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Amino Acid Content and Effect of Different Preservation Methods on Some Biochemical Properties in Black Myrtus communis L. Fruits

Meltem ÇAKMAK[a], Büşra BAKAR[a], Dursun ÖZER[a], Fikret KARATAŞ[b*], Sinan SAYDAM[b]

[a]*Department of Chemical Engineering, Faculty of Engineering, Fırat University, Elazığ, Turkey*
[b]*Department of Chemistry, Faculty of Science, Fırat University, Elazığ, Turkey*

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Corresponding Author: Fikret KARATAŞ, E-mail: fkaratas@firat.edu.tr
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ABSTRACT

In this study, fruits of black *Myrtus communis* L. were subjected to different preservation methods [frozen, sun and microwave (MW) dried] and, its biochemical properties were examined. All measurements were triplicated. It was observed that the vitamin levels decreased in sun and MW dried samples ($p<0.05$). On the other hand, total phenolic compounds, flavonoids and antioxidant capacity of fresh and frozen *M. communis* L. fruits were found to be higher in sun and MW dried fruits ($p<0.05$). The amount of oxidized glutathione and malondialdehyde (MDA) increased while the amount of ghrelin and reduced glutathione decreased in the dried fruits ($p<0.05$). Obtained results indicate that, it is rich in terms of vitamins, amino acids, and some elements. These results suggest that the black *M. communis* L. is a balanced source of amino acids in terms of the total essential amino acid/total amino acid ratio. Experimental findings show that the most suitable preservation method for *M. communis* L. fruits is freezing. In addition, MW drying seems more advantageous than sun drying in terms of vitamin loss and time.

Keywords: *Myrtus communis* L., food preservation, phenolic substance, antioxidant capacity, elements, HPLC

1. Introduction

The use of plants for human health is a well-established tradition and medicinal plants are in the center of interest around the world. *M. communis* L., commonly known as "myrtus", are grown as mainly black and white types. While white fruits of *M. communis* L. are generally consumed fresh, on the other hand the black fruits are consumed as dried. The myrtus fruits have been reported to have a higher level of vitamins, phenolic compounds, and antioxidant (Çakmak et al. 2021). The leaves and fruits of the plants are also used for curing constipation, haemorrhoids, gum infections, urinary tract infections, and chest diseases (Fadda & Mulas 2010). Generally, colored fruits have higher phenolic compounds therefore they might have higher antioxidant capacity led to health benefits (Karadeniz et al. 2005). The black myrtus fruits have no commercial values, but in recent years it drew attention because of health benefit due to the higher antioxidant capacity.

Vitamins are the nutrients that required for many biochemical functions, must be taken alongside diet. Plants are the main sources of vitamins and deficiencies of vitamins can cause various diseases (Asensi-Fabado & Munne-Bosch 2010). Lycopene is an essential carotenoid found in fruits and vegetables such as tomatoes, watermelon, and grapefruit (Yapaing et al. 2002). Elements selected in the study have functions such as antioxidants, cofactor of enzyme, stabilizers of cell membranes, structural components of metallo-enzymes and metallo-proteins, and protection against toxicity (Soetan et al. 2010). Amino acids are involved in neurotransmitter and biosynthesis processes in biological systems. Dietary supply of essential amino acids is necessary for protein synthesis, so it is important to determine the amount of amino acids in foods (Garlick 2004; Davidson 2019).

M. communis L. is a seasonal fruit, to be able to consume it all year around different preservation methods applied. Preservation techniques have an important effect on the nutritional value and medicinal benefits of the fruits. Although sun drying is widely used natural drying technique, different drying methods such as drying in ovens, drying tunnels and vacuum as well microwave (MW) drying which is a relatively new technique also be used for many foodstuffs (Ahmed et al. 2013). Temperature, time, light intensity, and humidity's are important factors on the nutritional content of fruits (Maisnam et al. 2016).

Since the black *M. communis* L. is a seasonal fruit, to consume all year around, various preservation procedures are being applied. Vitamin content of fruits depends on many factors. The importance of these factors is genetic and ecological including the manner in which the fruit is collected, the preservation methods and the shelf life. It is reported that some biochemical contents of fruits change depending on different preservation methods (Kamiloglu et al. 2015). To our knowledge, the study here is the first to investigate the biochemical content of black myrtus fruit to this extent in relation to preservation methods.

The study is based on the investigation of effect of different preservation methods (sun or MW drying and freezing) on the vitamins (A, B, C, E), carotenoids (β-carotene and lycopene), functional peptides (glutathione, ghrelin), oxidative stress markers [oxidized glutathione (GSSG) and malondialdehyde (MDA)], total phenolics and flavonoids, antioxidant capacity [α-diphenyl-β-picrylhydrazyl (DPPH), trolox equivalent antioxidant capacity (TEAC)]. In addition, some elements such as Se, Cu, Fe, Mn, Zn and amino acids also determined.

2. Material and Methods

Ripe black *M. communis* L. fruits were harvested from Osmaniye, Turkey (37.269020 N, 36.120391 E), in December 2019. The fresh fruit samples were analysed after the fruits were collected. During the study, 2.5 kg of samples were processed beforehand for each treatment. Dried samples were stored in desiccator. Frozen (-20 °C) samples were analysed in ten days after drying. 20.0 gram of each homogenized by the blender and used throughout the analysis. All measurements were triplicated.

2.1. MW and sun drying

The fresh fruit samples were dried either in MW or under the sun. For the MW drying, each portion of sample exposed to MW radiation 6 times for 5 min at full power (800 watts), while in sun-drying they were kept in a well-ventilated indoor area under sunlight for 5 days until 60% weight loss of the total weight in both cases.

2.2. Determination of vitamins A, E, β-carotene, and lycopene

1.0 g of homogenized *M. communis* L. fruit samples were introduced to a tube then, 5.0 mL C_2H_5OH was added, and the sample was sonicated for 10 minutes then vortexed and centrifuged for 6 minutes at 7,500 rpm. After that, 1.0 mL n-hexane was added to the sample and vortexed followed by the extraction of n-hexane phase and this process was repeated twice. The collected n-hexane phases were dried under vacuum, the residue was dissolved in 1.0 mL of methanol and analysed using a Supelcosil LC-18 column (25.0 cm x 4.6 mm x 5.0 µm), methanol: acetonitrile (ACN): water (63:33:4.0 v/v) as mobile phase (Mukhtar et al. 2019). Vitamin A is the sum of retinol and retinoic acid. A sample chromatogram is given in Figure 1 for the fat-soluble compounds.

2.3. Determination of B vitamins

The filtrate obtained in material section was used for the analysis of vitamins B, utilizing a Supelcosil LC-18-DB column (150 mm x 4.6 mm ID, 5 µm). 5.0 mM sodium heptanesulfonate: 0.1% TEA at 25:75 (v/v) and pH adjusted pH 2.8 by H_3PO_4 used as the mobile phase (Amidžić et al. 2005).

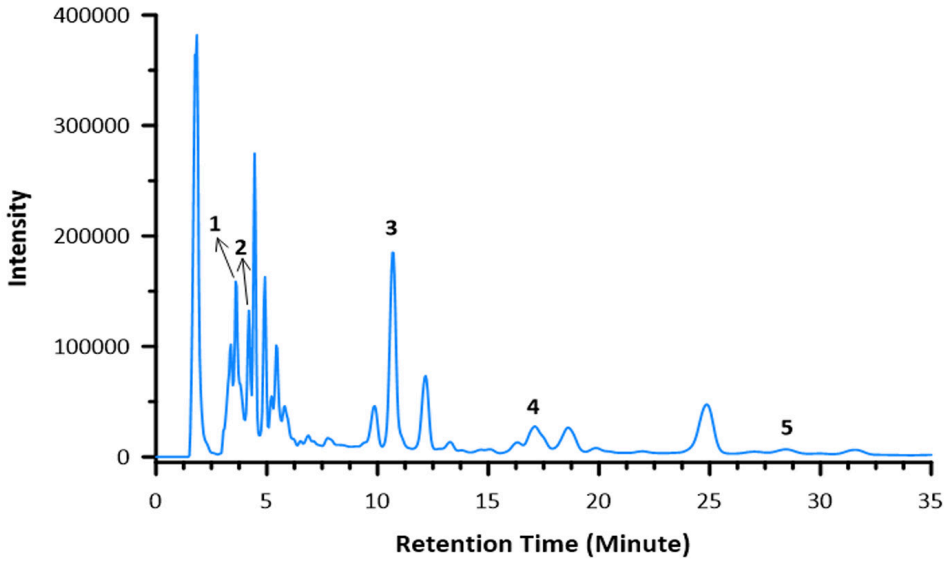

Figure 1- Sample chromatogram for fat soluble vitamins, (1: Retinol, 2: Retinoic acid, 3: Vitamin E, 4: Lycopene, 5: β-carotene)

2.4. Determination of Vitamin C, ghrelin, glutathione and MDA

Vitamin C, ghrelin, glutathione and MDA were determined, by using high performance liquid chromatography (HPLC) (Mukhtar et al. 2019; Dogan et al. 2016).

2.5. Extraction of M. communis L. fruit

The *M. communis* L. fruits samples, were subjected to different pre-treatments, were homogenized then 7.50 grams of sample extracted with 150 mL methanol in Soxhlet apparatus for 4 hours. Then methanol was removed by the rotary evaporator at 40 °C at a reduced pressure. The extract was dried, weighed dissolved in 75 mL of CH_3OH and the solution was stored in the fridge at 4 °C until analysis (Wang & Weller 2006)

2.6. Determination of total phenolic content

Total phenolic substance was determined spectrophotometrically according to Folin-Ciocalteu method modified by Dewanto et al. (2002). 0.50 mL of distilled water, 0.250 mL of sample or gallic acid and 0.125 mL of Folin-Ciocalteu reagent were mixed and shaken. After 6 minutes, 1.250 mL of 7% sodium carbonate solution was added and the total volume completed to 3.00 mL with distilled water. After 90 minutes, the absorption was measured at 760 nm by a ultraviolet-visible spectrophotometer. A working graph of gallic acid solutions prepared at different concentrations was established. The total phenolic content of the samples was determined, and the results were given as µg gallic acid per g dry weight sample (µg GAE/g dw).

2.7. Determination of total flavonoids

The total flavonoid substance was determined spectrophotometrically as described by Dewanto et al. (2002). 0.025 mL sample or quercetin, 1,250 mL distilled water and 0.075 mL 5% sodium nitrite solution, 0.150 mL 10% solution of aluminium chloride were mixed in a glass tube and allowed to stand for 5 minutes then 0.500 mL 1.0 M sodium hydroxide solution was added and total volume was completed to 2,500 mL with distilled water followed by measurement of absorbance at 510 nm. A working graph was formed with quercetin solutions prepared in different concentrations. The total phenolic content of the samples was determined using the working graph and the results were given as µg QE/g dw.

2.8. Total antioxidant capacity

Total antioxidant capacity was determined according to two different methods, DPPH and TEAC.

2.9. DPPH method

The antioxidant capacity was measured according to the method based on the scavenging activities of the stable DPPH free radical as described by Nile et al. (2013). A solution of 25 µg ml^{-1} DPPH in methyl alcohol was prepared, and the absorption of DPPH solution was measured at 510 nm. Then different amount of the sample extracts was added to DPPH solution and kept in dark for 30 minutes before measurement of absorbance at 510 nm. Results were given as IC_{50} (µg/mL), which indicates the concentration of the antioxidant substance that inhibits 50% of the DPPH radical in the medium. Low IC_{50} values indicate high antioxidant activity.

2.10. TEAC method

The ABTS free radical-scavenging activity was determined according to the method described by Re et al. (1999). The stock solutions including 7.0 mM ABTS solution and 2.4 mM potassium persulfate allowed to stand in the dark at room temperature for 12-16 h. The ABTS•+ solution was diluted with phosphate buffer (pH=7.4) to obtain an absorbance of 0.800±0.010 at 734 nm. Then 20 µL of the sample or Trolox standard was added to 2.0 mL ABTS•+ solution and allowed to stand at room temperature for 15 minutes then absorption was measured at 734 nm. Previously prepared ABTS•+ solution used as the control group. The antioxidant capacity of the sample was calculated as Trolox equivalent as µmol Trolox/g dw.

2.11. Analysis of selenium

20.0 grams of fresh *M. communis* L. fruit was homogenized and 2.5 g was transferred to a Teflon bomb then 6.0 mL of HNO_3: $HClO_4$ mixture (1: 4, v/v) was added and kept at 100 °C for 12 hours. After that, 2.0 mL of concentrated H_2O_2 was added, allowed to stand at room temperature for 24 hours. Mixture was transferred into tubes and a 4.0 N HCl concentration was achieved by adding concentrated HCl. The mixture was held at 90 °C for 15 min to reduce Se(VI) to Se(IV). To this mixture, 2 mL 2.5 M formic acid, 4 mL 0.1 M EDTA and 1.5 mL freshly prepared 3,3-diaminobenzidine solution were added and the pH was adjusted to 1.7 with the addition of 4 M NH_3. This was left to stand in the dark for 1.5 h for the formation of a metal-ligand complex. Selenium was analysed fluorimetrically according to the method of Dogan et al (2016).

2.12. Analysis of Cu, Fe, Mn and Zn

3.0 g of homogenized *M. communis* L. fruit sample was taken and 5.0 mL of $HCLO_4$ and HNO_3 mixture (1:4 v/v) was added, vortexed and sonicated for 30 minutes then left to stand for 24 hours followed by the addition of 2.0 mL H_2O_2. Final volume was completed to 25 mL with 1.0% triton-X 100 solution then the metal contents were determined by a flame AAS (Tüzen 2003).

2.13. Determination of amino acids

Hydrolysis: Approximately 2.0 grams of ground fresh fruit samples were taken into a glass tube and 5.0 mL 6.0 N HCl was added and vortexed thoroughly then, samples were kept at 110 °C for 24 hours to break peptide bonds (Elkin & Wasynczuk 1987). After that the samples cooled to room temperature, filtered and the filtrate volume was completed to 25 mL with distilled water.

Derivatization: Standard amino acid solutions were prepared using 0.10 N HCl at different concentrations between 1.0 to 5.0 µg/mL. Fifty µL standard amino acid solutions or hydrolysed fruit samples transferred to 5.0 mL glass tubes and dried under vacuum at 65 °C. Then 50 µL of "reagent 1" solution [(2: 2: 1 mixture of ethanol: water: Triethylamine (TEA) (v/v)] was added and vortexed and dried again under vacuum at 65 °C. The dried samples were vortexed by adding 50 µL of "reagent 2" solution [7: 1: 1: 1 mixture of ethanol: water: TEA: phenyl isothiocyanate (v/v)] and left at room temperature in the dark for 30 minutes for complex formation. At the end of this period, the samples were dried again under vacuum at 35 °C (Kwanyuen & Burton 2010). 1.0 mL mobile phase A and ACN mixture (8: 2 v/v) was added to each dried sample, vortexed and the samples were taken into HPLC vials for analysis.

2.14. Chromatographic procedure for amino acid analysis

Amino acid analysis was performed by HPLC by modifying Elkin and Wasynczuk (1987) with Kwanyuen and Burton (2010) methods. Nucleodur 100-5 C18 column (250x4.6 mm, 5 µm) was used. The analyses were carried out by applying the gradient program at 40 °C. The mobile phase consists of eluent A and eluent B mixture with a flow rate of 0.8 mL/min and measured at 254 nm (Table 1). Eluent A is 0.07 M $CH_3COONa \cdot 3H_2O$ (pH=6.4 with CH_3COOH) and eluent B is a mixture of ACN and water (60:40 v/v)

Table 1- Gradient program used for the separation of Phenylthiocarbamyl-amino acids

Time (minute)	*Flow rate (mL/min)*	*% Eluent A*	*% Eluent B*
0.01	0.8	90	10
12.00	0.8	70	30
16.00	0.8	65	35
16.01	0.8	50	50
25.00	0.8	100	0
30.00	0.8	50	50
30.01	0.8	10	90
35.00	0.8	10	902

2.15. Equipment and chemicals

Experiments were carried out by SHIMADZU HPLC, Prominence-I LC- 2030C 3D Model equipped with PDA detector, Perkin-Elmer, LS 55 fluorescence spectrophotometer and Perkin-Elmer flame atomic absorption spectrophotometer (AAnalyst-400), Sonicator (Wise Clean, WUC-AO3H, 170 W), Blender (Fakir Hausgrate 220 W), and Vestel brand MW (M.D-60x30, with 800-W power). Double distilled (H_2O) water was used throughout the work. All the chemical used are reagent or analytical grade and obtained from Merck or Sigma-Aldrich.

2.16. Statistical analysis

All measurements were triplicated and mean ± standard error was determined. The results were subjected to One-Way ANOVA by SPSS 10.0 for Windows. Differences between the group's means were analyzed for significance using Turkeys HSD test. The level of statistical significance was expressed as $p<0.05$. Insignificant change was indicated as $p>0.05$. Statistical difference indicated in table and figures with the different letter while the same letter indicates no statistical difference.

3. Results and Discussion

The amounts of vitamins, lyco *communis* pene ghrelin, stress biomarkers, total phenolic and flavonoids substance with total antioxidant capacity, selected elements and amino acids were measured in black *M. communis* L. fruits. The results are given in Table 2 and Figures 2-6.

Experimental results indicate that, there is no significant difference in the amount of vitamins and lycopene in fresh and frozen *M. communis* L. fruits ($p>0.05$). The lowest amounts of vitamins and lycopene lost were found in frozen fruit samples, while the highest lost found in sun-dried samples. Vitamin loss of black *M. communis* L. fruits dried in the sun and MW, ranged from 30 to 51 percent, (Table 2 and Figure 2).

The deterioration of vitamins is particularly dependent on the process condition such as temperature, presence of oxygen, light and time. Photochemical and enzymatic reactions can cause vitamins to decompose during sun drying process (Marszałek et al. 2015). Vitamin loss of sun-dried *M. communis* L. fruit samples are higher than MW-dried samples due to the longer exposure to sunlight during drying process. This can be explained by higher energy of sunlight to break down the vitamins and longer exposure time to dry (Sheraz et al. 2014). Because of shorter process time required for MW drying, lesser extend of vitamin loss is obtained and therefore it can be said that MW drying have an advantage over sun-drying process.

Table 2. The biochemical properties examined in wild black M. communis L.

Biochemical properties	*Fresh*	*Frozen*	*Sun-dried*	*Microwave-dried*	*MSE*
Vitamin A (µg/g dw)	2.15±0.06^{a}	1.92±0.07^{b}	1.10±0.05^{c}	1.18±0.05^{c}	0.015
Vitamin E (µg/g dw)	186.96±7.05^{a}	180.21±7.66^{a}	95.17±3.77^{b}	102.25±4.41^{b}	145.7
β-Carotene (µg/g dw)	7.00±0.12^{a}	6.70±0.11^{a}	4.16±0.18^{b}	4.87±0.15^{b}	0.089
Lycopene (µg/g dw)	9.34±0.31^{a}	8.95±0.26^{a}	5.92±0.19^{b}	6.42±0.25^{b}	0.292
Vitamin B_1 (µg/g dw)	78.72±3.03^{a}	73.59±2.47^{a}	48.91±1.97^{b}	54.59±1.92^{b}	25.67
Vitamin B_2 (µg/g dw)	118.20±5.52^{a}	112.00±4.39^{a}	60.14±2.38^{b}	63.48±3.26^{b}	74.22
Vitamin B_3 (µg/g dw)	344.27±14.60^{a}	336.81±10.84^{a}	176.16±8.54^{b}	188.71±7.24^{b}	499.2
Vitamin B_6 (µg/g dw)	40.80±1.36^{a}	36.42±1.45^{a}	26.82±0.98^{b}	27.00±1.23^{b}	7.231
Vitamin B_9 (µg/g dw)	5190.0±55.2^{a}	5125.0±29.2^{a}	2550.0±9.0^{b}	2568.0±7.1^{b}	4534
Vitamin B_{12} (µg/g dw)	71.39±2.05^{a}	69.61±1.65^{a}	36.21±1.27^{b}	38.69±2.14^{b}	14.8
Vitamin C (µg/g dw)	1323.0±31.9^{a}	1278.0±26.2^{a}	669.0±14.6^{b}	697.0±11.7^{b}	2310
Ghrelin (µg/g dw)	34.40±1.22^{a}	31.4±1.65^{a}	24.90±0.87^{b}	26.30±1.10^{b}	6.95
GSH (µg/g dw)	806.10±8.83^{a}	815.50±7.69^{a}	436.70±17.25^{b}	440.40±21.30^{b}	999.1
GSSG (µg/g dw)	190.10±6.27^{a}	196.70±6.59^{a}	232.70±5.26^{b}	216.20±5.90^{a,b}	163.3
MDA (µg/g dw)	5.32±0.23^{a}	6.00±0.28^{a}	6.80±0.20^{a,b}	7.00±0.14^{b}	0.210
Total phenolic substance (µg GAE/g dw)	43.01±1.97^{a}	40.94±1.73^{a}	38.63±1.36^{a}	39.94±1.71^{a}	39.17
Flavonoid (µg QE/g dw)	23.42±0.90^{a}	22.94±0.79^{a}	20.0±0.87^{a,b}	18.68±0.83^{b}	9.64
IC_{50} (µg/mL)	28.49±1.21^{a}	29.76±1.45^{a}	35.29±1.79^{a,b}	33.65±1.59^{b}	10.45
TEAC (µmol Trolox/g dw)	271.34±8.79^{a}	252.32±7.58^{b}	231.07±6.49^{c}	251.30±7.62^{b}	264.2
Selenium (µg/g dw)	0.63±0.01				
Zinc (µg/g dw)	268.71±9.13				
Iron (µg/g dw)	156.36±7.37				
Copper (µg/g dw)	46.55±1.94				
Manganese (µg/g dw)	29.76±0.95				

The letters in the table were used to compare the treatments in the rows at the 5% significance level for each characteristic examined, MSE: Mean square of error

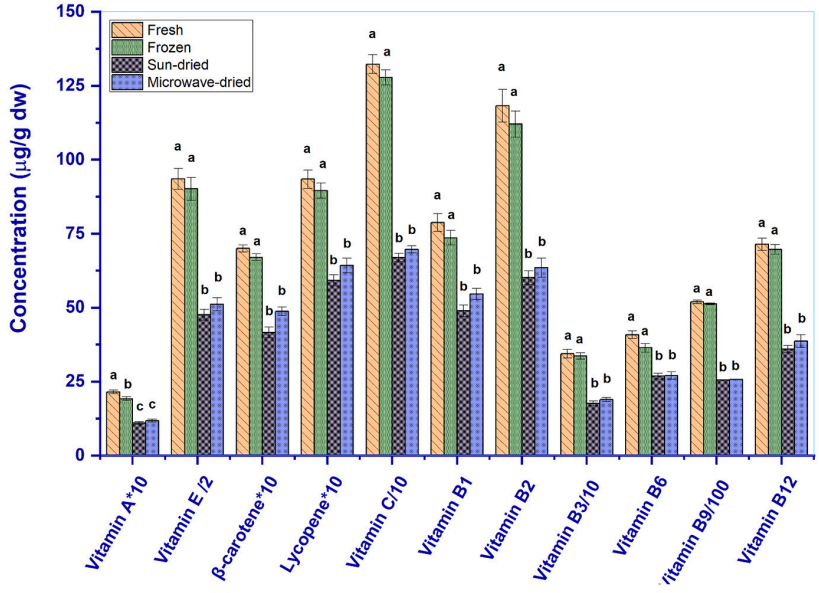

Figure 2- Fat and water soluble vitamins and lycopene content in black *M. communis* L. fruits (Vitamin A, β-carotene and lycopene values multiplied by 10, vitamin E values divided by 2, vitamin C and vitamin B_3 divided by 10, vitamin B_9 divided by 100)

It was reported that the amounts of vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_6, and vitamin B_9 in some fruit and vegetables (carrot, brinjal, okra, spinach, banana, and guava) were found between 0.2-1.8, 0.16-2.0, 0.1-1.0, 0.6-2.8 and 0.16-1.9 µg/g, respectively (Ismail et al. 2013).

The results obtained showed that vitamin content in black myrtus fruits (Table 2 and Figure 2) were higher than *Opuntia ficus-indica* fruits Bakar et al. (2020), monkey apple Onivogui et al. (2014), sweet cherry and sour cherry Ferretti et al. (2010), avocado and apricot Płonka.

It has been reported that ghrelin hormone contributes to antioxidant defence in blood and brain (Omrani et al. 2015). Once it has been considered as an animal origin, the ghrelin hormone reported to be found in fruits as well Aydin et al. (2006). Experimental results show that; the least ghrelin was found in sun-dried samples, on the other hand the highest found in fresh fruit (Table 2 and Figure 3).

The amount of ghrelin in the fruits of *Crataegus laevigata* were reported to be in the range of 18.96±6.73 to 79.96±12.14 µg/g (Mukhtar et al. 2019).

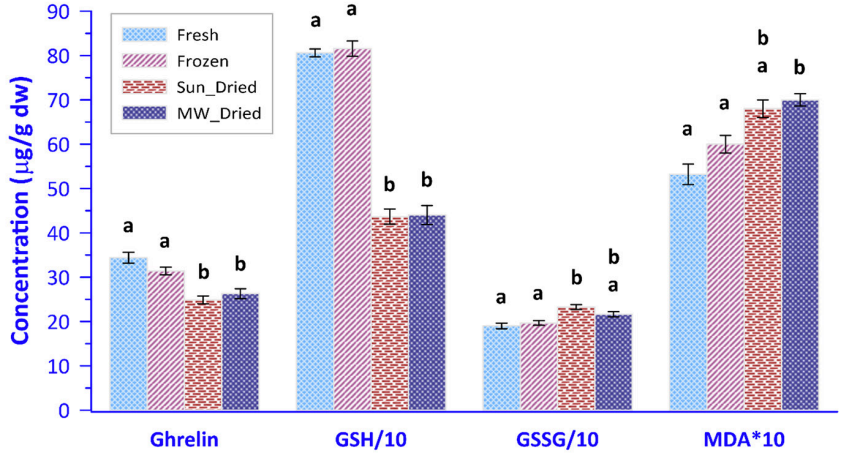

Figure 3- Ghrelin, GSH, GSSG and MDA content in black *M. communis* L fruits (GSH and GSSG values divided by 10, MDA values multiplied by 10)

Glutathione is required for the immune system of cells. Reduced glutathione is an important intracellular antioxidant molecule that is effective for transporting amino acids in metabolism and reducing sulfhydryl groups in proteins (Esterbauer et al. 1992). As seen from Table 2 and Figure 3, the amount of GSH in fresh black *M. communis* L. fruit was found to be 806.10±15.28 µg/g dw. Çakmak et al. (2021), reported that the GSH in wild white myrtus was found to be 609.90±25.80 µg/g dw. Agoreyo et al. (2017) reported that the level of glutathione in Musa *paradisiaca* L. (plantain) had 54.10±0.60 µg/g.

GSSG is an indicator of oxidative stress, the increase in the amount of GSSG inhibits protein synthesis in cells (Cnubben et al. 2001). The amount of GSSG in fresh black *M. communis* L. fruit was found to be 190.10±10.84 µg/g dw. While drying processes cause the decrease the amount of GSH, amount of GSSG had increased ($p<0.05$) (Table 2 and Figure 3).

Jones et al. (1992) reported that the amount of reduced glutathione in asparagus, avocado, apple, pear and strawberry fruits was 218, 206, 15, 33, 69, while the oxidized glutathione amount was 283, 277, 33, 50, 71 µg/g dw, respectively. GSH/GSSG ratio is used as stress biomarker, which decreases significantly as a result of drying of fruit samples ($p<0.05$) (Table 2).

Both glutathione and ghrelin are known as peptides. Preservation methods applied to foods can significantly affect the biological activity of peptides. Ultrasound, heat, and irradiation processing might affect protein structure and functions. In addition, these processes may cause Maillard reactions in food (Davis et al. 2001). As a result of the factors mentioned above, might leads to changes in the amount of peptides.

Free radicals cause lipid peroxidation by effecting the unsaturated fatty acids, resulting, MDA which is a biomarker of stress (Gaweł et al. 2004). According to the applied procedures, amount of MDA in black *M. communis* L. fruits was observed in the range between 5.32±0.40 to 7.00±0.25 µg/g dw (Table 2, Figure 3). Karatas and Kamisli (2007) reported that the drying apricot by MW and infrared increased the MDA level. The changes in the amount of MDA obtained by the drying process are consistent with the literature. Drying of *M. communis* L. fruits, cause to increase MDA level while GSH/GSSG ratio decrease which indicates the oxidative stress.

Since the moisture content of the fruits is reduced below a certain amount by drying, the shelf life of the fruits increases as they are more resistant to chemical, enzymatic and microbiological spoilage under normal atmospheric conditions. However, as a result of physical, chemical, biochemical and microbiological changes due to the effect of heat during the drying of fruits, there are losses in nutritional value.

The fact that the temperature is reduced along with the water activity in the freezing of fruits reduces the speed of chemical and biochemical reactions and microbial activities. As a result, the losses in the nutritional value of the fruits are less than in the drying process (Rickman et al. 2007).

Phenolic compounds consist of different organic molecules such as simple flavonoids, complex flavonoids, phenolic acids, and anthocyanins (Babbar et al. 2014). Total amount of phenolic substance was found to be 43.01±3.40 µg GAE/g dw in fresh black *M. communis* L. fruit. While the total phenolic substance in frozen black *M. communis* L. fruit was found to be 40.94±3.00 µg GAE/g dw, and 38.63±2.35 µg GAE/g dw in sun dried sample (Table 2 and Figure 4).

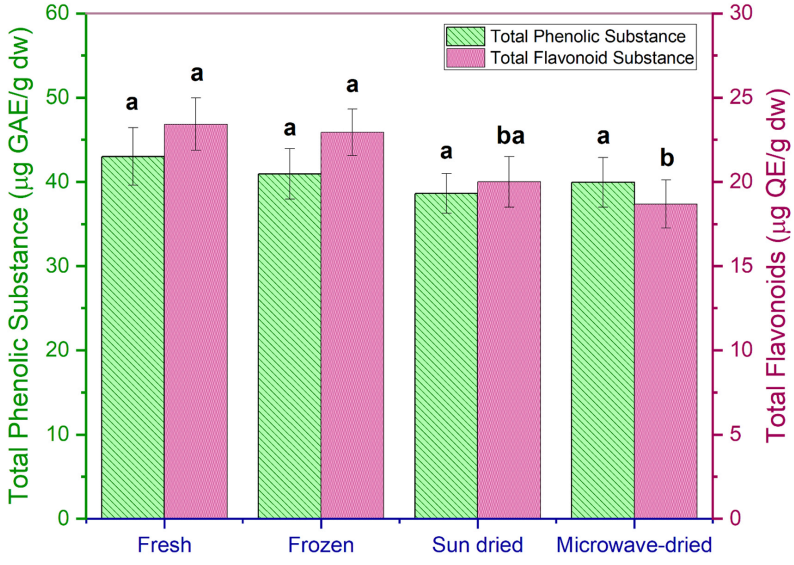

Figure 4 - Total phenolic compounds and flavonoids in black *M. communis* L fruits

The change in the amount of total phenolic compounds by different preservation methods in black *M. communis* L. fruit were found to be statistically insignificant ($p>0.05$). Zanoelo et al. (2006) reported that, after drying process the total amount of phenolic substances decrease, on the other hand, Carranza-Concha et al. (2012) found increase of phenolic compound but, Dewanto et al. (2002) reported that the total phenolic compound unchanged. As a result of the heat treatment, some phenolic compounds might be decomposed or formed a new type of phenolic compounds. Therefore, the change of the total amount of phenolic substance for different products might not be the same after drying process (Miletic et al. 2013). Flavonoids are the substances that cause the coloring of fruits and vegetables and involve in the activity of some enzymes (Panche et al. 2016). The amount of total flavonoid in fresh, frozen, sun and MW-dried black *M. communis* L. were found to be 23.42±1.55, 22.94±1.37, 20.00±1.50 and 18.68±1.43 µ*g QE/g* dw. (Table 2 and Figures 4). Hahm et al. (2015), reported that the amount of total flavonoid in *Opuntia ficus-indica* fruit as 1.91±0.29 (mg QE/g DM). The maximum loss of flavonoids was observed in MW drying process ($p<0.05$), while the least of flavonoid loss was observed in frozen fruit ($p>0.05$). This may be explained by the high temperature cause to decompose some of flavonoids during MW drying process. Drying of different vegetables and fruits under different conditions, may cause to decrease in the total flavonoid amount between 3% and 96% (Kamiloglu et al. 2015). Antioxidants are compounds that inhibit the formation of free radicals or neutralize them by transferring electrons to free radicals. Antioxidants are molecules produced from natural sources usually containing phenolic groups (Su et al. 2007).

DPPH and TEAC methods were used to determine the antioxidant capacity of the *M. communis* L. fruits. As seen from Table 2 and Figure 5, IC_{50} values in black *M. communis* L. fruits range from 28.49±2.10 to 35.29±3.10 µg/mL. Low IC_{50} values indicates high antioxidant activity. The least antioxidant capacity found in sun dried and the highest antioxidant capacity found in fresh fruit samples ($p<0.05$).

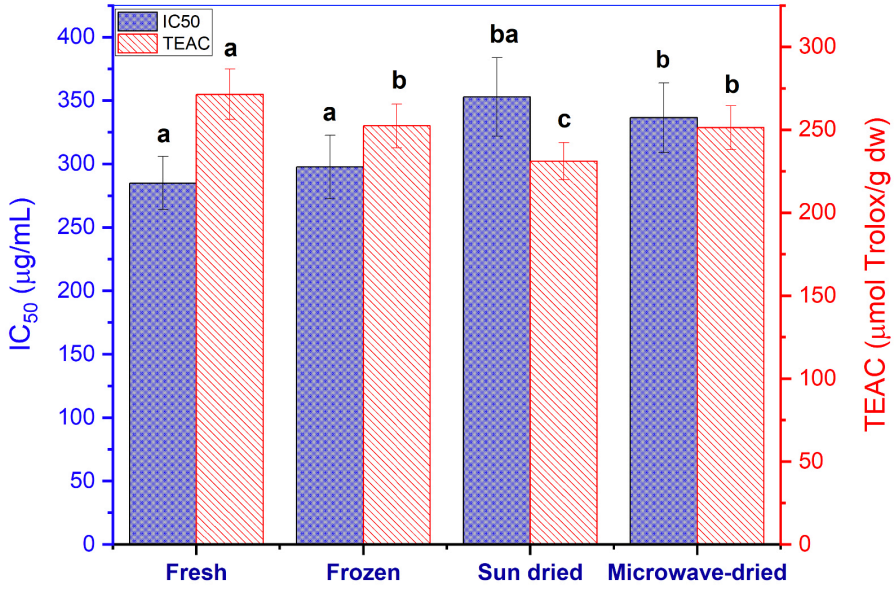

Figure 5- Total antioxidant capacity (TEAC and IC_{50} value) in black *M. communis* L fruits (IC_{50} values multiplied by 10)

IC_{50} values of mangosteen, orange, pamelo, grape and papaya fruits ranged from 11.18 to 32.80 mg/mL (Surinut et al. 2005). TEAC value of fresh black *M. communis* L. fruit was found to be 271.34±15.20 µmol troloxs/g dw. The difference of experimentally measured TEAC values of dried fruit samples in the MW was insignificant ($p>0.05$) whereas the decrease in TEAC values of sun-dried samples was significant ($p<0.05$) (Table 2 and Figure 5). Su et al. (2007) reported that the value of the antioxidant activity of rosehip fruit was 190±4.81 µmol TEAC/g. The black *M. communis* L. fruit showed higher trolox equivalents than rosehip fruit (Su et al. 2007). Major elements are found in many tissues, including the structure of bones, blood and liver, while trace elements serve as cofactors in the structure of many enzymes. Selenium is an essential trace element of several major metabolic pathways, including thyroid hormone metabolism, antioxidant defence systems, immune function. Zinc is one of the integral parts of a wide range of enzymes that is responsible catalytic, structural, and regulative role. Iron is vital for all living organisms, and it has a broad role in all metabolic activities, including oxygen transfer. Copper is one of the trace elements in the human body, and play an important role in biochemical processes. Manganese is one of the vital elements and is available in metallo proteins, such as carboxylase pyruvate and in the glial cytoplasmic enzyme, glutamine synthase (Al-Fartusie & Mohssan 2017).

The amounts of selenium, zinc, iron, copper and manganese in black *M. communis* L. were found as 0.63±0.021, 268.71±15.80, 156.36±12.75, 46.55±3.35 and 29.76±1.65 µg/g dw respectively (Table 2). Reported amount of selenium in apples, oranges, mangoes, and figs is 11, 28, 5 and 32 ng/g, respectively (Al-Ahmary 2009). Onivogui et al. (2014), reported that the amounts of zinc, iron, copper, and manganese in monkey apple (*Anisophyllea laurina R.* Br ex Sabine) were found to be 8.8, 141.4, 2.9 and 23.7 µg/g, respectively.

Essential amino acids used in protein synthesis and in metabolism must be taken with diet. Amount of amino acids determined in plants, vegetables, and fruits become important subject because one of the main source of amino acids in developing countries. In this study, the amount of essential amino acids in black myrtus fruit was found in the following order. Arginine > threonine > leucine > histidine > valine > lysine > isoleucine > methionine > phenylalanine > tryptophan. The essential amino acid content in black myrtus fruit ranges from 2.00±0.15 to 0.56±0.04 mg/g dw, while the total essential amino acid content was found to be 12.37±0.98 mg/g dw (Figure 6). Order of non-essential amino acids in the same fruit is glutamic acid > serine > aspartic acid > pyroline > glycine > alanine > cysteine > tyrosine > asparagine > glutamine. The amount of non-essential amino acids ranges from 2.69±0.20 to 0.43±0.03 mg/g dw. The total non-essential amino acid was found to be 16.04±1.38 mg/g dw.

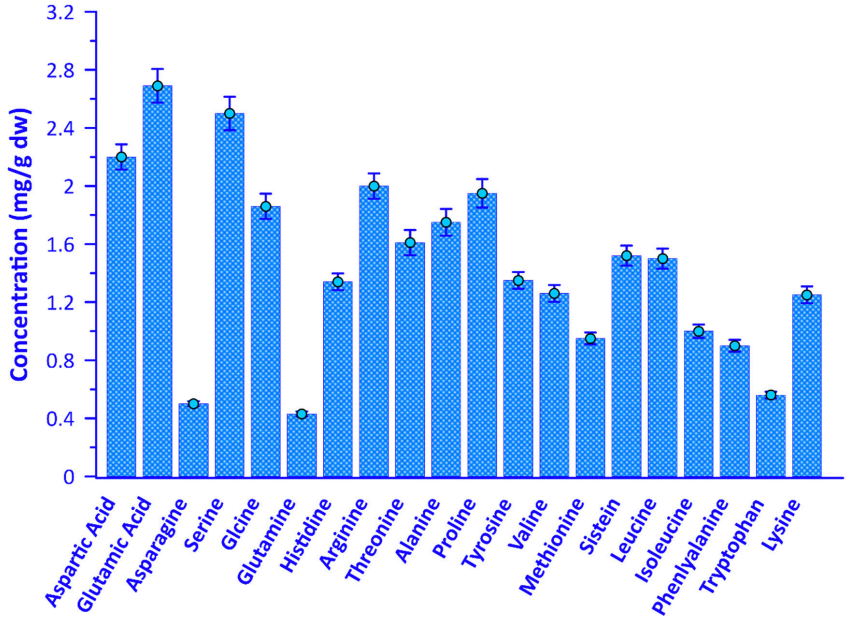

Figure 6- Amino acid composition in black *M. communis* L fruits

Glew et al (1997), reported that the amino acid content varies in between 2.64 and 0.26 mg/g, dw in *Vitex doniana* fruit and 3.94 to 0.18 mg/g dw in *Adansonia digita* (monkey bread pod). Zhou et al. (2019) studied *Nitraria tangutorum Bobr* pulp and peel grown in different regions, they reported that the total amount of essential amino acids ranged from 44.39±0.81-53.51±0.90 mg/g dw, and the total amount of non-essential amino acids ranged from 65.65±1.34-71.41±1.45 mg/g dw.

According to the Food and Agriculture Organization and the World Health Organization, the total essential amino acid/total amino acid ratio in a good protein source should be over 40%, while the total essential amino acid/total non-essential amino acid ratio should be over 60% (Zhou et al. 2019). Our results showed these ratios to be 43.5% and 77% for black myrtus fruit respectively. These results led us to say that black myrtus fruit, is a good source of essential and non-essential amino acids source.

4. Conclusions

The result obtained here in this work suggest that black *M. communis* L. fruit is a good source of nutrients in terms of fat and water-soluble vitamins, carotenes, lycopene glutathione, ghrelin, examined elements, amino acids and have a high antioxidants capacity. Content of fat and water-soluble vitamins, the total phenolic, flavonoids and antioxidant capacity in fresh and frozen black *M. communis* L. fruit samples have higher than the sun and MW-dried fruits samples.

It is also found that, while GSH/GSSG ratio is decreased, MDA level was increased in dried fruits. It can be said that drying process, induces stress to the fruits and as a result lipid peroxidation occurs. From the experimental findings, it can be said that the most suitable method for preserving *M. communis* L. fruits is freezing. In addition, MW drying seems more advantageous than drying under the sun in terms of vitamins and nutrient loss.

Data availability: Data are available on request due to privacy or other restrictions.

Authorship Contributions: Concept: D.Ö., F.K., Design: S.S., Data Collection or Processing: M.Ç., B.B., D.Ö., Analysis or Interpretation: M.Ç., B.B., D.Ö., F.K., S.S., Literature Search: M.Ç., B.B., Writing: D.Ö., F.K., S.S.

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