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Assessment of the nutritional potential and content of specialized metabolites of citrus fruits

Procjena nutritivnog potencijala i sadržaj specijaliziranih metabolita nekih vrsta agruma

Sandra VOĆA[1], Jana ŠIC ŽLABUR[1] (✉), Tomislav JURKOVIĆ[1], Jasmina DRUŽIĆ[2], Martina SKENDROVIĆ BABOJELIĆ[1], Marin ROJE[2]

[1] University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia

[2] Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb

✉ Corresponding author: jszlabur@agr.hr

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ABSTRACT

The aim of this study was to assess the nutritional composition of fruits of some citrus species (sweet orange, grapefruit, clementine and kumquat) and to determine their basic physicochemical parameters, bioactive compounds content, and antioxidant capacity. According to the analyzed data, kumquat fruits had the highest dry matter content (18.71%) and total acid content (3.08%), while the highest values of total soluble solids (13.41%), pH value (3.92), vitamin C (53.69 mg/100 g FW), total phenols (162 mg GAE/100 g FW), β-carotene (1053.17 μg/100 g FW), and antioxidant capacity (1.70 mmol TE/kg) were determined in clementine fruits. Considering the nutritional composition of citrus fruits, it can be concluded that they are a rich source of various bioactive compounds, especially vitamin C, carotenoids, and polyphenols, and are characterized by their high antioxidant capacity, which is important for health. Moreover, the cultivation of citrus fruits is an important factor in the Croatian economy, which should be promoted in the future, and the potential of the southern part of Croatia for the cultivation of these citrus fruits should be exploited to the maximum.

Keywords: sweet orange, grapefruit, clementine, kumquat, bioactive compounds, antioxidant capacity

SAŽETAK

Cilj ovog istraživanja bio je valorizirati nutritivni sastav plodova određenih vrsta citrusa: slatke naranče, grejpa, klementine i kumkvata, analizirajući njihova osnovna fizikalno-kemijska svojstva, sadržaj bioaktivnih spojeva i antioksidacijski kapacitet. Prema analiziranim podacima, plodovi kumkvata imali su najviše vrijednosti ukupne suhe tvari (18,71%), ukupnih kiselina (3,08%), dok su istodobno u plodovima klementine utvrđene najviše vrijednosti topljive suhe tvari (13,41%), pH vrijednosti (3,92), vitamina C (53,69 mg/100 g FW), ukupnih fenola (162 mg GAE/100 g FW), β-karotena (1053,17 μg/100 g FW) i antioksidacijskog kapaciteta (1,70 mmol TE/L.) Temeljem analiziranih parametara nutritivnog sastava plodova različitih vrsta citrusa može se zaključiti kako su agrumi bogat izvor različitih bioaktivnih spojeva, posebice vitamina C, karotenoida i polifenola, pa ih stoga karakterizira i visok antioksidacijski kapacitet, a što je od iznimne važnosti i za zdravlje ljudi. Štoviše, uzgoj agruma važan je čimbenik u hrvatskom gospodarstvu, koji bi trebalo dodatno promovirati, dok potencijal južnog dijela Hrvatske za uzgojem ovih agruma treba maksimalno iskoristiti.

Ključne riječi: slatka naranča, grejpfrut, klementina, kumkvat, bioaktivni spojevi, antiokidacijski kapacitet

INTRODUCTION

Citrus fruits occupy an important place in the human diet, mainly due their nutritional value and contribution to human health. Citrus fruits exhibit strong antiviral, anti-inflammatory, and antioxidant properties. They are rich in vitamins, especially vitamin C which stands out as an important factor in boosting the body's immunity (Fenech et al., 2019). The recommended daily amount of vitamin C for adults is in the range of 30-60 mg, and this requirement is approximately met by consuming one orange per day. In addition to vitamin C, citrus fruits contain other vitamins such as vitamin B complex, provitamin A, biotin, folic acid, riboflavin, thiamine, and niacin (Ramful et al., 2011; Siche et al., 2016). They are also rich in bioactive compounds such as phenolics, carotenoids, and volatile compounds from essential oil (Zahoor et al., 2016). Phenolic compounds are important for the organoleptic and nutritional properties of fruits as they are involved in the formation of colour, astringency, bitterness, aroma and odour of fruits. The primary flavonoid present in sweet oranges is hesperidin, while grapefruit is dominated by naringin, which gives it bitterness. The content of phenolic compounds depends on the species, variety, and ripeness of the fruit (Moulehi et al., 2012; Sarrou et al., 2013, Skendrović Babojelić and Fruk, 2016; Zhang et al., 2018). Phenolic compounds (especially flavonoids) are considered one of the most potent antioxidants, which is why they are of great importance for human health. Indeed, antioxidants inhibit the suppression of free radicals and oxidation processes in the human cell, thus protecting the body from various chronic and degenerative diseases such as cancer, cardiovascular disease, diabetes, and Alzheimer's disease (Siche et al., 2016; Haraoui et al., 2020). Plant pigments are chemically responsible for the colour of the peel and endocarp of the fruit, and one of the most important for citrus are carotenoids, which give the fruit its characteristic yellow, orange, and red colour. Citrus fruits are a rich source of carotenoids, with β-carotene being one of the most abundant and important for human health, where it acts as a precursor of vitamin A, which is known for its beneficial effects on eye health (Katalinić, 2006; Solomun, 2008; Jabrikaouri and Marzouk, 2013; Lado et al., 2019). The highest concentration of total carotenoids in orange fruits is found in the peel, ranging from 50 to 75%, while red grapefruits are dominated by lycopene, which gives the fruit its characteristic pink to red colour (Ladaniya, 2008; Lado et al., 2015). Citrus fruits are valued for their pleasant aroma. Sugars are most abundant in citrus fruits and are considered, along with acids, to be the basic component of the flavour of the raw material. The following sugars are found in citrus fruits: glucose, fructose and sucrose, with a percentage ranging from 1 to 9%, the most important factor in the sugar content being the ripeness stage of the fruit. As for the composition of acids, they are also a characteristic of citrus fruits and they are classified as acid fruits precisely because of their significant content of organic acids (citric acid and malic acid) (Siche et al., 2016).

The overall chemical composition of the fruit, including the characteristic phytochemicals or bioactive compounds, varies considerably depending on various factors such as: species, variety, maturity, environmental factors (temperature, rainfall, light, etc.), soil quality and type, agrotechnical measures, harvesting method, as well as postharvest procedures from transport to possible processing, and it is therefore important to know the origin of the raw material to ensure the traceability of its quality (Malik et al., 2021; Morales Alfaro et al., 2021). Citrus fruit cultivation in the Republic of Croatia has a long tradition and is especially widespread in the Dubrovnik-Neretva County. Due to the favourable ecological factors (temperature, rainfall, light, etc.) as well as the quality of the soil in this part of Croatia, the above-mentioned types of fruit are grown and supplying the market. Although various research on bioactive compounds have been conducted all over the world, so far no systematic researches of this kind have been conducted in Croatia. Only a few have approached this problem and studied the chemical composition of citrus fruits, especially oranges from the Dubrovnik area (Bakarić, 1998), limiting themselves to the study of some chemical properties (dry matter, total acids, ratio of total soluble solids / acids). The aim of this study was to assess the nutritional composition of fruits of some citrus species: sweet orange, grapefruit, clementine, and kumquat, determining their basic physico-chemical parameters, bioactive compounds content and antioxidant capacity.

MATERIALS AND METHODS

Plant material

The study was conducted on fruits of sweet orange, grapefruit, clementine, and kumquat. Of sweet orange (*Citrus sinensis* (L.) Osb), the cultivar 'Washington Navel' (Fig. 1a), of clementine (*Citrus clementina* Hort. ex Tan.) the cultivar 'Sra 29' (Fig. 1b), of grapefruit (*Citrus paradisi* Macf.) the cultivar 'Star Ruby' (Fig. 1c), and of the genus *Fortunella*, the fruits of kumquat (*Citrus japonica* Thunb.) of unknown cultivar (Fig. 1d) were analyzed. Fruit samples were collected at the stage of optimal ripeness (late December) in private citrus orchards in the area of Opuzen along the Neretva River valley (location Luka 43°01'15.6" N 17°33'53.8" E and Modrič 43°01'45.4" N 17°30'11.0" E). In the orchards, classical conventional cultivation is practiced with regular use of agrotechnical and pomotechnical measures. This area is influenced by the Mediterranean climate, characterized by mild and rainy winters and hot and dry summers. The climate and other natural elements make it very suitable for the cultivation of citrus fruits. In addition, according to the data collected by Statistical Yearbook of the Republic of Croatia (Ostročki, 2018) and the IUSS WRB Working Group (2014), the basic meteorological data and the predominant soil type in the Opuzen area were determined. According to the available data, the average annual air temperature was 16.2 °C, relative humidity 60%, average annual precipitation 692.4 mm and estimated number of sunny days 145, while the dominant soil type was leptic chromic cambisols. Before analysis, all citrus fruits were peeled, the fruit pulp (mesocarp) with white membrane (albedo) was separated and homogenized (IKA Ultra Turrax T-18, Staufen, Germany) and such fruit pulp was used for the purposes of chemical analysis. Harvesting was done in the early morning during dry weather. The optimum harvest time for each citrus species analyzed was determined by monitoring the standard external characteristics of the fruit (skin colour) according to species and other objective methods to determine the optimum harvest time: determination of soluble solids content (TSS), acidity (TA) and their ratio (TSS/TA). Immediately after harvesting, the fruits were transported in cardboard boxes under normal conditions, without the use of refrigeration, as it is possible to maintain the optimum temperature for the storage of citrus fruits during transport (optimum storage conditions for citrus fruits are temperature range from 5-9 °C and 70-80% relative humidity according to Štambuk, 2006). Upon arrival to the laboratory of the Department of Agricultural Technology, Storage and Transport at the University of Zagreb Faculty of Agriculture, the fruits were inspected and those with possible damage (mechanical or visible signs of spoilage) were removed. The citrus fruits were stored in the refrigerator at an average temperature of 8 °C and relative humidity of 84% for 1 day until the scheduled chemical analysis.

Determination of physicochemical properties

Standard methods were used to determine the physicochemical properties of citrus fruits: total dry matter content (DM, %) by drying to constant mass at 105 °C according to AOAC (1995), total soluble solids content (TSS, % Brix) by digital refractometer (Mettler Toledo, Refracto 30PX, SevenMulti Company, Switzerland) (AOAC, 1995), total acidity (TA, %) by potentiometric titration according to AOAC (1995) and pH by digital pH meter (Mettler Toledo, SevenMulti, Switzerland) (AOAC, 1995).

Determination of bioactive compounds content and antioxidant capacity

Vitamin C content was determined by titration with 2,6-dichlorindophenol (DKF) according to AOAC (2002). Vitamin C was isolated from the citrus fruits by homogenizing 10 g ± 0.01 of the fresh plant material with 100 mL of 2% (v/v) oxalic acid. The prepared solution was filtered through Whatman filter paper and the filtrate was obtained in a volume of 10 mL. This was then titrated with freshly prepared 2,6-dichlorindophenol until pink

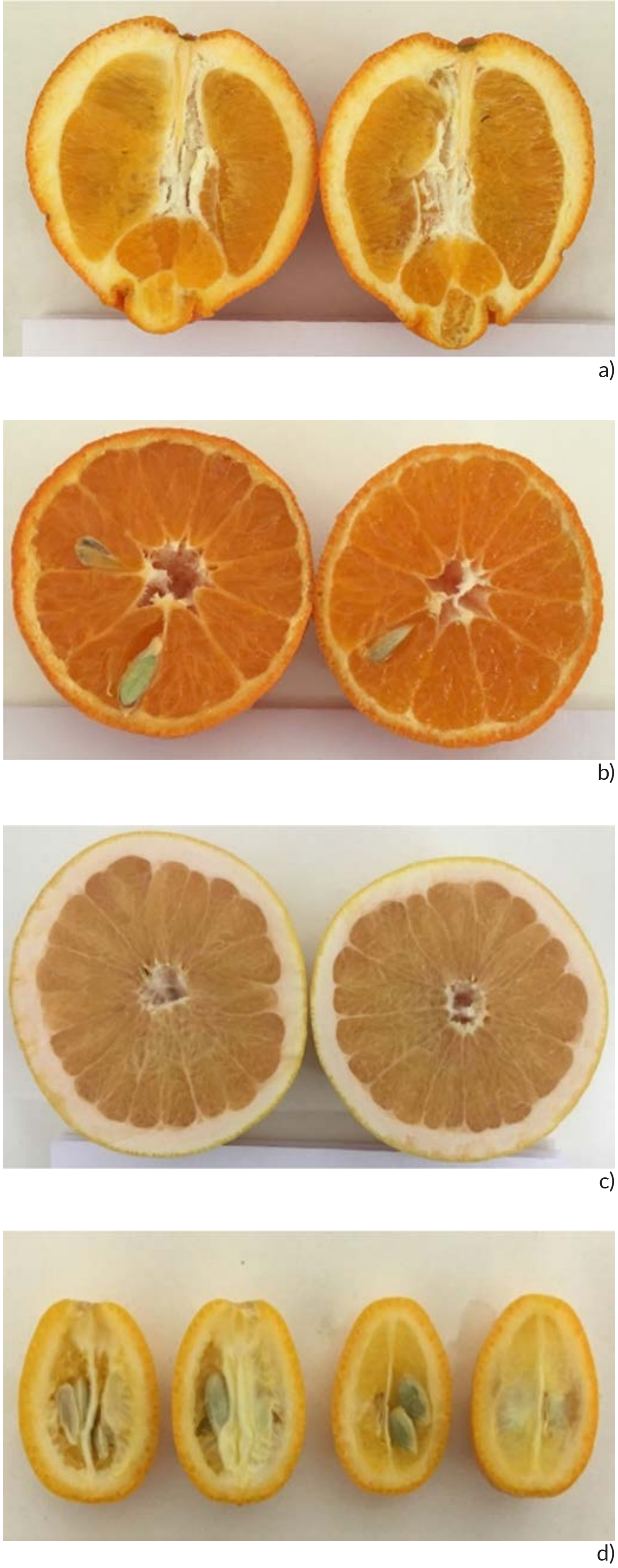

Figure 1. Different citrus species analyzed in this research: a) *Citrus sinensis* var. 'Washington Navel'; b) *Citrus clementina* var. 'Sra 29'; c) *Citrus paradisi* var. 'Star Ruby'; d) *Citrus japonica*

coloration appeared. The final vitamin C content was calculated according to equation (1) and expressed as mg/100 g fresh weight (FW)

$$Vitamin\ C = \frac{V\ (DKF) \times F}{D} \times 100$$

where V (DKF) is volume of DKF (mL), F is factor of DKF and D is sample mass used for titration.

Total phenolic content (TPC), including flavonoid content (TFC) and non-flavonoid content (TNFC), were determined spectrophotometrically (Shimadzu, UV 1650 PC) using a method based on a color reaction that phenols develop with Folin-Ciocalteu reagent, measured at 750 nm (Ough and Amerine, 1988). Extraction of polyphenolic compounds was carried out according to the following procedure: 10 g ± 0.01 of fresh citrus fruit were weighed into an Erlenmeyer flask and the first 40 mL of 80% EtOH (v/v) was added; the prepared sample was heated to boiling point and refluxed for a further 10 min. After 10 min, the sample was filtered through Whatman filter paper into a volumetric flask of 100 mL. After filtration, the rest of the sample was transferred to the Erlenmeyer flask and another 50 mL of 80% EtOH (v/v) was added and the procedure was repeated under reflux for 10 min. The sample was filtered into the same volumetric flask; the filtrates were combined and the flask was made up to the mark with 80% EtOH (v/v). The extract thus prepared was used for the reaction with freshly prepared Folin-Ciocalteu reagent. To a volumetric flask of 50 mL, 0.5 mL of the phenol ethanolic extract was added and the following chemicals were added: 30 mL of distilled water (dH_2O), 2.5 mL of the prepared Folin-Ciocalteu reagent (1:2 with dH_2O) and 7.5 mL of saturated sodium carbonate solution (Na_2CO_3); the flask was filled up to the mark with dH_2O and the prepared sample was allowed to stand at room temperature for 2 h with intermittent shaking. The same ethanolic extracts prepared for TPC determination were used for TNFC content determination, and TNFC separation was performed according to the following procedure: 10 mL of the phenol ethanolic extract was added to the 25 mL volumetric flask, then 5 mL of HCl (1:4, v/v) and 5 mL of formaldehyde were added. The prepared samples were aerated with nitrogen (N_2) and left at room temperature for 24 h in a dark place. After 24 h, the same Folin-Ciocalteu reaction was performed as for TPC. The absorbance of blue color in both TPC and TNFC reactions was measured spectrophotometrically at 750 nm using distilled water as a blank. Gallic acid and catechol were used as external standards and the concentration of TPC and TNFC content was expressed as mg GAE/ 100 g fresh weight (FW). TFC content was mathematically expressed as the difference between total phenols and non-flavonoids.

The determination of β-carotene content was carried out according to the method described by Booth (1957) with minor modifications in the isolation procedure. In a glass laboratory test tube, 0.5 g ± 0.01 of the citrus fruits were weighed and a total volume of 15 mL of petroleum ether (40-70 °C): acetone (1:1) was added three times. After each addition of solvent, the samples were homogenized using a laboratory homogenizer (IKA, UltraTurax T-18, Staufen, Germany). Distilled water was used to elute the acetone from the samples, while the samples were passed through anhydrous sodium sulphate for the purpose of water removal. Final separation of β-carotene from the isolated carotenoid layer was performed by column chromatography using a specific adsorbent. Eluted β-carotene was transferred to a volumetric flask with a volume of 10 mL, the concentration was read spectrophotometrically at 450 nm using petroleum ether (40-70 °C) as a blank, the final results were converted and expressed as μg/100 g fresh weight (FW).

The ethanolic extracts obtained from total phenol determination were used for antioxidant capacity (Ant_cap) determination. Antioxidant capacity was determined spectrophotometrically using an ABTS radical cation (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) at 734 nm according to the method described by Re et al. (1999). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, St. Louis, MO, USA) was used as an antioxidant standard. Trolox was prepared as a stock standard and appropriate dilutions for the preparation of the calibration curve were also prepared. To obtain an ABTS radical (ABTS-1), 5 mL of ABTS solution (7 mM) and 88 mL of potassium persulfate solution (140 mM) were mixed and allowed to stand for 16 h in the dark at room temperature. On the day of analysis, a 1% ABTS-1 solution (in 96% ethanol) was prepared. A total of 160 μL of the extract was injected directly into the cuvette and mixed with 2 mL of stable ABTS-1. Final results were expressed as mmol TE/L.

Statistical analysis

Thirty fruits of each species were randomly harvested from a total of 5 trees in an orchard. Laboratory analyzes of physical-chemical properties and bioactive compounds content were performed in triplicate for each sample. According to a randomized block design (with three replicates), to determine the significance of differences within the factor studied, ANOVA and Duncan's multiple range tests were performed using statistical software SAS (ver. 9.3, 2010). Means were compared using an LSD test, and p= 5% was considered the statistical significance level. In addition to the results, the tables show different letters indicating significant statistical differences between the different treatments at $P \leq 0.0001$. The average deviation of the results from the mean for each parameter studied with the standard deviation values is also shown.

RESULTS AND DISCUSSION

Physical-chemical properties of different citrus fruit species

The results of physicochemical properties of fruits of different citrus species are presented in Table 1 and include total dry matter content (DM, %), total soluble solids content (TSS, %), total acids (TA, %), and pH.

When the values of physicochemical properties were compared, significant statistical differences ($P \leq 0.0001$) were found among the studied citrus species. The highest DM content (18.71%) as well as TA (3.08%) were recorded in kumquat fruits, while the highest TSS content (13.41%) as well as pH (3.92) were determined for clementines.

In general, the DM content for the analyzed fruits of different citrus species ranged from 11.01 (grapefruit) to 18.71% (kumquat). The highest values were obtained for kumquat fruit (18.71%), followed by clementine (14.19%), orange (12.17%) and finally grapefruit (11.01%). Comparing the values by individual fruit varieties, kumquat had a 41% higher DM content compared to grapefruit, about 35% higher than orange and 24% higher than clementine. In a study conducted by Bakarić (1998) on orange fruits from plantations near Dubrovnik, the DM values ranged from 10.9 to 11.8%, which are similar results to those reported in this study. The author group Machado et al. (2011) reported values of DM content averaging 6.5% for grapefruit cv. 'Star Ruby' grown in Brazil, which is almost twice as low as the values obtained in this study for the same cultivar. The DM content in clementine fruit of cultivar 'Sra 29' was 14.19%, which is in agreement with the literature data of Norac Kljaju (2008) whose DM values averaged 13.53%, and also similar to the values of Levaj et al. (2009) who reported DM values of 13%. The results of DM content of kumquat fruits obtained in this study were in agreement with the data reported by Yildiz Turgut et al. (2015) and Oliveira et al. (2015).

The TSS content in the fruits of the orange cultivar 'Washington Navel' was 10.65% and the results obtained were significantly different from those reported by the group of authors Ramful et al. (2011). In fact, the mentioned group of authors TSS reported content of orange variety 'Washington Navel' (grown in Africa) on average 6.64%, which is significantly lower than the values obtained in this study for the same variety of sweet oranges. The analysis of grapefruit cv. 'Star Ruby' carried out in this study determined a TSS content of 10.3%. According to Ozeker et al. (2000), the University of California study (www.citrusvariety.ucr.edu) determined a TSS content of 10.1% for grapefruit of the same cultivar, suggesting that the results of this study are in agreement with the above. In clementine cv. 'Sra 29', the TSS content was 13.42%, while the author Sinclair (1972) reported values between 13 and 13.7%, which are in agreement with the results of this study. Moreover, the result of TSS content in clementine fruits from this study was compared with the results of author Norac Kljaju (2008) who reported average TSS values of 12%, which is a slightly lower value compared to the variety investigated in this study. In addition, authors Ramful et al. (2011) TSS reported content of grapefruit 7.84%, which is almost twice lower than the value obtained in this study. Since these are significantly different locations (climatically different continents), the reasons for this discrepancy are expected. The TSS content of the kumquat fruits analyzed in this study averaged 12.68%, and these results are also in agreement with the results obtained by the authors Yildiz Turgut et al. (2015).

The TA content ranged from 0.41 (clementine) to 3.08% (kumquat). For the analyzed orange cultivar 'Washington Navel', the TA content was found to be 0.46%, which is a significantly lower value compared to the results obtained by Bakarić (1998), in the range of 1.11 to 1.2%, but is a similar result compared to the results obtained in the study by Ramful et al. (2011), on average 0.74%. The TA -content of grapefruit cv. 'Star Ruby' obtained in this study was 1.92%, which is very similar to results reported by Sinclair (1972), or Ozeker (2008). Also, the results of TA content obtained in this study for clementine cultivar 'Sra 29' are slightly lower compared to those reported by Sinclair (1972), Norac Kljaju (2008) and Ramful et al. (2011). The TA content in the kumquat fruits obtained in this study (3.04%) are significantly higher compared to the results obtained by Ramful et al. (2011) from fruits harvested in Africa (1.34%), while they are slightly lower compared to the TA content (about 4%) obtained by Maarse (1991).

The pH values were similar for clementine and orange fruits (3.93; 3.70) and grapefruit and kumquat fruits (2.98; 2.72) (Table 1). The highest pH value was obtained for clementine fruits followed by oranges, grapefruits and kumquats. The obtained pH values of the studied orange fruit cv. 'Washington Navel' were similar to the other literature data (Sinclair, 1972; Rapisard et al., 2008; Siche et al., 2016). The recorded pH values of other citrus fruits analyzed (clementine, grapefruit and kumquat) in this study were also in agreement with the data obtained

Table 1. Physicochemical composition of different citrus fruit species

Species	DM (%) *P*≤0.0001	TSS (%) *P*≤0.0001	TA (%) *P*≤0.0001	pH *P*≤0.0001
Sweet orange	12.17c±0.22	10.65c±0.1	0.46c±0.02	3.70b±0.01
Clementine	14.19b±0.14	13.41a±0.03	0.41c±0.001	3.92a±0.02
Grapefruit	11.01d±0.03	10.30c±0.17	1.92b±0.03	2.89c±0.01
Kumquat	18.71a±0.44	12.68b±0.4	3.08a±0.25	2.72d±0.03

DM – dry matter content; TSS – total soluble solids; TA- total acids content. Different letters show significant statistical differences between means

by other authors with possible minor variations (Sinclair, 1972; Ozeker, 2000; Norac Kljaju, 2008; Machado et al., 2011; Yildiz Turgut et al., 2015; Oliveira et al., 2015).

Based on the obtained results of physicochemical properties of the studied fruits of different citrus species and comparison with other relevant scientific data, it can be concluded that one of the most important factors affecting the chemical composition is the pedoclimatic factors of each location and varietal or genetic characteristics. All the studied citrus species differ significantly based on the analyzed physicochemical parameters, indicating that genetic traits are one of the most important factors affecting the final content of the specific parameters of chemical composition. It is also important to emphasize that all examined fruits of different citrus species grown in the Dubrovnik area have a favourable composition and ratio of individual components of physicochemical composition, and therefore can be considered as a significant food raw material for fresh consumption and for numerous possibilities of further processing.

Bioactive compounds content and antioxidant capacity of different citrus fruit species

The content of analyzed bioactive compounds in the fruits of different citrus species is presented in Table 2. The obtained results show significant statistical differences (*P*≤0.0001) between citrus species for each of the studied bioactive compounds.

The values of vitamin C content ranged from 36.95 to 53.69 mg/100 g FW, with the highest recorded for clementine, followed by lower values in orange fruit (39.14 mg/100 g FW), grapefruit (36.95 mg/100 g FW), and kumquat (27.82 mg/100 g FW). Considering the values obtained, in clementine fruit even 37% higher vitamin C content than in orange fruits, 45% more than for grapefruit and even 93% more than kumquat was determined.

Studies by other authors on the nutritional composition of different citrus species have found different values of vitamin C content, but all are consistent with the values found in this study. For example, Ramful et al. (2011) reported values of vitamin C content in fruits of the orange cv. 'Washington Navel' ranging from 300 to 500 µg/mL, while Marti et al. (2009) reported an average vitamin C value for the same orange cultivar of 41.7 mg/100 mL. Machado et al. (2011) reported values of vitamin C in the grapefruit cv. 'Star Ruby' of 57.66 mg/100 g FW, which is somewhat higher value than those recorded in this study. Furthermore, Marti et al. (2009) reported vitamin C content in the fruits of clementine variety 'Sra 85' on average of 53.1 mg/100 mL, which coincides with those obtained for the fruits of clementine variety 'Sra 29' analyzed in this study.

In this research the lowest value of vitamin C content was determined for kumquat which deviates from the values established by other authors. For example, group of authors Yildiz Turgut et al. (2015) reported vitamin C content in kumquat fruit up to 53.75 mg/100 g FW, while Oliveira et al. (2015) reported an average value of up to 86.45 mg/100 mL, which are several times higher than that obtained in this study.

Possible deviations in the results of vitamin C content for the analyzed citrus species in this study compared to those of other authors can be explained by several reasons. Namely, vitamin C is an extremely unstable vitamin subject to the negative influence of numerous factors, especially the influence of unfavourable pedoclimatic conditions, as well as agronomic measures such as unbalanced fertilization (Wang et al. 2008). Also, numerous studies indicate a significant influence of genetic traits on vitamin C synthesis, as well as maturity stage, harvest date, and location of cultivation (Fenech et al., 2019).

Total phenolic content (TPC) of fruits of different citrus species ranged from 135.32 to 162 mg GAE/100 g FW (Table 2), while the highest content was found in clementine fruits (162 mg GAE/100 g FW), then in orange fruits (151.01 mg GAE/100 g FW), kumquat fruit (143.52 mg GAE/100 g FW), and grapefruit (135.32 mg GAE/100 g FW).

The obtained results of polyphenolic compounds content were compared with the data of other literature citations, e.g., Ramful et al. (2011) found TPC in orange fruits of cv. 'Washington Navel' ranging from 750 to 950 µg/g, then Castro-Vazquez et al. (2016) found a TPC content of 49.14 mg GAE/g DW in yellow endocarp grapefruits, and 27.95 mg GAE/g DW in red fruits. Levaj et al. (2009), in turn, determined the TPC content in the clementine fruit of 1646.88 mg/100 g FW, and Palma and D'Aquino (2018) in the kumquat fruit TPC contents ranging from 250 to 293 mg GAE/100 g.

The TFC content values obtained within this research ranged from 62.81 to 88.87 mg CTH/100 g FW, and were the highest in orange (88.87 mg CTH/100 g FW), followed by clementine (85.73 mg CTH/100 g FW), grapefruit (73.79 mg CTH/100 g FW), and kumquat (62.81 mg CTH / 100 g FW). The values of TNFC content ranged from 61.52 to 80.71 mg GAE/100 g FW, the highest were recorded in kumquat (80.71 mg GAE/100 g FW), followed by clementine (76.27 mg GAE/100 g FW), orange (62.14 mg GAE/100 g FW), and grapefruit (61.52 mg GAE/100 g FW). Comparing the obtained results with other studies, significant differences are notable. Ramfula et al. (2011) recorded in a study for the orange cv. 'Washington Navel' TFC values ranging from 400 to 600 µg/g, while for TNFC average values of 62.14 mg GAE/100 g fresh mass. Dragović-Uzelac et al. (2009) determined TNFC content of same orange cultivar of 519 mg GAE/kg. According to the study of Ramful et al. (2011) the TFC content in clementine fruit was less than 400 µg/g; while in study by Levaj et al. (2009) clementine contained 163.83 mg/100 g of TFC, which is a significant deviation from the results of this study. Also, Ramful et al. (2011) recorded TFC content in the fruits of kumquat cv. 'Nagami' of 400 µg/g.

In general, based on the results of other researchers, there are significant variations in the obtained values of TPC, TFC, and TNFC, proving that genetic characteristic and pedoclimatic conditions are one of the most important factors influencing the content of phytochemicals in citrus fruits.

From the group of carotenoids, β-carotene within this study was only determined in the fruits of clementine and kumquat (Table 2). Indeed, no β-carotene content was detected in orange and grapefruit, which is expected since β-cryptoxanthin and lycopene are carotenoids which predominate in those citrus species (Alquezar et al. 2013). Significant statistical difference ($P \leq 0.0001$) in the content of β-carotene was found between the clementine and kumquat. The β-carotene content of clementine cv. 'Sra 29' was 1053.17 µg/g. The obtained result was compared with the results of Čeh (2005), who reported the β-carotene content for the cultivar 'Sra 63' as 0.74 mg/kg DW, and with the results of Dhuique-Mayer et al. (2005), who reported the β-carotene content for clementines as 1.45 mg/mL, which is significantly different from the results of this study. The results of β-carotene content in kumquat fruit (240.42 µg/g) were compared with the data obtained by authors Schirra et al. (2008), who found a β-carotene content of 0.33 mg/kg, which is significantly lower value than that recorded in this study. Since pigments are chemically responsible for the colour of the skin and endocarp of the fruit, and one of the most

Table 2. Bioactive compounds content and antioxidant capacity of different citrus fruit

Species	VIT_C P≤0.0001	TPC P≤0.0002	TFC P≤0.0002	TNFC P≤0.0001	β- carotene P≤0.0001	Ant_cap P≤0.0001
Sweet orange	39.14b±2.34	151.01b±3.8	88.87a±5.85	62.14c±2.66	NDc	1.48b±0.36
Clementine	53.69a±0.99	162.00a±0.84	85.73a±2.51	76.27b±1.93	1053.17a±0.9	1.70a±0.12
Grapefruit	36.95b±0.95	135.32d±0.76	73.79b±0.86	61.52c±0.71	NDc	1.51b±0.16
Kumquat	27.82c±0.73	143.52c±6.55	62.81c±5.45	80.71a±1.25	240.41b±1.1	0.92c±0.18

VIT_C – vitamin C content (mg/100 g FW); TPC – total phenol content (mg GAE/100 g FW); TFC – total flavonoid content (mg CTH/100 g FW); TNFC – total non-flavonoid content (mg GAE/100 g FW); β- carotene - content of β- carotene (μg/g); Ant_cap – antioxidant capacity (mmol TE/L). ND – not determined. Different letters show significant statistical differences between means

important for citrus are carotenoid pigments that give a characteristic yellow, orange and red colour, they are also varietal traits, but also their content is highly dependent on biological and, in general, environmental factors. Therefore, the reasons for this variation can be explained by differences in terms of genetic characteristics, but also the specific location of cultivation.

The determined values of antioxidant capacity ranged from 0.92 to 1.70 mmol TE/kg, with the highest value determined in clementine (1.70 mmol TE/kg), then in grapefruit (1.51 mmol TE/kg), orange (1.48 mmol TE/kg) and the lowest in kumquat (0.92 mmol TE/kg). Based on the determined values of all analyzed bioactive compounds, the values of antioxidant capacity determined in each citrus species are expected. Namely, since the content of almost all bioactive components was the highest in clementine (vitamin C, TPC, TFC and β-carotene), the highest value of antioxidant capacity was also determined. Compounds such as polyphenols, especially flavonoids, then vitamins, especially vitamins C and E, and carotenoids show significant antioxidant properties due to their characteristic molecular structure, i.e. they effectively inhibit and reduce the effect of harmful free radicals, thus protecting human cell from oxidation (Haraoui et al., 2020).

The obtained results of antioxidant capacity were compared with literature data. Arena et al. (2001) found 5.18 mmol TE/L in the endocarp of red oranges, while in yellow colour, for example, values ranged from 2.19 to 2.97 mmol TE/L, thus establishing that genetic characteristics significantly affect the antioxidant capacity of fruits. Castro-Vazquez et al. (2016) reported data on antioxidant capacity of grapefruit cv. 'Star Ruby' amounting to 122.34 mg TE/g DW for yellow fruits and 99.46 mg TE/g DW for red fruits, also finding significant differences in relation to variety. Compared to the results of the study by Solomon (2008), the clementine fruits from this study showed significantly higher antioxidant capacity, while they showed slightly lower values compared to the results of the study by Levaj et al. (2009). The determined antioxidant capacity of kumquat fruits from this study was comparable with the results obtained by Chen et al. (2017).

CONCLUSIONS

The main objective of this study was to evaluate the nutrient composition of fruits of some citrus species and to determine their basic physicochemical parameters, bioactive compounds content, and antioxidant capacity. It can be concluded that there is a significant variation in the nutrient composition of fruits depending on citrus species variety. Kumquat fruits had the highest values of total dry matter content, total soluble solids, and total acids content, while in clementine fruits the highest values of analyzed bioactive compounds were determined, as well as the highest antioxidant capacity. Considering the obtained data, all the studied species of citrus fruits are characterized by high nutrient composition, and therefore the specific location of their cultivation (the area of Opuzen) is optimal for their growth and development by ecological conditions. Thus, the cultivation of different citrus species in the Opuzen area (Neretva valley) should be further encouraged and research on the chemical composition and content of some individual components of the nutritional composition should be continued in order to gain further knowledge about the quality of the raw materials produced in this location. Rich nutritional composition of selected citrus species studied in this research can point out this species as favorable raw material for consumption in fresh state, but also as a high-quality material for further processing.

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