



Determination of Biochemical Contents of Myrtle (*Myrtus Communis* L.) Fruits at Different Maturity Levels

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Abstract

The aim of this study was to determine some physical properties and biochemical contents of myrtle fruits at three different maturity stages. For this purpose, the fruits were harvested on 3 October (pre-maturity), 1 November (beginning of maturity) and 29 November (fully mature) in 2015. In the study, L^* and b^* values, which are among the skin color values of the fruits, did not change with maturity, while the a^* value increased with maturity from -8.21 (1st period) to 4.40 (3rd period). While the total soluble solid content of the fruits increased with maturity, the titratable acid content decreased. Total phenolic content of myrtle fruit was found to be 1408 , 794 and 757.6 mg 100 g⁻¹ in the 1st, 2nd and 3rd periods, respectively. The quercetin content of myrtle decreased significantly with maturity. While the quercetin content in fruits was 10.20 µg g⁻¹ in the 1st period, it decreased to 6.33 µg g⁻¹ in the 3rd period. It was determined that the tannic acid content of myrtle fruits also decreased statistically significantly with maturity. In addition, malic and citric acids contents in fruits increased statistically significantly with maturity, from 790.5 and 86.6 µg g⁻¹ in the 1st period, respectively, to 1037.6 and 219.8 µg g⁻¹ in the 3rd period, respectively. The tartaric acid content in myrtle fruits, on the other hand, decreased statistically significantly with maturity, from 2063.3 µg g⁻¹ (1st period) to 754.1 µg g⁻¹ (3rd period). As a result, it was determined that myrtle fruits differed significantly in terms of biochemical content according to the ripening level.

Keywords Fruit quality · Fruit size · Organic acid · Phenolics · Tannin

Introduction

Myrtle (*Myrtus communis* L.) is a shrub-shaped, evergreen fruit species with medicinal and aromatic properties in the Myrtaceae family. In addition to the coastlines of Mediterranean countries, it naturally spreads in temperate regions of Austria, the Middle East and North America (Baytop 1999; Jamoussi et al. 2005; Mendes et al. 2001). The myrtle plant, which grows wild in the coastal regions of Morocco, Turkey and Tunisia, is cultivated in Spain, Italy, Iran and Corsica (Jamoussi et al. 2005). Myrtle, which has a wide ecology, can grow in Turkey at an altitude of 500–600 m above sea level, on the entire coastline, especially in the Mediterranean and Aegean Regions (Kaya and Aladağ 2009; Yildirim et al. 2015).

Myrtle, which has black and white fruits, is referred to as ‘common myrtle’, ‘murt’ and ‘hambeles’ in the southern parts of Turkey. Its fruits are consumed fresh or dried. Myrtle is a fruit that has positive effects on health thanks to the tannins, essential oils, organic acids, anthocyanins and phenolic compounds it contains. Phenolic compounds are known to have antibacterial, antidepressant, antidiabetic, antiviral, anti-inflammatory and immunostimulatory properties. Due to its antioxidant properties, it also reduces the risk of many diseases such as cancer (Proestos et al. 2006; Aydın and Özcan 2007; Şan et al. 2015a, 2016; Yanguia et al. 2021).

Some researchers state that the nutrient content of fruit species is affected by genotype, environmental factors, cultural practices and processing methods (Howell et al. 2001; Pehlivan and Güleriyüz 2004; Gao et al. 2012; Alkaltham et al. 2021). In recent years, research on increasing the nutritional content of fruits has been given importance (Rossi et al. 2003; Küçüker et al. 2015; Kui et al. 2020). It is also known that the biochemical contents of fruits change with the time of harvest and the degree of ripening. For this reason, it is important to harvest the fruits at the ap-

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appropriate time when they are rich in biochemical content. Phenolic compounds are one of the most important biochemical substances in fruits, which are affected by genetic or external factors. It has been reported that phenolic components decrease during fruit ripening, while the amount of anthocyanins increases in fruit species such as jujube, pomegranate and myrtle (Wang et al. 2016; Babou et al. 2016; Bayar and Şan 2017). In this respect, consumers and food industry organizations need important information about the changes in the bioactive substance content of fruit species and their antioxidant activities at different maturity stages. As in all fruit species, it is necessary to know the changes in biochemical contents of myrtle fruits according to different maturity levels. In this study, changes in some physical properties and biochemical contents of myrtle fruits harvested at different maturity periods were investigated.

Materials and Methods

Materials

In the study, the fruits of the myrtle genotype in the farmer's garden in the Serik district of Antalya province were used as material. The genotype of myrtle used in the study is a type with white and large fruit, propagated by grafting by producers and sold especially in local markets. This genotype is locally referred to as 'Aşı Mersin' (Şan et al. 2015b). Fruits belonging to the genotype were harvested on 3rd October (pre-maturity), 1st November (beginning of maturity) and 29th November (fully mature) in 2015.

Method

Determination of Physical Properties

Fruit width and fruit length were measured with a digital caliper in harvested fruits, and fruit weight was measured with precision scales. Skin color values of fruits were measured with Minolta CR-400 colorimeter (Osaka, Japan) and expressed as L*, a*, b*.

Determination of Biochemical Contents

In order to determine their biochemical properties, the fruits were squeezed and the juice was filtered with the help of

filter paper. The pH of the juice was determined with the help of a pH meter. The water-soluble solids content was measured with a hand-held refractometer. Titratable acidity content was analyzed according to the method stated by Karaçalı (2010) and the results were calculated as % malic acid.

Analysis of Total Phenolic Content

The total phenolic content of the fruits was determined according to the Folin-Ciocalteu method described by Singleton and Rossi (1965). For this purpose, 1 g of myrtle fruit flesh was taken and completely crushed in 4 ml of ethanol. After the samples were kept in a hot water bath for 10 min, they were centrifuged at 8000 rpm for 15 min. Then, 4 ml of 80% ethanol was added to the filtered samples and after they were kept in a hot water bath for 10 min again, the samples were made up to 20 ml with 80% ethanol. A total of 0.5 ml of the extract was taken and 2.5 ml of Folin-Ciocalteu (0.2 M) solution (Sigma-Aldrich, Steinheim, Germany) and 1 ml of distilled water were added to this. After the samples were kept for 10 min, 1 ml of sodium carbonate (0.7 M) was added and incubated in the dark for 2 h. Absorbance values were read in a spectrophotometer (T70 Plus Dual Beam/Arlington, USA) set at a wavelength of 760 nm. Results are expressed as mg GAE 100 g⁻¹ of fresh fruit. In order to determine the standard calibration curve, 0, 20, 40, 60 and 80 mg/l gallic acid (Merck, Darmstadt, Germany) solution was prepared and obtained by reading the absorbance values in the spectrophotometer with the application of the same method.

High Pressure Liquid Chromatography Analysis of Phenolic Compounds

Phenolic compound analysis were carried out using Shimadzu brand high pressure liquid chromatography (HPLC) device (Shimadzu Inc., Kyoto, Japan) by the modified method of Caponio et al. (1999). For this purpose, 5 g of myrtle fruit was weighed and 10 ml of methanol was added. It was mixed at room temperature for about 18 h at 100 rpm in a shaker. After the samples were filtered with coarse filter paper, they were again passed through a filter with a pore diameter of 0.45 µm (Vivascience AG, Hannover, Germany). Then, 20 µl of sample was injected into the HPLC device equipped with a DAD detector ($\lambda_{max}=278$) and on a column of Agilent Eclipse XDB-C18 (250 × 4.60 mm,

Table 1 Gradient program used in the high pressure liquid chromatography device for the analysis of phenolics in myrtle fruits

Time (min)	0.1	20	28	35	50	60	62	70	73	75	80	81
A (%)	93	72	75	70	70	67	58	50	30	20	0	93
B (%)	7	28	25	30	30	33	42	50	70	80	100	7

Solvent A: 3% acetic acid, solvent B: methanol

5 μm) operated at 30 °C. Detection and quantification were performed with a SIL-10AD vp auto sampler, a SCL-10 Avp system controller, a DGU-14A degasser, a LC-10AD vp pump and a CTO-10Avp column oven. The elution solvents were 3% acetic acid (A) and methanol (B). The flow rate was 0.8 ml/min. The gradient program used in the HPLC device is given in Table 1. Peak identification was performed according to the standards (gallic acid, chlorogenic acid, resveratrol, myricetin and quercetin) purchased from Sigma-Aldrich Chemical Co (Steinheim, Germany). The amounts of phenolics were evaluated according to their peak areas. Concentrations were expressed as $\mu\text{g g}^{-1}$ FW.

High Pressure Liquid Chromatography Analysis of Tannic Acid

Tannic acid analysis were carried out using a Shimadzu HPLC device by modifying of the methods described in Caponio et al. (1999) and Verzele and Delahaye (1983). For this purpose, 5 g of myrtle fruits were weighed and crushed. Then, 20 ml of methanol was added to this and shaken in a magnetic shaker for 1 h. After the samples were filtered with coarse filter paper, they were again passed through a filter with a pore diameter of 0.45 μm (Viva-science AG, Hannover, Germany). A total of 20 μl of the filtered sample was injected into the HPLC device equipped with a DAD detector (280 nm) and on a column of Luna Silica (2) (250 \times 4.60 mm, 5 μm) operated at 30 °C. Detection and quantification were performed with an SCL-10 Avp system controller, a DGU-14A degasser, a LC-10AD vp pump and a CTO-10ACvp column oven. The elution solvents were 0.2% phosphoric acid in distilled water (A) and 0.04% phosphoric acid in acetonitrile (B). The flow rate was 1.2 ml/min. The gradient program used in the HPLC device is given in Table 2. Peak identification was performed according to the standard of tannic acid purchased from the Sigma-Aldrich Chemical Co (Steinheim, Germany). The amounts of tannic acid were evaluated according to the peak area. Concentrations were expressed as $\mu\text{g g}^{-1}$ FW.

High Pressure Liquid Chromatography Analysis of Organic Acids

Organic acid analysis were performed using a Shimadzu HPLC device by modifying the methods described in Al-

Table 2 Gradient program used in the high pressure liquid chromatography device for tannic acid analysis in myrtle fruits

Time (min)	0.1	15	20	22	23	30
A (%)	100	90	0	0	100	Termination
B (%)	0	10	100	100	0	–

Solvent A: 0.2% H_3PO_4 in distilled water, solvent B: 0.04% H_3PO_4 in acetonitril

hendawi et al. (1997) and Krapez et al. (2001). Before the analysis measurements were made, the Supelco C18 solid phase cartridge was first conditioned with 3 ml of methanol and then washed with 10 ml of distilled water. After weighing 10 g fruit samples, 20 ml of H_3PO_4 (2%) was added. The samples were shaken for 5 min in a magnetic shaker and filtered with coarse filter paper. Then, 1 ml of the filtrate was taken and 1 ml of extraction solution was added to this. As the extraction solution, 0.01 M KH_2PO_4 (pH, 8) was used. A total of 1 ml of this solution was passed through the cartridge and the solution was taken into a tube. Then, after the cartridge was washed with 1 ml of extraction solution again, the solutions were combined and 20 μl of sample was injected into the HPLC device equipped with a SPD-10Avp UV-VIS detector (210 nm) and on a Prodigy ODS-2 column (250 \times 4.6 mm, 5 μm) operated at 30 °C. Analysis of organic acids was performed with a SIL-20AC prominence auto sampler, an LC-20AT prominence system controller, a DGU-20A5 prominence degasser, an LC-20AT prominence pump and a CTO-10ASvp column oven. The elution solvents was phosphoric acid (pH: 2.2). The flow rate was 0.8 ml/min. Peak identification was performed according to the standards (tartaric acid, ascorbic acid, malic acid, citric acid and succinic acid) purchased from Sigma-Aldrich Chemical Co. The amounts of organic acid were evaluated according to their peak areas. Concentrations were expressed as $\mu\text{g g}^{-1}$ FW.

Experimental Design and Data Analysis

The experiment was planned according to the randomized plot design with three replications. The obtained data were subjected to analysis of variance using the Minitab 17 software program (Minitab Inc.) and the differences between the means were determined at the 5% error level with the TUKEY comparison test and given with different letters.

Results and Discussion

Determination of Physical Properties

In the study, fruit weight, fruit width and fruit length values of myrtle fruits increased statistically significantly with the progress of maturity ($p < 0.05$). It was observed that the increase in fruit length was higher than the increase in fruit width in the last 4-week period. In our study, fruit width increased by 14% and fruit length increased by 19% in the last 2 months of maturity. This difference also affected the fruit shape index value. Fruit shape index values, which is a value dependent on fruit width and fruit length parameters, showed similar results with all other parameters as expected. The results obtained were similar to

Table 3 Some physical and biochemical properties of fruits at different maturity stages

Property	3 October	1 November	29 November
Fruit weight (g)	0.73 ± 0.03c*	1.01 ± 0.01b	1.22 ± 0.02a
Fruit width (mm)	14.10 ± 0.3c	15.07 ± 0.2b	16.12 ± 0.3a
Fruit length (mm)	11.03 ± 0.2b	12.76 ± 0.4a	13.17 ± 0.3a
Shape index	0.78 ± 0.02b	0.84 ± 0.02a	0.81 ± 0.01ab
L*	82.96 ± 0.6	84.15 ± 1.1	82.38 ± 0.3
a*	-8.21 ± 0.6c	-0.86 ± 0.9b	4.40 ± 0.3a
b*	31.72 ± 1.0a	25.27 ± 0.9b	26.18 ± 2.1b
Total soluble solids (%)	14.33 ± 0.5b	15.20 ± 0.2b	17.10 ± 1.0a
Fruit juice pH	4.37 ± 0.03b	5.39 ± 0.04a	5.51 ± 0.4a
Titrateable acidity (%)	0.40 ± 0.02a	0.12 ± 0.004b	0.11 ± 0.02b

*The difference between the means shown with different letters on the same line is statistically significant ($p \leq 0.05$)

the study of Fadda and Mulas (2010). Between October 3 and November 29, fruit weight increased by approximately 67%. Similar to our results, Aidi-Wannes et al. (2019) reported that fruit width and length increased by 5–19% and 16–28% respectively, in the last 2 months of maturity based on genotypes. While a significant increase was detected in a* value with maturity according to fruit skin color, b* value decreased. There was no statistical difference in the L* value, which expresses the brightness (Table 3). It is known that there is a positive relationship between a* value and anthocyanin content. As a matter of fact, it is reported that anthocyanin content increases in parallel with maturity (Fadda and Mulas 2010).

Titrateable Acidity, Total Soluble Solids and PH of Fruit Juice

The highest pH value (5.51) in fruit juice was determined in the full maturity period (29th November). Accordingly, the titrateable acidity content also decreased as maturity progressed. As expected, the content of total soluble solids of fruit juices increased regularly as maturity progressed, and this increase was statistically significant (Table 3). The total soluble solids of the fruits harvested at full maturity was found to be 17.10%. Similar to our findings, Kui et al. (2020) reported that as the maturity of myrtle progressed, the content of total soluble solids increased, while titrateable acidity decreased. It is also stated that the titrateable acid content of myrtle fruits, which was 0.53% after 30 days of full bloom, decreased to 0.16% after 210 days after full

bloom (Fadda and Mulas 2010). Additionally, it was reported by Mezni et al. (2019) that the total soluble solids and titrateable acidity contents vary significantly according to the cultivars. Researchers have reported that the content of total soluble solid varied between 4 and 10%, and the titrateable acid content varied between 0.3 and 0.7%, depending on the genotype and applications.

Organic Acid Contents

It was determined that citric and malic acid ratios increased in parallel with the progress of maturity in fruits, while tartaric acid decreased significantly. While the malic acid content of the fruits was 790.5 µg g⁻¹ in the pre-maturity period (3rd October), this value increased to 1037.6 µg g⁻¹ in the full maturity period (29th November). It was determined that the citric acid content of the fruits increased by approximately 60% during the full maturity period (29th November) (Table 4). It was determined that malic and citric acid contents increased with maturity. However, the total organic acid ratio decreased with maturity. In support of our study, Güçlü et al. (2022) found that malic acid content in medlar fruit first decreased with maturity and then increased again. Researchers reported that the tartaric acid content of medlar fruits also decreased with maturity, similar to our results. Contrary to our results, it was determined that the citric acid content of passiflora and medlar fruits decreased with maturity (Zhang et al. 2021; Güçlü et al. 2022). The organic acid contents differ according to the species. As a matter of fact, it has been reported that the malate content

Table 4 Organic acid contents of fruits harvested at different maturity stages

Organic acid	3 October	1 November	29 November
Malic acid (µg g ⁻¹)	790.5 ± 73b*	663.2 ± 70b	1037.6 ± 13a
Citric acid (µg g ⁻¹)	86.6 ± 10c	156.6 ± 19b	219.8 ± 23a
Tartaric acid (µg g ⁻¹)	2063.3 ± 56a	1263.3 ± 17b	754.1 ± 11c
Total organic acids (µg g ⁻¹)	2940.4	2083.1	2011.5

*The difference between the means shown with different letters on the same line is statistically significant ($p \leq 0.05$)

Table 5 The content of total and individual phenolics of fruits harvested at different maturity stages

Phenolics	3 October	1 November	29 November
Total phenolic content (mg 100 g ⁻¹)	1408.0 ± 299a*	794.0 ± 109b	757.6 ± 94b
Gallic acid (µg g ⁻¹)	146.5 ± 18	110.6 ± 14	111.9 ± 15
Quercetin (µg g ⁻¹)	10.20 ± 0.85a	9.50 ± 0.44a	6.33 ± 0.43b
Myricetin (µg g ⁻¹)	8.06 ± 1.0ab	11.10 ± 1.1a	6.93 ± 2.0b
Tannic acid	52.23 ± 15a	24.5 ± 4b	Nd

Nd Not detected

*The difference between the means shown with different letters on the same line is statistically significant ($p \leq 0.05$)

increases with maturity in apple, banana, grape, kiwifruit, lime, peach, plum and strawberry. It was stated that citrate increased with maturity in guava, lemon, lime, orange, plum, strawberry and pineapple, and decreased in papaya and peach (Batista-Silva et al. 2018). It has been reported that the decrease in organic acid content in many fruits is due to the increase in fruit size rather than organic acid metabolism. In addition, it has been reported that changes in organic acid content in fruit species are significantly affected by environmental conditions such as light, temperature, and cultural practices (Famiani et al. 2015).

Total and Individual Phenolics

Total phenolic content of myrtle juice was found to be higher in immature fruit than in mature fruit (Table 5). In the study, the highest phenolic content (1408 mg 100 g⁻¹) was detected in immature fruits (3rd October) and decreased with maturity. Aidi-Wannes and Marzouk (2013) found the total phenolic content of myrtle fruit to be 1370 mg 100 g⁻¹, similar to our study. Some researchers also stated that the total phenolic content appears at high levels after fruit set and decreases as development progresses (Vvedenskaya and Vorsa 2004; Renard et al. 2007). It was also stated by Aidi-Wannes et al. (2019) that the total polyphenol in myrtle fruits increased until the ripening period of the fruits and decreased after maturity. It is reported that the total phenolic content of jujube fruits is highest during the white maturity period and decreases with the progress of maturity (Wang et al. 2016).

There were no statistically significant differences in terms of gallic acid content in fruits harvested at different maturity periods. It was determined that there was a statistically significant decrease in quercetin contents with maturity ($p < 0.05$) (Table 5). Yeğın and Uzun (2015) found the quercetin content of myrtle fruit to be 1.18 mg 100 g⁻¹ (11.8 µg g⁻¹), which supports our findings. It was determined that the myricetin content of the fruits increased until the ripening time and decreased again with the progress of ripening. Yeğın and Uzun (2015) found the myricetin content in myrtle fruits to be approximately two to 20 times higher than our results. Researchers stated that myricetin content in fruits is between 1.99 and 25.36 mg

100 g⁻¹ based on genotypes. It is thought that the variability in the fruits of myrtle genotypes is due to factors such as ecology, climate and maturity level (Fadda and Mulas 2010; Barboni et al. 2010; Medda et al. 2021). As a matter of fact, it has been reported that the phenolic content of myrtle fruits varies based on the cultivars, negatively affected by high temperature, and the anthocyanin content increases with altitude (Medda et al. 2022). In addition, it was stated that the phenolic and flavonoid contents of wild-grown myrtle fruits were higher than the cultured genotypes (Çakmak et al. 2021).

While the highest tannic acid content of 52.23 µg g⁻¹ was determined in fruits harvested in the immature period (3rd October), this value decreased to 24.5 µg g⁻¹ at the beginning of maturity, and tannic acid was not detected in fruits harvested in the following period (29th November). It was observed that the tannic acid content of myrtle fruits decreased statistically significantly with maturity. Similar to our results, Fadda and Mulas (2010) stated that the tannin content of myrtle fruit regularly decreases with the progression of maturity. Researchers stated that while the tannin content was 223.55 mg 100 g⁻¹ in the fruits harvested at 30 days after full bloom, it decreased to 6.84 mg 100 g⁻¹ in the fruits harvested at 210 days after full bloom. On the other hand, it was stated by Aidi-Wannes et al. (2019) that the tannic acid content of myrtle fruits increased until maturity and decreased after maturity. There is a relationship between the amount of tannic acid in the myrtle fruit and the bitterness in the fruit flavor. In this case, it is preferred that the amount of tannic acid in the fruits is low (Mezni et al. 2019). It has been reported that the amount of tannic acid in myrtle fruit may differ based on cultivars (Mezni et al. 2019), ecology and precipitation (Medda et al. 2022).

Conclusions

As a result, it was determined that the biochemical contents of myrtle fruits vary significantly according to the degree of ripening. In this respect, it was determined that while the total soluble solid content of myrtle fruits increased, the titratable acidity decreased with the progression of maturity. The total organic acid and tartaric acid content of the fruits

decreased regularly with maturity. However, malic and citric acid contents of fruits increased with maturity. It has been determined that immature fruits are richer in phenolic substance than ripe fruits. In particular, it was determined that the content of total phenolics, quercetin and gallic acid decreased by approximately 46, 38 and 24%, respectively, with maturity. It was determined that the tannic acid content, which gives a bitter taste to the fruits, decreased to a trace amount in the full maturity period.

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Declarations

Conflict of interest B. Çelik and B. Şan declare that they have no competing interests.

Ethical standards For this article no studies with human participants or animals were performed by any of the authors. All studies mentioned were in accordance with the ethical standards indicated in each case.

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