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The Effect of Enzyme and Sonicator Application of Biochemical Properties of Raw and Ripe *Myrtus communis* L. Juice

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Abstract

In this study, the effects of enzyme and sonicator usage on the juice amount and antioxidant activity of raw and ripe *Myrtus communis* L. fruit were investigated. Raw and ripe myrtle fruits collected from the same tree were divided into 4 groups. After crushing by adding equal weight of water, the first group was affected with a sonicator and, the second group with hydrolytic enzyme mixture. The third group with a sonicator after the enzyme mixture and the fourth group is the control group in which no application was made. After the fruit juices were filtered, their amounts, turbidity and antioxidant activities were compared with the control group and each other. According to the results obtained, the amount of fruit juice was higher only in the enzyme-activated group than in the others. The use of sonicator caused a decrease in the amount of raw myrtle juice. Turbidity values of only enzyme-treated myrtle juices were lower than all other samples. In order to determine antioxidant activities, total phenolic content (TPC), total flavonoid content (TFC), iron reducing power (FRAP) and radical scavenging activities (ABTS) were determined, and it was observed that raw myrtle had higher antioxidant activity than mature myrtle. In addition, the use of enzymes and sonicators in general led to an increase in TFC value as 70.7 % and 283.6% for raw and ripe juices, respectively whereas in the ABTS activity, 29 % and 28 % increament was observed.

Keywords:

Myrtus communis L., sonication, enzyme, turbidity, antioxidant properties

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Introduction

The myrtle plant, called *Myrtus communis* L., is also known for its black or white colored, waxy-covered fruits with a distinctive flavor. M. communis, one of the typical native plants of the Mediterranean Basin; It is found naturally in Adana, Antalya, İçel, Çanakkale, İstanbul, Zonguldak, Sinop, Ordu, Trabzon, İzmir, Samsun, Muğla and Hatay provinces in Turkey (Söke & Elmacı, 2015). *M. communis* L. (Myrtaceae) has been used since ancient times for medicinal and food and purposes. *M. communis* also showed several pharmacologic, biologic and medical activities such as antifungal, antibacterial, anti-inflammatory, antiviral, analgesic, antimutagenic, anti-hemorrhagic, antioxidant, hepatoprotective, wound healing and anti-hyperglycemic activities (Asgarpanah & Ariamanesh, 2015). Antioxidant activity of essential oils of *Myrtus communis* L. has been extensively studied in the literature (Chryssavgi et al.,, 2008; Snoussi et al.,, 2011; Medda et. al., 2021; Odeh et al.,, 2022; Roozitalab et al., 2022). This medicinal and aromatic plant received great attention in the last years for the large appreciation of the liqueurs produced by its berries (red liqueur) and leaves (white liqueur) (Medda et al.,, 2021). Since fruit production takes place in a limited period of the year, obtaining fruit juice or extract is a very common way to benefit more from the fruits. Various processes are applied to increase efficiency and biological benefit in fruit juice production. In recent years, interest in sonication applications in the production of fruit juice has increased, and the changes in the properties of fruit juice due to sonication have been investigated (Nguyen & Nguyen, 2018; Hussain et al.,, 2021; Lopez-Martinez et al.,, 2022; Silva & Sulaiman, 2022). It has been suggested that processing of fruit tissue by sonication prior to juice extraction may disrupt the cell wall and increase juice yield, as well as increase the recovery of water-soluble compounds. (Kidon &Narasimhan, 2022). So, sonication is widely accepted as a green, innovative, cost-effective and rapidly developing technique in industrial production, and also offers the potential for widespread use in freezing, extraction, separation, emulsification, softening, crystallization, drying, filtration, etc. (Gupta et al., 2021). Numerous studies have been reported investigating the effects of enzymatic treatment on fruit juice quality (Wang et al., 2009; Heffels et al., 2017; Babagil & Nadaroglu 2022; Alagöz et al., 2022; Sheladiya et al., 2022; Ozyilmaz & Gunay 2023). It commonly uses enzymes to achieve higher yield and clarity in the production of fruit and vegetable juices. These enzymes break down the cell walls of fruits and vegetables, enabling high juice yield and high recovery of cell content. Of course, many studies have been conducted on how both sonication and enzyme treatment affect the properties of the fruit juice (Lieu & Le, 2010; Radziejewska-Kubzdela et al., 2020; Redd et al., 2020; Kidon &Narasimhan, 2022; Gupta et al., 2021).

In this study, it was investigated how the amount of juice, turbidity and antioxidant properties changed as a result of sonication, treatment with hydrolytic enzyme mixture and sonication after enzyme treatment in the extraction of *Myrtus communis* L. juice. Raw and ripe myrtles were collected from the same tree from Samandağ, Hatay, Turkey.

Materials and Methods

Enzymes as α-amylase from *Aspergillus oryzae* (800 FAU/g), cellulase from *Trichoderma reesei* (700 U/g) and pectinase from *Aspergillus aculeatus* (3800 U/ml), Trolox (6-hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid), ABTS (2,20-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), 3.5- Dinitrosalicylic acid (DNS), glucose anhydrous, were purchased from Sigma. All other chemicals were of analytical grade and all aqueous reagents were prepared using deionized water. *Myrtus communis* L. (known as hambeles) was obtained from the same tree in Samandağ district of Hatay province in the south of Turkey.

Extraction of Myrtle Juice

In the study, freshly picked myrtles were washed and 20 grams of fruit crushed and mixed with 20 grams of water. 4 groups were formed in this way. The applications made to these groups are given in Table 1.

Table 1. Applications for myrtle fruit pulp before juice extraction

Groups	Application
C	Control
S	Sonication
E	Incubation with enzyme mixture for 2 hours
E+S	Incubation with enzyme mixture for 2 hours then sonication

For S groups, myrtle mash containing water was sonicated for 2 min applying 10 sec impuls. Afterwards, the mash was filtered with a muslin cloth. For E group 500 U amylase, cellulase and pectinase containing enzyme solution was added to myrtle mash and kept at 35°C for 2 hours, then the mash was filtered with a muslin cloth. For E+S group, first, the procedure applied to the E group was performed, then sonication was performed as in the S group. The fruit juice yield was calculated with the formula given in Eq. 1 by weighing the amount of juice obtained for each group.

$$Juice\ Yield\ \% = \frac{Amount\ of\ juice}{amount\ of\ mash} x100 \qquad \text{(Eq. 1)}$$

Determination of Juice Properties

Turbidity

Turbidity of juices were determined spectrophotometrically at 600 nm. Standard formazin solution (1000 NTU) was used to get standard curve and results were given at NTU unit.

Total Phenolic Content (TPC)

The total phenolic contents of the myrtle juice extracts were determined by the Folin Ciocalteau method (Gutfinger, 1981). To 0.5 ml of juice, 2.5 ml of water and 0.25 ml of Folin reagent were added. After 5 min 750 µl of 20% Na_2CO_3 was added and mixture was kept in dark for 2 hours. Absorbance values were measured at 750 nm and TPC values were calculated as mg gallic acid equivalent per ml of juice.

Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined by the aluminum chloride method using quercetin as the reference compound (Kumaran & Karunakaran, 2006). To this, 0.5 ml of juice was diluted by 2 ml of water and then 150 µl of 5% $NaNO_2$ aqueous solution was added. After 5 minutes, 150 µl of 10% $AlCl_3$ aqueous solution was added and waited for 1 min. Finally, 1 ml of 1 M NaOH was added and then the absorbance was measured at 510 nm. Standard curve was obtained using quercetin and TFC values were calculated as mg quercetin per ml of juice.

Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing antioxidant power was determined according to Kamtekar et al., (2014). To the 2.5 ml of extract, 1ml of 0.2 M phosphate buffer pH 6.6 and 1 ml of 1 % $K_3[Fe(CN)_6]$ was added. The reaction mixture was incubated at 50°C for 20 minutes. After 2.5 ml of 10 % TCA adding, mixture was centrifuged for 10 minutes. 2.5 ml of supernatant was taken and 2.5 ml of distilled water and 0.5 ml of 0.1 % $FeCl_3$ solution was added. After 10 min, absorbance value was measured at 593 nm. Trolox was used as standard and results were given as mg trolox per ml of juice.

The Radical Scavenging Activity (ABTS)

Trolox equivalent antioxidant capacity (TEAC) or 2,20-azinobis (3-ethylbenzothiazolline-6-sulfonic acid (ABTS) assay was carried out according to Arnaou et al., (2001). This method is based on the scavenging of ABTS radical cation by antioxidants in juice. ABTS radical is formed by mixing 7 mM ABTS and 2.45 mM $K_2S_2O_8$ in a 2:1 ratio and keeping it in the dark for 16 hours. The prepared radical is diluted with ethanol until its absorbance is fixed at 0.7 at 734 nm and 100 µl of juice sample was added in 3 ml of the ABTS radical. After 5 minutes absorbance value was measured at 734 nm. Inhibition percentage was calculated using Eq.2.

$$Inhibition\ \% = \frac{A_{final}}{A_o} x100 \qquad \text{(Eq.2)}$$

Where, A_{final} is the absorbance value at the end of the 5 min, Ao is the initial absorbance (0.7).

Trolox solutions prepared at different concentrations and the inhibition % values were calculated. A standard curve was created by plotting the % inhibition values against trolox concentration. The % inhibition values calculated for fruit juices were evaluated using the standard curve and the radical scavenging activities of juices were calculated as trolox equivalent in mg trolox/ml juice.

Results

Juice yields of *Myrtus communis* L (it is also known as hambeles) samples treated with sonication and enzyme are given in Table 2. As seen in Table 2, the fruit juices obtained in the fruit juice extraction studies (Group E and Group E+S) in which the enzyme use was performed were higher than the results of the control group and the study in which only sonication was performed. It can be concluded that the enzymes used especially disrupt the cell wall network and decrease the viscosity in relation to the breakdown of the macromolecules in the cell composition and increase the juice yield.

Table 2. Yields of myrtle samples

	Juice Yield %	
	Raw	**Ripe**
Control	70.44	75.76
Sonicator	70.47	73.43
Enzyme	74.73	78.66
Enzyme+Sonicator	71.61	78.42

Compared with the control group, sonication did not affect the amount of raw juice, but reduced the amount of ripe juice. Turbidites of juice samples are given in Figure 1. As seen in Figure 1, turbidity values decreased by 52% and 41% in raw and ripe myrtle juices, respectively, in the extraction process using only enzymes. When only sonication was applied, the turbidity of both raw and ripe juices did not change.

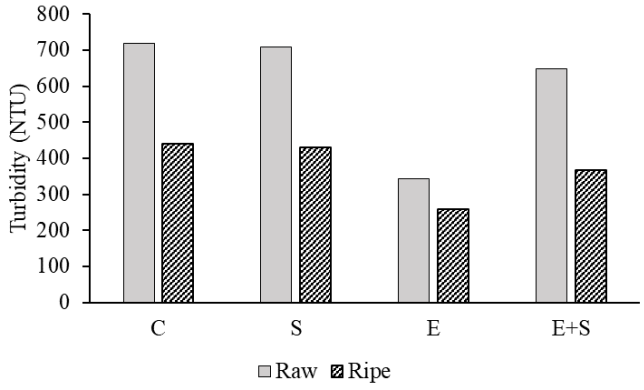

Figure 1. Turbidity values of raw and ripe myrtle juices

Total phenolic contents (TPC) of myrtle juices are represented in the Figure 2. As seen in Figure 2, TPC values in the ripe myrtel juice treated with only sonication or enzyme alone did not differ from the control group, while, the TPC value of the sample treated with enzyme plus sonication was approximately 18% higher than that of the control group. TPC values of raw myrtle juices were higher than that of the ripe myrtle juice. TPC values in the enzyme-treated myrtle juice were relatively lower than those of the other samples. TPC values of C, S and E+S samples did not differ much.

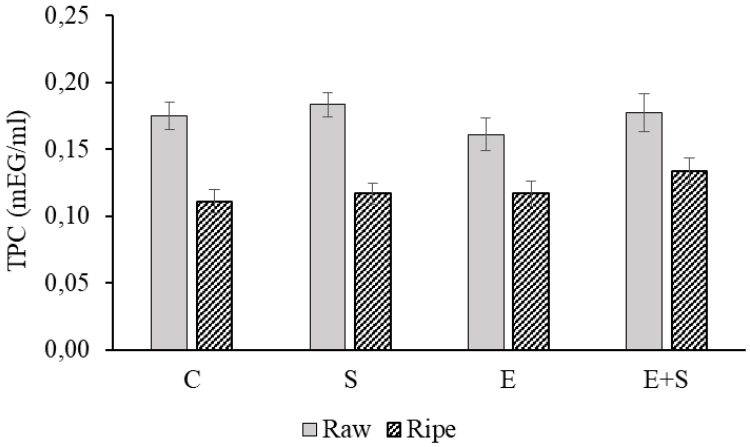

Figure 2. TPC values of raw and ripe myrtle juices

Figure 3 shows the total flavonoid contents (TFC) values of the myrtle juices. As seen in Figure 3, TFC values of raw myrtle was increadibly higher than that of ripe myrtle. With the application of enzyme plus sonication, both raw and ripe myrtle TFC values increased and the values became very close to each other. Ripe myrtle TFC values did not increase much with the use of enzyme alone, while the TFC value increased from 0.692 mg Quercetin/ml to 1.542 mg Quercetin/ml with E+S. As can be seen from the results, the combined application of enzyme and sonication in the production of ripe myrtle juice provide a great increase in TFC value.

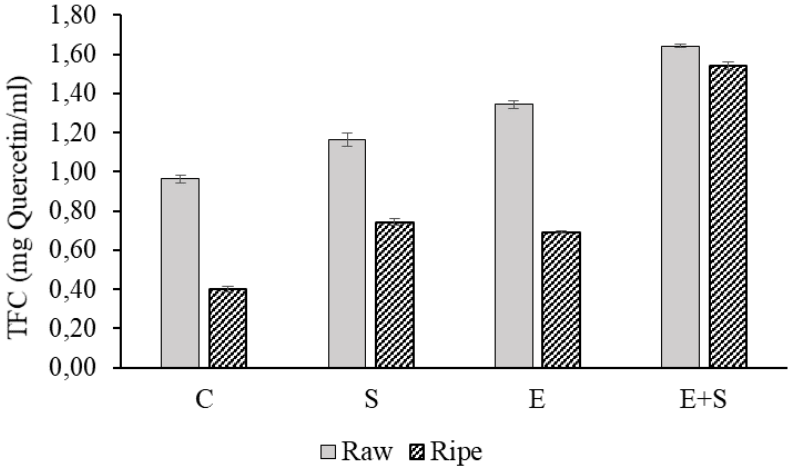

Figure 3. TFC values of raw and ripe myrtle juices

When comparing to other assays based on measurement of inhibition of free radicals, the FRAP assay is the one that direct measures antioxidants (or reductants). The values obtained from the FRAP assay provide information on the amount of electron donating antioxidants that can contribute to the reduction of $Fe^{3}+$ to $Fe^{2}+$. (Halvorsen et al., 2002). The FRAP values are given in Figure 4, and as seen, the FRAP values of myrtle juices treated only with sonication did not differ from the control group. However, a decrease in FRAP values was observed as a result of the application with the enzyme.

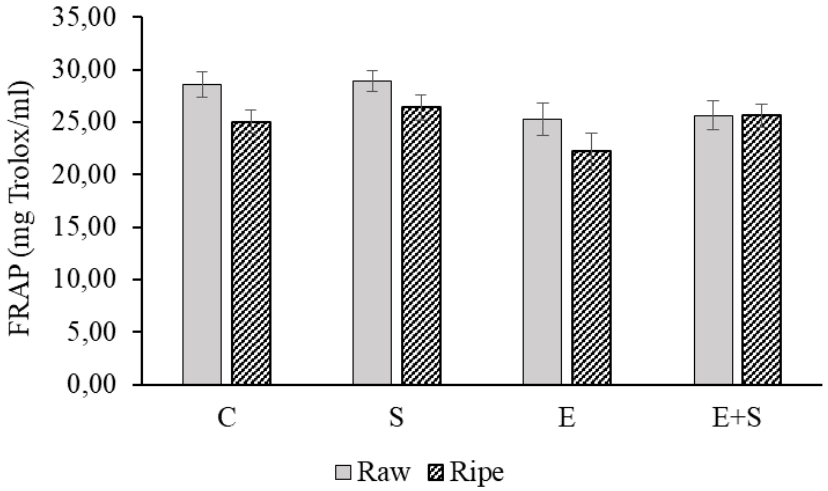

Figure 4. FRAP values of raw and ripe myrtle juices

Radical scavenging activities of raw and ripe myrtle juices are summerized in Table 3. It was clear from the results that ABTS radical scavenging activity of E and E+S samples increased significantly compared to C group. However, it was observed that the sonication application did not cause an additional improvement over the enzyme application. While the radical scavenging effect of raw myrtle juice was 0.085 mg trolox/ml, this value was found to be 0.092, 0.113 and 0.110 mg trolox/ml with S, E and E+S applications, respectively. The enzyme-treated raw myrtle juice showed the greatest increase, about 33%, compared to the control group. In case of ripe myrtle juice, while the radical scavenging effect of C group was 0.107 mg trolox/ml, this value was determined as 0.115, 0.140 and 0.137 mg trolox/ml in S, E and E+S groups, respectively. As can be seen, the ABTS activity of the ripe myrtle juice treated with the enzyme was approximately 31% higher than the control group. Another result obtained is that ABTS activity of ripe myrtle juice is higher than that of raw myrtle.

Table 3. Radical scavenging activities (ABTS) of raw and ripe myrtle juices

	Raw		Ripe	
	Inhibition %	**mgTrolox/ml**	**Inhibition %**	**mgTrolox/ml**
C	60.67	0.085	73.43	0.107
S	65.10	0.092	78.29	0.115
E	77.05	0.113	92.86	0.140
E+S	75.14	0.110	90.95	0.137

In Figure 5, the reducing sugar concentrations of myrtle juices determined by the DNSA method are given. The reducing sugar concentrations of all samples were higher than those of the control group. Depending on the treatments, the reducing sugar concentration increased in S, E, E+S samples, respectively. In the control group, the reducing sugar levels of raw and ripe myrtle juice were very close to each other. However, the reducing sugar concentration of ripe myrtle juice increased greatly than raw myrtle juice in the treatments. While the reducing sugar content of raw and ripe myrtle juices is around 18.7 mM, these values in S, E and E+S samples were 19.5, 21.1 and 21.4 mM values respectively in raw hambeles; mature hambeleste increased to 21.9, 25.4 and 27.3 mM, respectively. As a result of E+S application, the highest reducing sugar concentration was reached, and the reducing sugar concentration in raw and ripe myrtle juices increased by approximately 14% and 46%, respectively, compared to the control group.

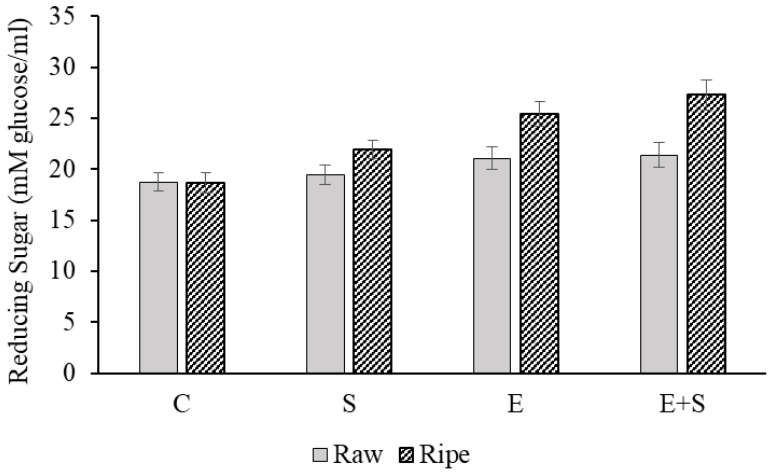

Figure 5. Reducing sugar concentrations of raw and ripe myrtle juices

Discussion

Myrtus communis L., which is widely found in the majority of Mediterranean countries, warm regions of North America and different parts of Australia, grows in the wild, mainly on the coasts of Tunisia, Morocco, Turkey and France (Çınar & Aksay, 2022). Although *M. communis* has been used for a long time in traditional medicines for the treatment of lung diseases and as an antiseptic,

anti-inflammatory, mucolytic, carminative, etc., it has recently been shown to have antioxidant, analgesic, antibacterial and antifungal activities and larvicide, insecticide and repellent effects (Asgarpanah & Ariamanesh, 2015). Therefore, investigation of antioxidant properties is of great importance. Antioxidants are extremely interesting components, as they help protect the human body against damage caused by reactive free radicals that occur during atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease, and even aging (Asgarpanah & Ariamanesh, 2015). Today, interest in natural antioxidants is increasing in order to prevent and treat many diseases and increase the quality of life (Arslan et al., 2022). Fruits are foods rich in antioxidants. Fruit juice, on the other hand, is a process product that ensures efficient consumption of fruits. Enzymatic treatment of fruit juice has many advantages such as an increase in juice yield, enhanced clarification, increased total soluble solids in juice, improved pulp liquefaction, and decreased turbidity and viscosity (Ramadan, 2019). In this study, the use of enzymes increased the juice yield. However, while sonication does not affect the yield of raw myrtle juice, it reduced the ripe myrtle juice yield. There are studies in the literature reporting that the use of sonication in fruit juice extraction increases the yield. Nguyen & Nguyen (2018) treated mulberry mash with sonication in the production of mulberry juice and reported that the juice yield increased by 26%. However, as in our study, there are also studies reporting that sonication alone does not increase or slightly increased juice yield (Reddy et al., 2022; Kidon & Narasimhan, 2022). In our study, while the sonication process did not affect the turbidity much, the enzyme application caused a decrease in turbidity. Studies in which turbidity increased after sonication have been reported in the literature (Bhat & Goh, 2017; Campoli et al., 2018). In our study, turbidity in fruit juices decreased with enzyme application, and these results were consistent with the literature (Wang et al., 2009; Ceretti et al., 2017; Lachowicz et al., 2018; Ozyilmaz & Günay, 2023). TPC values did not change much with sonication, but a decrease in raw myrtle juice was observed with enzyme application. Studies in which TPC increased after sonication and (Bhat & Goh 2017; Kidon & Narasimhan, 2022) enzyme application (Wang et al., 2009, Reddy et al., 2020, Radziejewska-Kubzdela et al., 2020) have been reported in the literature. In our study, when the FRAP value was compared with the control group, it did not change much at the end of sonication, but FRAP values decreased after enzyme treatment. However, radical scavenging activities (ABTS) increased significantly after enzyme treatment. In the literature, there are studies reporting that the antioxidant activity of fruit juice increases as a result of sonication or enzyme treatment (Nguyen & Nguyen 2018; Lachowicz et al., 2018; Kidon & Narasimhan, 2022). In our study, the reducing sugar level was higher in the S, E and E+S groups compared to the control group, which is a desirable result as it contributes to the sweetness of the product (Reddy et al., 2020).

In this study, the differences in yield, turbidity and antioxidant properties of raw and myrtle juice compared to the control group were investigated as a result of the individual and sequential application of sonication and enzyme use. In the light of the data obtained, it can be said that the use of the enzyme mixture alone is more advantageous in terms of yield, turbidity and ABTS activity. However, it is seen that they do not have a significant effect on TPC and FRAP values.

E+S application gave better results in terms of TFC value and reducing sugar concentration. The combined use of enzyme and sonication in the study, performing sonication before and after the enzyme application and comparing the results with the control group is considered as a subject worth investigating. It is thought that the rate of interaction of macro-biomolecules with enzyme in fruit juices treated with enzyme after sonication may increase.

Author Contributions

All authors read the final version of the manuscript and approved it for publication.

Conflict of Interest

The authors declare that they have no conflict of interest.

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