood vessel walls and gastric mucosa. There has been a study only on phenolic composition and antioxidant activity of blood orange varieties grown in Turkey (12). To our knowledge, this is the first detailed quantitative study of the distribution of fruit quality characteristics. Chemical composition in fruits varies greatly due to their growing conditions, different varieties, cultural practices, etc. Therefore this study aimed to investigate the variation among three blood orange varieties in terms of total phenolics, vitamin C, sugars, organic acids, fatty acids, total lipid, aroma, total phenol and flavonoid.

MATERIALS AND METHODS

Plant materials

The citrus varietieswere grown in the experimental field of the Subtropical Fruits Research and Application Center at University of Çukurova, Adana-Turkey. In 2006–2007 growing period, the fruits were harvested at the commercial ripe stage from 12 years old trees. Totally 75 fruits were used for each varieties with three replicate. Freshly hand-squeezed orange juices of samples of experimental were used for chemical analysis.

Aroma extraction

The juice was obtained from approximately randomly selected twenty five fruits for each replicate and immediately mixed with 5 g NaCl for 50 mL of fruit juice. The liquid-liquid extraction technique was used. 150 mL of dichlorometane were used as an organic solvent and shaked for 1 min using separated glass funnel. The organic phase was dried on anhydrous Na_2SO_4 and evaporated under nitrogen to 1 ml.

GC-MS Analysis

Volatilecompoundswereanalyzedonan HP-GCDapparatus equipped with an HP-5 MS (30 m × 0.25 mm × 0.25 µm) fused-silica capillary column. Helium (1ml/min) was used as a carrier gas. The injector temperature was 250 °C, set for split less injection. The oven conditions were set to 50 ^{0}C for 1 min and then the temperature was increaseed to 200 ^{0}C at a rate of 4 ^{0}C/min. Thermal desorption was allowed for 1.5 min. The detector temperature was 280 ^{0}C. The components were identified by comparison of mass spectra and retention time data with those of authentic samples and complemented with a Wiley and NIST GC-MS library (15).

Extraction of sugars and organic acids

Approximately 500 mL of fruit juice was used with each replicate was used separately, then from this homogenized material 1 ml sample with 10 ml of aqueous ethanol (80 %, v/v) was placed in to a screw cap Eppendorf tube. Reaction mixture was placed in an ultrasonic bath and sonicated for 15 min at 80 °C then filtered through the regular filter paper and the extraction procedure was repeated 3 more times. All filtered extracts were combined and evaporated to dryness on boiling water bath. The residue was dissolved with 2 ml of distilled water and filtered (Whatman nylon syringe filters, 0.45 um, 13 mm, diam.) before HPLC analysis (16). Organic acids from the same homogenate (1 ml of frozen sample) was weighed and powdered with liquid nitrogen in a mortar and mixed with 20 mL of aqueous *meta*-phosphoric acid (3 %) at room temperature for 30 min using a shaker. This mixture was filtered and made up to 25 mL with the same solvent, then used for HPLC analysis (17).

HPLC of organic acids and sugars

The liquid chromatographic apparatus (Shimadzu LC 10A*vp*) consisted of an in-line degasser, pump and controller coupled to a photo diode array detector (Shimadzu SPD 10A*vp*) equipped with an automatic injector (20 mL injection volume) interfaced to a PC

running Class VP chromatography manager software (Shimadzu, Japan). Separations were performed on a 250 mm × 4.6mm *i.d.*, 5um, reverse-phase Ultrasphere ODS analytical column (Beckman) operating at room temperature with a flow rate of 1 ml/min. Detection was carried out with a sensitivity of 0.1 a.u.f.s. between 200–360 nm wavelengths. Elution was isocratic with 0.5 % aqueous *meta*-phosphoric acid. Components were identified by comparison of their retention times to those of authentic standards under analysis conditions and UV spectra with our in-house PDA-library. A 10 min equilibrium time was allowed between injections.

The same apparatus (Shimadzu LC-10A*vp*) was used for separation of sugar with and separations were performed on a 150 mm × 4.6mm *i.d.*, 5um, reverse-phase Nucleosil NH2 analytical column (Shimadzu, Japan) at room temperature with a flow rate of 1 mL/min. Elution was isocratic with 75 % aqueous acetonitrile. Components were identified by comparison of their retention times with those of authentic standards under analysis conditions. A 10 min equilibrium time was allowed between injections.

Quantitative Analyses

All the samples were directly injected to the reverse phase chromatography column. For the stock solution of the organic acid standards, L-ascorbic acid, malic acid, citric acid, were dissolved in methanol at a concentration of 1 mg/ml and the sugar standards, glucose, fructose, sucrose, were dissolved in water at a concentration of 30 mg/mL. All the samples and standards were injected three times each and mean values were used.

FAME analyses

Lipid extraction was carried out according to Bligh and Dyer (18). Boron trifluoride/methanol was used for preparation of fatty acid methyl esters.

Gas chromatographic condition

The fatty acid composition was analyzed by GC Clarus 500 with auto sampler (Perkin Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m × 0.32 mm, ID × 0.25 µm, BP20 0.25 UM, USA). The oven temperature was 140 °C, held 5 min, raised to 200 °C at a rate of 4 °C/min and to 220 °C at a rate of 1 °C/min, while the injector and the detector temperature were set at 220 °C and 280 °C, respectively. The sample size was 1 µL and the carrier gas was controlled at 16 ps. The split used was 1:100. Fatty acids were identified by comparing the retention times of FAME with a standard 37 component FAME mixture (Supelco). Triplicate GC analyses were performed and the results were expressed in GC area % as a mean value and ± standard deviation.

Total phenol analysis

Total phenols were estimated as gallic acid equivalents (GAE), expressed as mg gallic acid/L. To *ca.* 6.0 mL H_2O, 100 µL sample was transferred to a 10.0 mL volumetric flask, to which was subsequently added 500 µL undiluted Folin-Ciocalteu reagent. After 1 min, 1.5 mL 20 % (*w*/*v*) Na_2CO_3 was added and the volume was made up to 10.0 mL with H_2O. After 2 h incubation at 25 °C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. The data are presented as the average of triplicate analyses.

Total flavonoids

Total flavonoids were determined according to Miliauskas et al. (19). One ml of fruit extract in methanol (10 g/L) was mixed with 1 mL aluminium trichloride in ethanol (20 g/L) and diluted with ethanol to 25 mL. The absorption at 415 nm was read after 40 min at 20 °C. Blank samples were prepared from 1 ml plant extract and 1 drop acetic acid, and diluted to 25 mL. The rutin calibration curve was prepared in ethanolic solutions with same procedure. All determinations were carried out in quadruplicate and the mean values were used.

Statistical analyses

Statistical analyses were carried out by SAS (20).

RESULTS AND DISCUSSION

Sugars, organic acids, total sugars and vitamin C

Table 1 shows the content of sugars, organic acids, total sugars and vitamin C in three blood orange varieties. Sucrose, fructose, and glucose were identified as the major sugars in blood orange varieties (Table 1). Sucrose (28.36–38.58 g/L) was always higher than fructose (25.80–31.61 g/L) and glucose (24.13–28.12 g/L) levels. The proportion ofsucrose, glucose, and fructose in fresh Florida orange juice is about 2:1:1. Sucrose was the predominant sugar in Cara Cara and Moro Blood varieties (38.58–29.67 g/L), while fructose was predominant in cv. Sanguinello (31.61 g/L). The statistically important differences ($p < 0.01$) were observed among varieties in terms of sucrose, glucose, and fructose content (Table 1). The sugar profile of blood orange juice is an important component of chemical composition and provides valuable information regarding the authenticity of fruit juices. It also has an effect on the sensory properties and nutritional value of fruit products (7).

Table 1: Sugars, organic acids, vitamin C and total phenols and total flavonoid contents content of blood orange varieties

Varieties	Fructose (g/L)	Glucose (g/L)	Sucrose (g/L)	Total sugars (g/L)	Malic acid (g/L)	Citric acid (g/L)	Vitamin C (mg/100 mL)	Total phenols (mg/L)	Total flavonoids (mg/L)
Cara Cara	25.80 b	24.13 b	38.58 a	88.51^{NS}	0.71 c	4.21 b	34.24 b	441.0 b	121.70c
Moro Blood	29.50 ab	27.85 a	29.67 b	87.11	0.79 b	5.61 ab	31.83 c	527.0 a	239.80a
Sanguinello	31.61 a	28.12 a	28.36 b	88.09	0.82 a	6.36 a	41.59 a	465.6 b	196.56b

Different letters within same columns indicate the statistical differences ($p<0.01$). NS: Non significant.

No significant differences ($p < 0.01$) existed in total sugars content, which ranged from 87.11 (cv. Moro Blood) to 88.55 g/L (cv. Cara Cara). Compared with the other varieties, the total sugar content of blood oranges varied between 104–128 g/L in another previous study and the results indicating higher values than our results. The sugar profile and total sugar content contents of oranges are influenced by genotype, harvest time and environment (7). Evidence for strong genetic control of total sugar content is encouraging in establishing blood orange breeding goals for increased sweetness or sugar production. Although sugars are not alone in accounting for variation in the sweetness of blood oranges, higher sugar levels and increased sweetness are desirable factors for improving blood orange quality (21).

Vitamin C content of blood orange varieties varied between 31.83 and 41.59 mg/100 mL, being lowest in cv. Moro Blood and highest in cv. Sanguinello varieties (Table 1). Vitamin C is highly bioavailability and is therefore the most important water-soluble antioxidant in cells and efficient scavenger of reactive oxygen species. Kelebek et al., (12) reported 50–53 mg/100 mL ascorbic acid in blond orange varieties indicating slightly higher than our present result. The slightly higher values in the literature may due to the genotype, environmental conditions or analytical methods used. It was previously reported that the ascorbic acid content of oranges were between 25–59 mg/L by Niu et al., (7) which supports our findings. Ascorbic acid or vitamin C is hypothesized to prevent cancer by inhibiting the formation of N-nitroso compounds in the stomach and by stimulation of the immune system (22). Orange juice with high ascorbic acid content would be appreciated by consumers.

The organic acid profile of three blood orange varieties is presented in Table 1. The varieties had higher citric acid content (4.21–6.36 g/L) than malic acid (0.71–0.82 g/L). The differences among varieties for citric and malic acid were found significant ($p<0.01$). The organic acid composition of oranges is of interest because of its important influence on the sensory properties of fruits and fruit juices. Even though they are minor components of fruits, in combination with sugars, they are important attributes of the sensory quality of raw and processed fruits (23). Citrus fruits are also classified as acid fruits, since their soluble solids are composed mainly of organic acids and sugars which are used as the main index of maturity (24). The organic acid composition and content vary depending on the origin, climate, variety, and degree of maturity (25).

Total phenols and total flavonoids

The quantitative values for the content of total phenols and total flavonoids in blood orange varieties are listed in Table 1. The total phenolic contents of various blood orange varieties were in range from 441.0 to 527.0 mg GAE/L with and average of 477.5 mg GAE/L. There were significant difference at a level of $p<0.01$ in average content of total phenolics among blood orange varieties. Moro Blood was found to have the highest total phenolics among the 3 blood orange varieties, whereas Cara Cara had the lowest. Moro orange is the richest in anthocyanin variety of the blood orange group as previously reported. In Moro orange juices, only two anthocyanins were predominant cyaniding-3-glucoside and cyanidin3-(6"-malonyl glucoside). Total phenolic content of blood orange varieties were reported a wide range, 43–291 mg/L by Kelebek et al., (12). These findings are in accordance with previous studies on blood orange juices made on Moro and Senguinello varieties (10). The various levels of total phenolics may possibly result from varieties, geographic origin, growing seasons, other agricultural practices, and differences in analytical methods.

The range of total flavonoids in blood oranges varied between 121.90 to 239.80 mg/L. The total flavonoid content of cv. Moro Blood was ~2 times higher than that of cv. Cara Cara (Table 1). Previously total flavonoid content of sweet oranges was 20 mg/L which lower than blood oranges (25). Like total phenolics in blood oranges tested, the total flavonoids among the blood orange varieties resulted in significant difference at a significant level of $p<0.01$.

Total lipid and fatty acid content

Table 2 presents total lipid and fatty acid composition of three blood orange varieties. The highest lipid content

(2.01 %) was detected in cultivar Moro Blood, and followed by cv. Cara Cara (0.65 %) and cv. Sanguinello (0.59 %), respectively. Previously it was showed that total lipid content of citrus juices is negligible at a percentage of about 0.20 % (9).

Sixteen fatty acids were detected in blood orange varieties and among them four fatty acids such as linoleic acid, palmitic acid, oleic acid and linolenic acid were predominant. This result is in agreement with Moufida and Marzouk (9) who revealed abundance of 4 fatty acids (linoleic acid, palmitic acid, oleic acid and linolenic acid) in citrus juices.

C18:2 (linoleic acid) was predominant for all varieties ranged from 23.15 to 31.83 %. This fatty acids followed by palmitic acid (C16:0) ranged from 15.27–19.09 %, oleic acid (18:1) between 15.92–18.77 % and linolenic acid (18:3) ranged from 13.26 to 16.40 %, respectively (Table 2). The other important fatty acids detected in blood orange varieties were stearic acid (18:0; 0.93–4.15 %) and palmitoleic acid (16:1; 2.77–2.95 %), respectively. Undecanoic acid (C10:0), lauric acid (12:0), myristic acid (14:0), pentadecanoic acid (15:0) and eicosatrienoic acid (20:3) were detected in all blood orange varieties with a minor amount. Fatty acid components representing about 82.38 % (cv. Sanguinello)-91.12 % (cv. Moro Blood) of total lipid were characterized (Table 3). PUFAs (38.43–45.37 %) was the highest in all blood orange varieties, and followed by SFAs with a range 21.46–23.72 % and MUFAs (19.60–22.49 %), respectively. Linoleic acid (C18:2), oleic acid (C18:1) and palmitic acid (C16:0) were the most represented fatty acids in PUFAs, MUFAs and SFAs, respectively.

PUFAs are known to be improving the nutritional value and protect from diseases (26). Alpha-linoleic acid is an essential fatty acid that cannot be synthesized in the human body and must be obtained through the diet. The

Table 2: Total lipid (%) and relative fatty acid composition (%) of citrus varieties

	Varieties		
	Cara Cara	**Sanguinello**	**Moro Blood**
Lipid (%)	0,65±0,20	0,59±0,14	2,01±0,08
Fatty acids	Relative fatty acid content (%)		
C10:0	n.d.	3,80±0,22	2,01±0,71
C12:0	0,24±0,07	0,32±0,00	0,26±0,01
C14:0	0,50±0,11	0,63±0,13	0,59±0,10
C15:0	0,18±0,00	0,14±0,00	0,16±0,01
C16:0	18,65±0,78	15,27±3,81	19,09±0,31
C17:0	n.d.	0,13±0,02	0,25±0,02
C18:0	4,15±0,12	1,17±0,79	0,93±0,13
C20:0	n.d.	n.d.	0,19±0,03
∑ SFA	23,72	21,46	23,48
C16:1	2,77±0,02	2,95±0,02	2,77±0,19
C17:1	0,91±0,11	1,15±0,54	0,73±0,01
C18:1	15,92±1,11	18,39±0,69	18,77±0,48
∑ MUFA	19,60	22,49	22,27
C18:2 n6	25,92±0,64	23,15±6,26	31,83±1,35
C18:3 n3	16,40±0,52	14,93±7,18	13,26±0,81
C20:2 cis	n.d.	0,10±0,00	0,05±0,04
C20:3n6	0,36±0,17	0,25±0,00	0,17±0,24
C20:4 n6	0,15±0,01	n.d.	0,06±0,05
∑ PUFA	42,83	38,43	45,37
∑	86,15	82,38	91,12

Table 3: Identified aroma compounds of three blood citrus varieties

Alcohols (aliphatic and monoterpene)	**Cara Cara**	**Moro Blood**	**Sanguinello**
Ethanol	+	+	+
Butanol	+	n.d.	n.d.
2-Hexanol	+	+	+
3-Hexen-1-ol	+	n.d.	+
3-dodecanol	+	n.d.	+
Linalool	+	+	+
Nerol	+	+	+
Phenol	+	+	+
l-stigmestrol	+	+	+
Monoterpene hydrocarbons			
Alpha-farnesene	+	n.d.	n.d.
Alpha-pinene	+	+	+
Beta-caryophyllene	n.d.	+	+
Beta-Ocimene	+	+	n.d.
Terpinolene	+	n.d.	+
Sabinene	+	+	+
Myrcene	+	+	n.d.
Limonene	+	+	+
Carboxylic acids			
Benzoic acid	+	n.d.	+
Benzeneacetic acid	+	n.d.	+
Butanoic acid	n.d.	+	+
Hexanedioic acid	+	+	+
Tetradecanoic acid	+	n.d.	+
1,2-benzenedicarboxylic acid	+	n.d.	n.d.
Mandelic acid	n.d.	n.d.	+
9-octadecenoic acid	+	+	+
n-Hexadecanoic acid	+	n.d.	+
Aldehydes and ketones	+	+	+
Citronellal	+	+	+
Decanal	+	+	+
Hexanal	+	+	+
Trans-2-hexanal	+	+	+
Heptanal	n.d.	+	+
Octanal	+	+	+
Citral	+	+	+
z-citral	+	+	+
2-pentanone	+	+	+
Sesquiterpene hydrocarbons			
Beta-sitosterol	+	n.d.	+
Dihydro-beta-ionone	+	n.d.	+
Gamma-terpinene	+	+	+
Gamma-sitosterol	+	+	+
3-octadecene	+	n.d.	+
6-octenal	+	n.d.	n.d.
Valencene	+	+	+
Esters			
Linalyl butyrate	n.d.	+	+
10-undecen-1-ol acatate	+	n.d.	n.d.
1,2-benzenedicarboxylic acid 2-methylpropyl ester	+	+	+

n.d: not detected.

U.K. Department of Health recommends an ideal ratio of n6/n3 of 4.0 at maximum (27). Values higher than the maximum value are harmful to health and may promote cardiovascular diseases (28). In this study, the ratio of n6/n3 was found to be ranging from 1.55 (cv. Sanguinello) to 2.40 (cv. Moro Blood) (Table 2).

Aroma compounds

The character of citrus aroma is also very important in commercial flavors and fragrances. Citrus essential oils are widely used in various products, including foods, beverages and cosmetics. Three different profiles were distinguished on the basis of aroma compounds: Sanguinello, Cara Cara and Moro Blood. The aroma content of blood orange varieties is shown in Table 3. A total of 41 aroma compounds were determined in cv. Cara Cara juice, 39 aroma compounds in Sanguinello juice and 30 aroma compounds in cv. Moro juice.

Brat et al., (28) noted that the alcohols (aliphatic and monoterpene), monoterpene hydrocarbons, carboxylic acids, aldehydes and ketones, sesquiterpene hydrocarbons and esters were main group in orange juices. Among these 6 main groups, monoterpene hydrocarbons are predominant. Approximately 70–75 % of aroma compounds of three varieties were limonene (data not shown). The following monoterpene hydrocarbons were found in all the aromas analyzed: Alpha-pinene and sabinene (Table 3). Brat et al., (28) showed that monoterpene hydrocarbons constitute the main volatile group, representing 80–90 % of total volatiles in orange juices. In an early report, limonene is found the most abundant component of orange fruit juice and its contents vary from 63 % in the blood orange aroma to about 90% in bitter orange aroma (9). Tonder et al., (29) also showed that limonene is the most abundant compound in aroma of orange juice (88 %). Valencene as sesquiterpene hydrocarbons was also found in all blood orange varieties nearly 2–4 %.

Oranges can be divided three groups in terms of aroma quantity. Sweet oranges had the highest aroma quantity, blood oranges had moderate aroma quantity and bitter oranges characterized by the lowest aroma quantities (9).

Nevertheless, the same compound was not recorded for all blood orange varieties. For example as alcohols, 2-hexanol was detected only in cv. Cara Cara. The Beta-ocimene was absent in sweet orange and cv. Sanguinello. In the same way, myrcene was also missing in cv. Senguinello. However, mandelic acid was only identified in cv. Senguinello. In general all varieties had aldehydes and ketones with one exception heptanal were missing in cv. Cara Cara. cv. Moro is also missing most of the sesquiterpene hydrocarbons (Table 3) .

CONCLUSION

This investigation clearly shows the potential value of blood orange varieties. Among them the fruits of Moro Blood variety reflect significant source of fat and fatty acids, phenolic and flavonoid compounds compare to the other varieties, severely. The big differences among genotypes in terms of chemical compounds in different fruit species have been well known (30, 31, 32). Malic acid, citric acid and Vitamin C content of Sanguinello was found to be the higher than the others while the fruits of Cara Cara variety was detected the sweetest and the more aromatic. Therefore, these characteristics can be considered for further studies. The blood varieties can potentially be used in food and nutraceutical supplement formulations as well. Meanwhile, the present study demonstrated that the blood orange varieties possess improved quality and sensory attributes and therefore they can be used in order to take advantages of its nutraceutical compenents.

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