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ANALYSIS OF NUTRITIONAL CONSTITUENTS IN DIFFERENT FRUIT TISSUES IN FOUR CITRUS SPECIES FROM SOUTH ALBANIA REGION

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Abstract

Statistical Office of the European Communities (Eurostat) ranks Albania sixth in Europe for fresh orange production in 2018, and the mandarin production was greater than the global and EU average yields, indicating a high-performing subsector. It is essential to examine the chemical composition of citrus fruits cultivated in Albania to fully benefit from their advantages, which was the motivation for this research. This study comprised four different groupings of citrus species (C. sinensis, C. limon, C. reticulata and C. paradisi) harvested in south Albania region. Among others, total polyphenol content (TPC), total flavonoid content (TFC), vitamin C content (VCC), and antioxidant activity were the focus of our analyses. SPSS and Sigma Plot statistical programs were used to analyse the data. The percentages of different biochemical components varied not only across species, but also between different citrus fruit tissues (Sigma Plot, ANOVA- Tukey test, $p<0.05$). Citrus paradise shows clearly a significantly high TPC compared to other citrus species. Our findings indicate the presence of a significant amount of bioactive compounds in citrus peel and pulp, making them ideal candidates for use in antioxidant-rich diets and the pharmaceutical industry.

Key words*: Citrus species, bioactive compounds, Sigma Plot, SPSS.*

INTRODUCTION

Citrus family is one of the most significant fruit families for human consumption and are among the most studies due to hight antioxidant ability associated with phytochemical and nutrients composition (Liu et al., 2022).

Albania, as part of the Mediterranean region, has a strong tradition in citrus fruits production due to its favourable climate condition, especially in south costal region. In the genus *Citrus* L. of the family Rutaceae, subfamily Aurantioideae are included *Citrus sinensis*, *Citrus paradisi*, *Citrus limon* and *Citrus reticulata*, commonly known as orange, grapefruit, lemon, and mandarin (Wu et al., 2018). According to Eurostat, Albania in 2018 is ranked seventh in Europe to produce fresh oranges with 10,800 tons (Eurostat, 2018). Furthermore. Moreover, according to FAOSTAT, Albania's yield of mandarin production (36 tons/ha) was higher when compared to the world average as well as EU average yield, demonstrating a highly performing subsector (FAOSTAT, 2018). The region of Vlora produces over 59 percent of all citrus fruits in Albania (FAOSTAT, 2018). Also, according to the Institute of Statistics in Albania the highest level of citrus production was achieved in the prefecture of Vlora with 66.05% (32,497 tons) (INSTAT, 2020). If the proper policies are implemented and this sector receives the appropriate investment, the aforementioned data can be taken into consideration as a significant indicator that Albania will probably become one of the leading countries in Europe for the production and export of citrus. In the case of Albania, most citrus fruits are utilized to produce juice, whereas the waste treatment of citrus is underestimated and not been given the proper attention. This approach is in contradiction with numerous studies which evidence the fact that citrus fruit waste offers a lot of potential for use in the food and pharmaceutical industries, as well as for adding value to processed foods and other diets (Chavan et al., 2018; Casas Cardoso et al., 2022). Furthermore, based on the literature, citrus fruits are rich in numerous bioactive compounds, such as ascorbic acid, tocopherols, carotenoids, dietary fibre, minerals, and a few other compounds, such as flavonoids, phenolic acids, and tannins (Lv et al., 2015; Rafiq et al., 2018; Saini et al., 2022).

Most of these compounds have antioxidant properties. We chose to carry on with this study because, as far as we are aware, few research has been done to ascertain the chemical composition of citrus fruits and their antioxidant capacities that grown in Albania. The first preliminary date, published in 2015 (Lloha et al., 2015), where quite encouraging because they indicate a high presence in active natural metabolites that potentially provide benefits for human health as well as a wide range of possible applications in the food industry. In the frame of human health, different studies suggest that a dietary approach rich in fruits, vegetables, and whole grains, are valuable in preventing various chronic diseases (Medina-Remón et al., 2018; Zou et al., 2016).
Therefore, the main aim of this study was to evaluate the total mineral content (TMC), total phenolic content (TPC), total anthocyanin content (TAC), total vitamin A and C as well the antioxidant activities in different tissues of citrus fruits that grow in south Albania regions.

MATERIALS AND METHODS

Fruits belonging to four species of genus *Citrus*, respectively, *Citrus sinensis*, *Citrus limon*, *Citrus reticulata* and *Citrus paradisi*, judge by colour and flavour, were harvested during autumn 2018, in Vlora and Saranda region (Figure 1).

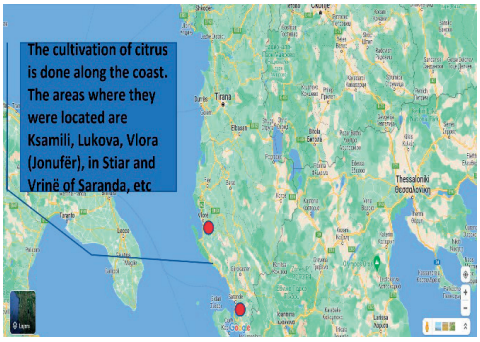

Figure 1: South Albania costal region where the citrus fruits samples were harvested (source google map).

Following collection and during transportation, samples are kept on ice and then were stored at 4^0C at the Laboratory of Food Analyses, Faculty of Biotechnology and Food. Before conducting any analysis in all the fruits was determined the physical characteristics such as dimension, weight, colour etc. About 1 kg sample of each citrus fruit, with fruits having the same dimensions, were analysed. All citrus fruits, of the four citrus species mentioned above, have been peeled and squeezed out and then placed on thermostat (WTB binder) in 80°C for 24 hours. Then the samples were analysed about the moisture content (thermostat) and the mineral content (Muffle furnace) of both fruit pulp and peel samples. After temperature treatment the peels and the dried pulps contacting the seed were grinded. An amount of 10 g of powder samples was extracted with 150 ml distilled water (Heidolph promax) within an interval 60 minutes. After extraction the samples were centrifuged 1500 rpm/min for 10 minutes and then filtered in a vacuum filtering apparatus. For the organic solvent extracts preparation, the powder samples were weighted and an amount of 10 g was extracted with petroleum benzene (50-70°C) in a soxhlet apparatus.
Total phenolic content (TPC) was calculated (Elfalleh et al., 2012) using Folin-Ciocalteu method, with some modification. Briefly, an amount of 150 µl was added with 1ml ethanol and 5 ml distilled water. After vortexes, into the solution was added 0.5 ml Folin-Ciocalteu reagent, and vortexes again for 3 minutes followed by added 1 ml sodium carbonate (Na_2CO_3 5 g/l). The solution was kept in a dark place for 60 minutes and then the absorbance was measured in a photospectrometer at 725 nm length wave. As blank was used distilled water and the results are expressed as gallic acid equivalent.
The total anthocyanin content (TAC) was determinate using the method proposed by Di Stefano et al., 1989. Briefly the samples were diluted (250 µl/5 ml, 500 µl/5 ml, 1000 µl/5ml) in a solution containing ethanol, distilled water and concentrated hydrochlorides acid (70/30/1 v/v/v). The absorbance was measured at a length wave 540 nm. The results are expressed as malvidine-3-glucoside equivalents based on the equation:
TA_{540nm} (mg/ml) = $A_{540\ nm}$ 16.7d (Di Stefano et al., 1989)
Total vitamin A determination was based on spectrophotometric methods (Rutkowski et al., 2007). Briefly 10 ml of each sample was

centrifuged, added with 10 ml KOH and vortexes for 1 minute. Then the flasks were placed on water bath at 60°C for 20 minutes and cooled. After cooling was added 10 ml xylene and vortexes again for 1 minute. Samples were centrifuged (1500 rpm for 10 minutes) and the absorbance (A_1) of the supernatant was measured at 335 nm length wave using xylene as blank. The samples were placed under UV radiation for 30 minutes and measured the absorbance (A_2) at 335 nm length wave using xylene as blank. The vitamin A concentration was determined based on the equation:

$C_x = (A_1\text{-}A_2) \times 22.23$ (Rutkowski et al., 2007)

A rapid and practical method for the total vitamin C content determination is the iodine titration method. Samples water extracts are centrifuged and filtrated and then 5 ml are added with 20 ml distilled water and 2 ml of a water solution containing 1% starch. The mixture is titrated with solution 0.01 N iodine. 1 ml mixture is equivalent of 0.88 mg ascorbic acid.

All samples were tested for the antioxidant activity using the DPPH (2,2-difenil-1-pikrilhidrazil) method (Malacrida et al., 2012) with some modification. Briefly, an amount of 50µ and 100µl of each water extract was placed in a laboratory flask and added with 3 ml DPPH methanol solution (0.04 g/l). After measuring the absorbance A_1 at 517 nm length wave the samples were placed in dark for 60 minutes, after 60 minutes was measured absorbance A_2 at 517 nm with methanol used as blank. The results are calculated using the formula:

$AA = (ABS_{control} - ABS_{sample}/ABS_{control}) \times 100$

All data are expressed as mean and standard deviation of six replicates.

All the data were analysed using statistical program Sigma Plot 12.5 and SPSS (IBM statistic 25.00). A One Way Anova and Tukey post hoc test were performed to observe if there is a significant difference between variables measured in different tissues, pulp and peal, of citrus fruits samples.

RESULTS AND DISCUSSIONS

The following results belong to four citrus species harvested in two regions of Albania, Vlora and Saranda. Total mineral content (TMC), total phenolic content (TPC), and total anthocyanin content (TAC) were determined from each sample extract of citrus fruits, harvested from the Vlora region, using the methods described above, as indicated below (Table 1). There is clearly a difference in value between peal and pulp tissues for all the parameters that are measured, which is in compliance with the literature (Barros et al., 2012). The highest value of TMC was noted in peal mandarin fruits followed by grapefruits and lemon. When compared to other citrus fruits, grapefruit and lemon have higher levels of TPC in peel and the difference is statistically significant ($p<0.01$) which is consistent with the results published from Czech in 2021 and Elkhatim in 2018 (Czech et al., 2021; Elkhatim et al., 2018). Furthermore, TAC value was higher in lemon pulp and peal followed by TAC value observed in orange.

A one-way ANOVA (Statistical programme sigma Plot 12.5) was performed to determine if there is any significance difference between the values of TMC, TPC and TAC extracted in peal and pulp of the four citrus species fruits harvested in the region of Vlora. The data show that there is a significance difference between TMC value in peal and pulp among citrus fruits ($F_{(7,\ 40)} = 7{,}414$; $p < 0.001$) as also there is a significance difference between TPC value in peal and pulp among citrus fruits ($F_{(7,\ 40)} = 4213.4$; $p < 0.001$). Furthermore, the data show that there is a significance difference between TAC value in peal and pulp among citrus fruits ($F_{(7,\ 40)} = 3.417$; $p<0.004$). A Tukey post hoc test was performed to analyse if there is any significant difference between different citrus fruits tissues, pulp, and peal (Table 1). Since both regions are characterized by a similar and very favourable climate for the growth of citrus fruits, quite the same data were extracted from the samples harvested in the region of Saranda. In comparison to the citrus fruits harvested in Vlora, TMC, TPC, and TAC are somewhat higher in peal and pulp for all citrus fruits harvested in Saranda (Table 2). Mandarin fruits harvested in Saranda has a higher level of TMC in peal compared to other citrus fruits. As for TPC value was higher in lemon pulp fruits followed by TPC observed in mandarin pulp fruits. Furthermore, TAC value was higher in

lemon pulp followed by TAC value observed in mandarin pulp fruits.

A one-way ANOVA (Statistical programme Sigma Plot 12.5) was performed to determine if there is any significance difference between the values of TMC, TPC and TAC extracted in peal and pulp of the four citrus species fruits harvested in the region of Saranda. The data show that there is a significance difference between TMC value in peal and pulp among citrus fruits ($F_{(7, 40)} = 5.463$; $p<0.001$) as also there is a significance difference between TPC value in peal and pulp among citrus fruits ($F_{(7, 40)} = 374.073$; $p<0.001$). Furthermore, the data show that there is a significance difference between TCA value in peal and pulp among citrus fruits ($F_{(7, 40)} = 232.910$; $p<0.001$). A Tukey post hoc test was performed to analyse if there is any significant difference between different citrus fruits tissues, pulp, and peal (Table 2).

Table 1. Citrus fruits index from the region of Vlora, Albania. The value of total mineral content (TMC), total phenolic content (TPC) and total anthocyanin content (TAC) in each citrus fruits are express in mean ± Std. Dev. measured as mg/ml samples for TMC, TPC, TAC and in % for the total moisture (TM)

Index	Tissues	*Citrus sinensis* Orange	*Citrus limon* Lemon	*Citrus reticulata* Mandarin	*Citrus paradisi* Grapefruit
TM	Peal	74.2700±0.75551	77.4533±0.87191	67.6517±1.07669	78.3967±0.89021
	Pulp	91.4117±0.86057	92.4000±0.85746	84.1067±0.51640	94.6800±0.69977
TMC	Peal	$0.0822±0.00615^{b}$	$0.1217±0.01169^{b}$	$0.6600±0.43973^{a}$	$0.2033±0.01033^{b}$
	Pulp	0.3217±0.29034	$0.0663±0.00403^{b}$	$0.0462±0.00360^{b}$	$0.0812±0.03508^{b}$
TPC	Peal	$1.08433±0.003011^{bcd}$	$1.38267±0.003077^{bc}$	$0.87400±0.003225^{bcde}$	$1.76267±0.002658^{b}$
	Pulp	$0.95550± 0.004637^{bcde}$	$1.88150±0.014639^{a}$	$1.08700±0.032961^{bcd}$	$0.86050±0.021230^{bcde}$
TAC	Peal	0.15200±0.002757	0.13300±0.005477	0.06283±0.005345	0.19500±0.004382
	Pulp	0.16583±0.237196	$0.23733±0.004502^{a}$	0.08750±0.004324	$0.05883±0.004622^{b}$

*All Pairwise Multiple Comparison Procedures with SigmaPlot 12.5 (Tukey post hoc Test) show a statistical significative difference ($P<0.01$) between parameters analysed in different tissues of citrus fruits.

Table 2. Citrus fruits index from the region of Saranda, Albania. The value of total mineral content (TMC), total phenolic content (TPC) and total anthocyanin content (TAC) in each citrus fruits are express in mean ± Std. Dev. measured as mg/ml samples for TMC, TPC, TAC and in % for the total moisture (TM)

Index	Tissues	*Citrus sinensis* Orange	*Citrus limon* Lemon	*Citrus reticulata* Mandarin	*Citrus paradisi* Grapefruit
TM	Peal	76.437 ± 1.312	82.953 ± 1.950	68.318 ± 1.616	82.397 ± 2.331
	Pulp	92.912 ± 1.511	93.067 ± 0.5166	84.440 ± 0.820	95.347 ± 1.273
TMC	Peal	$0.0983 ± 0.0147^{b}$	0.128 ± 0.0117	$0.515 ± 0.452^{a}$	0.217 ± 0.0186
	Pulp	$0.0628 ± 0.00560^{b}$	$0.0817 ±0. 0117^{b}$	$0.0467 ± 0.0137^{b}$	$0.118 ± 0.0204^{b}$
TPC	Peal	$1.118 ± 0.0189^{bde}$	$1.402 ± 0.0990^{bd}$	$0.973 ± 0.0116^{bde}$	$1.918 ± 0.0797^{c}$
	Pulp	$1.007 ± 0.0451^{bde}$	$1.974 ± 0.0460^{a}$	$1.198 ± 0.00662^{bde}$	$0.929 ± 0.0388^{bde}$
TAC	Peal	$0.171 ± 0.0112^{bc}$	$0.143 ± 0.0116^{bcd}$	$0.0738 ± 0.00725^{bcde}$	$0.212 ± 0.0125^{b}$
	Pulp	$0.0817 ± 0.00763^{bcde}$	$0.252 ± 0.0133^{a}$	$0.0882 ± 0.00479^{bcde}$	$0.0758 ± 0.0159^{bcde}$

*All Pairwise Multiple Comparison Procedures with SigmaPlot 12.5 (Tukey post hoc Test) show a statistical significative difference ($P<0.001$) between parameters analyzed in different tissues of citrus fruits.

The total vitamin A and C in both peal and pulp was quantified based on the method described above. The data show that the value for total vitamin A and C are higher in peal than pulp for all the citrus species and for both regions where the citrus fruits are harvested. According to our findings, the peel in general contains more polyphenols and vitamin C than other fruit sections, resulting in increased antioxidant activity. The results are comparable with the finding published for citrus fruits that grow in Cyprus, Greece (Goulas & Manganaris, 2012) with one exception, despite the high levels of TPC in the peal of grapefruits, our findings show that even the pulp of the *C. limon* and *C. reticulata* has the highest content of total polyphenols and the strongest antioxidant activity. We assume, based also on the

literature, that the presence of the presence of seed in dried pulp in samples had a significant impact in increasing the value of TPC in this tissue (Elkhatim et al 2018; Costanzo et al., 2020). The highest cumulative capacity to scavenge free radicals was measured in the peal of *C. paradisi* (87%) followed by the value of the pulp in *C. limon* (86%) and in the pulp of *C. reticulata* (75%). The lowest cumulative capacity to scavenge free radicals was measured in the peal of *C. reticulata* (62%). Statistical analyses of the data with SPSS show a clear correlation (Pearson 2-tailed) between TPC and antioxidant activities. Correlation is significant at the 0.05 level (2-tailed) between total phenolic content in peal of *C. paradisi* and antioxidant activities ($R^2 = 0.859$, $p = 0.028$) as it is a significant correlation at 0.01 level between total phenolic content in peal of *C. limon* and antioxidant activities ($R^2 = 0.929$, $p = 0.007$).

Table 3. Citrus fruits index from the region of of Vlora and Saranda, Albania. The value of total Vitamin A nd Vitamin C content, in each citrus fruits are express in mean ± Std. Dev. measured as mg/ml samples

	Fruits index	Tissues	***Citrus sinensis*** Orange	***Citrus limon*** Lemon	***Citrus reticulata*** Mandarin	***Citrus paradisi*** Grapefruit
Vlora	Total Vit. A	Peal	3.512 ± 0.134^{bc}	5.067 ± 0.134^{b}	5.500 ± 0.0754^{a}	2.460 ± 0.180^{bc}
		Pulp	0.565 ± 0.0579^{bc}	1.507 ± 0.0147^{bc}	1.628 ± 0.0155^{bc}	0.491 ± 0.0454^{bc}
	Total Vit. C	Peal	0.384 ± 0.0141^{b}	0.313 ± 0.0235^{bc}	0.341 ± 0.0102^{bc}	0.419 ± 0.0124^{a}
		Pulp	0.255 ± 0.00792^{bcd}	0.287 ± 0.00775^{bcd}	0.261 ± 0.00987^{bcd}	0.279 ± 0.00770^{bcd}
Saranda	Total Vit. A	Peal	3.930 ± 0.228^{b}	5.882 ± 0.506^{a}	5.985 ± 0.468^{a}	2.890 ± 0.157^{bc}
		Pulp	0.525 ± 0.0959^{bcd}	1.475 ± 0.0426^{bcd}	1.672 ± 0.0387^{bcd}	0.508 ± 0.0655^{bcd}
	Total Vit. C	Peal	0.398±0.0255b	0.338 ± 0.0412^{b}	0.341 ± 0.0486^{b}	0.484 ± 0.0407^{a}
		pulp	0.263 ± 0.0128^{bcd}	0.308 ± 0.0273^{bc}	0.282 ± 0.0250^{bc}	$0.283 \pm 0.0104b^{c}$

*All Pairwise Multiple Comparison Procedures with Sigma Plot 12.5 (Tukey post hoc Test) show a statistical significative difference ($P<0.001$) between parameters analysed in different tissues of citrus fruits.

To the best of our knowledge, this is the first report indicating the bioactive compound profile of four citrus fruits species that grown in south region of Albania along with antioxidant activities in different tissues of these fruits. The data included in this report indicate a high presence of TMC, TPC, TAC, vitamin C and A in both types of tissues analyzed. In general, from the two regions of southern Albania where the samples were collected, the highest values of the aforementioned parameters were identified in grapefruit samples, followed by lemon fruit samples.

Regardless of the data presented in this manuscript, further studies are warranted in the near future to have a full understanding of the bioactive compounds founds in citrus fruits that grow in south Albania region.

CONCLUSIONS

This study was carried out in four citrus fruits species that grow in south Albania region. The data showed a significant difference in the distribution of bioactive compounds between peal and pulp. When compared to other citrus fruits, grapefruit has a higher total phenolic and total anthocyanin content in its peel with excellent scavenging properties. Lemon and mandarin fruits have higher total phenolic content in their pulp. Total vitamin A and total vitamin C was significantly higher in peal in all citrus fruit's species. The value for the bioactive compounds, total mineral content and total vitamin A and C extracted from peal and pulp of citrus species that grow in south Albania region are comparable to findings from previous citrus research studies in other countries. Another result of this study is that we demonstrated the presence of the bioactive compounds in the waste products of the citrus juice producing industry. This indicates that these by-products can be used in various ways in order to exploit the presence of the nutrients in it.

The data presented in this research are very encouraging, nevertheless, the exploitation of citrus by-products in food industry is yet premature. Therefore, further research and

studies in citrus fruits that grow in Albania are needed before conducting these natural resources from waste to the food industries.

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