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Original research paper

IMPACT OF THERMAL AND ALTERNATIVE PROCESSING ON PHYTOCHEMICAL QUALITY AND ANTIOXIDANT ACTIVITY OF ALGERIAN *MYRTUS COMMUNIS* L. FRUIT

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Abstract: Myrtle (*Myrtus communis* L.) fruits serve as a crucial reservoir of biologically active and health-protective compounds. These fruits have gained increasing attention for their potential to promote human health due to their diverse range of bioactive phytochemicals. Drying, a common post-harvest treatment, can significantly affect the content and biological efficacy of these compounds. The objective of this study was to investigate the phenolic compound content and antioxidant capacity of myrtle (*Myrtus communis* L.) fruits as influenced by four different drying methods: FD (freeze drying), SD (sun drying), OD (oven drying), and MWD (microwave drying). Various bioactive compounds were quantified, including total phenolic content (TPC), total flavonoid content (TFC), total flavonol content, total condensed tannin content (CTC), and anthocyanin content (AC). Antioxidant capacity was assessed using four different tests: the DPPH radical scavenging assay (DPPH-RSA), the ABTS radical scavenging assay (ABTS-RSA), the ferric reducing power assay (FRP), and the phosphomolybdenum antioxidant activity assay (PAA). The results indicated that the drying process significantly affected the phytochemical composition and antioxidant capacity of myrtle fruit. Specifically, the freeze-drying (FD) method yielded the highest TPC, TFC, flavonols, CTC, AC, with values of 88.12 mg GAE/g DW, 12.05 mg QE/g DW, 29.99 mg RE/g DW, 75.40 mg CE/g DW, and 4.96 mg CGE/g DW, respectively. Furthermore, FD was associated with the strongest antioxidant activity, demonstrating DPPH-RSA of 143.37 mg TE/g DW, ABTS-RSA of 154.31 mg TE/g DW, FRP of 89.25 AAE/g DW, and PAA of 354.58 TE/g DW, all surpassing the other drying methods. In contrast, sun drying (SD) and oven drying (OD) had a moderate impact on phytochemical composition and antioxidant capacity, while microwave drying (MWD) resulted in the lowest levels of phytochemical content and relatively low antioxidant capacity. Additionally, the correlation test and Principal Component Analysis (PCA) confirmed the effectiveness of FD method in preserving the bioactive compounds and antioxidant activities of myrtle fruits. These findings suggest that FD is the most effective method for maintaining and enhancing the bioactive properties of myrtle fruits.

Key words: *Myrtle fruit, drying treatments, freeze drying, sun drying, oven drying, microwave drying , phenolic compounds, antioxidant capacity*

INTRODUCTION

Fruit consumption has been widely linked to health benefits, showing either protective or neutral effects against a range of chronic diseases. These include cardiovascular disease, hypertension, type 2 diabetes, certain cancers, respiratory conditions such as asthma, eye di-

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seases like cataracts, cognitive decline, mental health disorders including depression, as well as conditions affecting the digestive system and bone health (Fardet, Richonnet & Mazur, 2019). The protective effects of vegetables and fruits are attributed to various phytochemicals, particularly phenolic antioxidants. These compounds help protect humans against oxidative damage by inhibiting or neutralizing free radicals and reactive oxygen species (Mohd Zainol, Abdul-Hamid, Abu Bakar & Pak Dek, 2009).

Myrtle (*Myrtus communis* L.), as one of the most frequently cited medicinal plants in ancient books on traditional medicine, is an evergreen shrub widely growing in the Mediterranean area but also in America, Australia and Himalaya (Gorjian & Khaligh, 2023; Mahboubi, 2017). In Algeria this plant is called Rihan or Mersin and grows wild in the coastal Tell Atlas region (Benmarce et al., 2024).

A pharmacological and clinical studies found that *Myrtus communis* exhibits a wide range of biological activities, including anti-inflammatory, antimicrobial, antioxidant, antidiabetic, anticancer, dermatological, cardiovascular, neuroprotective, and gastrointestinal protecttive effects, among many others (Aykac et al., 2019; Azimi & Hasheminasab, 2020; Bagatin et al., 2023; Talebianpoor, Talebianpoor, Mansourian & Vafaiee-Nejad, 2019).

Recently, myrtle fruits have emerged as a significant interest as a valuable natural resource with potential applications in food, pharmaceuticals, and even pesticides (Aggul, Demir & Gulaboglu, 2022; Bouaoudia-Madi et al., 2022; Firooziyan et al., 2022; Kordali, Usanmaz, Cakir, Komaki & Ercisli, 2016). This growing interest is partly due to their strong antioxidant properties and rich nutrient content (Al-Maharik, Jaradat, Al-Hajj & Jaber, 2023; Taibi et al., 2025). The black myrtle fruit is gaining increasing attention due to its high antioxidant capacity. It is considered an excellent source of phenolic compounds, flavonoids, flavonols, and anthocyanins Since *M. communis* L. is a seasonal fruit, drying methods should be employed to take advantage of its therapeutic properties and richness in antioxidants year-round (Dinçer, Doğan & Erkan, 2022).

Drying is the oldest and most popular storage and preservation method that reduces water content, restricts microbiological activity, and increases the shelf life of food products (Pravallika, Chakraborty & Singhal, 2023). In addition, drying leads to the concentration of phenolic compounds, enhancing the fruit's value as a healthy product (Bassey, Cheng & Sun, 2024). Recent research has highlighted the significant influence of drying techniques on the preservation of bioactive compounds, particularly phenolics and flavonoids, in fruits and medicinal plants. Freeze drying (FD) has emerged as a preferred method, effectively retaining higher concentrations of these compounds compared to conventional methods such as sun drying (SD) and oven drying (OD). Studies show that FD minimizes thermal degradation and oxidation, preserving phenolic content (Nawawi et al., 2023; Zubia et al., 2023). In contrast, sun drying can lead to substantial losses due to prolonged exposure to heat and light, often resulting in a decrease of flavonoid levels (Ghorbani, Eghlima, Farzaneh & Rezghiyan, 2025). Moreover, microwave drying (MWD) has been recognized for its rapid processing time, yet findings indicate that it may not be as effective as FD in preserving phenolic compounds due to the high temperatures involved (Saifullah, McCullum, McCluskey & Vuong, 2019). Despite these advancements, gaps remain in understanding the long-term stability of these compounds post-drying and the impact of varying environmental conditions on their preservation. Continued exploration of innovative drying methods and their effects on bioactive compounds is essential for optimizing the quality of medicinal and aromatic plants.

Several drying methods have been proposed for drying fruits and vegetables. Freeze drying (FD) has emerged as an excellent method for drying products with heat-sensitive compounds, as it preserves the initial functional properties of these components nearly intact, resulting in a product with high aroma quality. However, its high cost, especially for large-scale commercial production, needs to be considered (Abouelenein et al., 2021; López-Parra et al., 2024).). Sun drying is still widely employed as a drying process due to its low cost, producing a product with rich color and a translucent appearance, but it comes with limitations. It is time-consuming, weather-dependent, labor-intensive, and highly exposed to potential environmental contamination (Arslan

& Özcan, 2010). Oven drying is commonly used as a traditional method for post-harvest processing and storage of various plant products due to its simplicity and efficiency. Nevertheless, the prolonged drying time and high temperatures often degrade product quality by diminishing nutritional and nutraceutical compounds, as well as affecting color and flavor. Additionally, the OD process is known for its high energy consumption and low productivity (Garcìa et al., 2021). Microwave drying (MWD) has emerged as a promising alternative drying method for various food products due to its numerous advantages, including faster drying times, lower costs, and high energy efficiency. Indeed, the volumetric heating penetrates the entire sample, unlike conventional methods. However, improper heat control and mass transfer can cause product damage. Therefore, combining microwave drying with pretreatment techniques is necessary to prevent product quality degradation (Calín-Sánchez et al., 2020)**.**

Despite their nutritional and health benefits, myrtle fruits are seasonal and perishable. Therefore, proper preservation is essential to ensure year-round availability and to minimize significant post-harvest losses. Drying process is the most common and easiest way of food preservation. Many studies have been conducted for profiling of phytochemical and antioxidant potential in myrtle fruits but limited knowledge is present regarding the effects of different drying methods on these fruits. Thus, the aim of this research was to assess the impact of different drying methods namely freeze drying (FD), sun drying (SD), oven drying (OD) and microwave drying (MWD) on phenolic compound content and antioxidant activity of Algerian *M. communis* L. fruits growing wild in Bejaia province, Algeria.

MATERIALS AND METHODS

Chemical reagents

Acetone was procured from Honeywell (Seelze, Germany). ABTS (2,2’ -Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and Trolox (6-hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid) were sourced from Sigma-Aldrich (Fisher scientific, Fair Lawn, NJ, USA). DPPH (2,2-diphenyl-1-picrylhydrazyl) disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), 4-hydroxy-3-methoxybenzaldehyde (vanillin), ferric chlorid ($FeCl_3$), sodium acetate ($C_2H_3NaO_2$), gallic acid, catechin, quercetin, ammonium molybdate ($(NH_4)_6Mo_7O_{24}$, $4H_20$) and Trichloroacetic acid were acquired from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Hydrochloric acid (HCl), Sulfuric acid (H_2SO_4) and Sodium carbonate (Na_2CO_3) were obtained from Prolabo (Loire, France). Folin–Ciocalteu’s reagent, Potassium ferricyanide ($C_6N_6FeK_3$) and chloride aluminium ($AlCl_3$), were supplied by Biochem-chemopharma (Loire, France). Analytical grade chemicals and reagents were used through the experimental study.

Plant material

Fresh, mature myrtle fruits were harvested in November 2019 from Merdj Ouamene city in Bejaia province, Algeria (northeast Algeria; latitude 36.642°; longitude 4.903°; altitude 96 m). The fruits were cleaned to remove impurities, washed thoroughly, and sliced into quarters using a knife. They were then divided into two portions: the first portion, representing the fresh fruits, was extracted immediately, while the second portion underwent various drying treatments.

Drying treatments

To investigate the effect of drying on the phenolic content of myrtle fruit, 100 g of fresh myrtle fruits were subjected to four drying methods: freeze drying (FD), sun drying (SD), oven drying (OD), and microwave drying (MWD). Drying continued until the weight stabilized over repeated measurements. Once the fruits were completely dried, they were ground into a fine powder. Each drying process was conducted in duplicate to ensure reproducibility.

Freeze drying (FD): The freeze-drying process was conducted using a laboratory-scale freeze dryer (D-37520, Christ, Germany). Sliced fresh *M. communis* L. fruits were initially frozen at –55 °C to solidify their water content. They were then subjected to primary drying under a vacuum pressure of –0.07 mbar, facilitating the sublimation of ice directly into vapor. The entire drying cycle lasted 20 hours, during which samples were weighed at regular intervals until a stable weight was achieved, indicating complete moisture removal. Secondary drying further reduced residual moisture

to minimal levels, enhancing the stability and shelf life of the freeze-dried product. All samples were processed in triplicate to ensure reproducibility and data reliability.

Sun drying (SD): The drying process involved exposing fresh *M. communis* L. fruits to natural sunlight under ambient conditions, with temperatures ranging from 25 to 27 °C. The fruits were evenly arranged in a single layer on clean drying trays to promote adequate air circulation and uniform drying. This process lasted for 120 hours (5 days), during which the samples were periodically turned to ensure consistent moisture removal and minimize the risk of microbial growth. Drying continued until the samples reached a stable weight, indicating complete moisture loss. All samples were processed in triplicate to ensure reproducibility and data reliability.

Oven drying (OD): Fresh *M. communis* L. fruits were washed, sliced, and uniformly arranged in a single layer on glass petri dishes for consistent drying. The samples were placed inside a ventilated drying oven (UF 55, Memmert GmbH, Germany) with controlled airflow to promote even heat distribution and moisture removal. Drying was conducted at a constant temperature of 50 °C for 9 hours, a condition selected to balance efficient moisture evaporation while minimizing thermal degradation of sensitive bioactive compounds. The fruits were weighed at regular intervals until a constant weight was reached, indicating the completion of drying. All experiments were performed in triplicate to ensure reproducibility.

Microwave drying (MWD): Microwave drying was carried out using a domestic microwave oven (Samsung ME6124T-1, Malaysia) operating at a constant power of 300 W. Fresh *M. communis* L. fruits were sliced and evenly spread in a microwave-safe glass container, positioned at the center of the oven to ensure uniform exposure to microwave radiation (2450 MHz).

The drying process lasted 30 minutes, during which internal moisture was rapidly removed through volumetric heating. This technique significantly reduced drying time compared to conventional methods by directly exciting water molecules within the sample. All experiments were conducted in triplicate, and mean values were recorded.

Determination of moisture content in fresh fruits

Moisture content in fresh fruits was determined in order to calculate phenolic concentrations on a dry weight basis. Briefly, in triplicate, each fresh fruit sample (10 g) was sliced into tiny pieces and was dried in an oven at 105 °C (Memmert, Germany) until weight stabilization (Correddu et al., 2019).

Phenolic compounds extraction

Both fresh and dried powdered myrtle fruits were subjected to phenolic compound extraction according to the optimized protocol of Taibi et al. (2024). Briefly, 500 mg of each sample was mixed with 20 ml of 50% acetone and extracted in a shaking water bath at 40 °C for 180 minutes. The extracts were then centrifuged at 5000 rpm for 10 minutes, filtered through Whatman paper, and stored at -20 °C before analysis. These myrtle fruit extracts were used to quantify total phenolic content (TPC), total flavonoid content (TFC), total flavonol content, total condensed tannin content (CTC), and anthocyanin content (AC), as well as to measure antioxidant capacity using various tests, including the DPPH assay, ABTS assay, ferric reducing power assay (FRP), and phosphomolybdenum antioxidant activity assay (PAA).

Evaluation of phytochemical content

Total phenolic content (TPC)

The method recommended by Singleton and Rossi (1965) was used to assess the phenolic content of both fresh and dried samples**.** This method is based on the reduction of the tungstate-molybdate complex by phenolic compounds, resulting in a color shift from yellow to blue-black, which can be measured to quantify the total phenolics present (Lawag, Nolden, Schaper, Lim & Locher, 2023).). For each extract, 0.2 ml was combined with 1 ml of 10% Folin-Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate.

The mixture was then kept in the dark at room temperature for 30 minutes, and absorbance was measured at 765 nm against a blank. The TPC of dried samples was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW), based on a calibration curve prepared with gallic acid.

Total flavonoid content (TFC)

Total flavonoid content (TFC) was evaluated using the protocol of Santas, Carbo, Gordon and Almajano (2008) with some modifications. The quantification of flavonoids was based on the formation of a flavonoid-aluminum chloride complex. This reaction causes a shift in the absorption spectrum towards longer wavelengths, resulting in an increase in light absorption at the new wavelength. The amount of light absorbed can be measured spectrophotometrically to determine the flavonoid content in the sample (Fernandes, Ferreira et al., 2012). To perform the assay, 1 ml of the sample extract was mixed with an equal volume of 2% $AlCl_3$. After incubating in the dark for 10 minutes at room temperature, the absorbance was read at 410 nm. Quercetin was used as a standard for the calibration curve, and flavonoid content was calculated as milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW).

Total flavonol content

Total flavonol content in dried and fresh fruits was estimated using the method of Yermakov, Arasimov and Yarosh (1987). To each 2 ml of extract, 2 ml of 2% aluminum trichloride and 3 ml of 50 mg/ml sodium acetate were added. After incubating for 2.5 hours at 20 °C, the absorbance was measured at 440 nm. A calibration curve with rutin as the standard was used, and total flavonol content was calculated as milligrams of rutin equivalent per gram of dry weight (mg RE/g DW).

Total condensed tannin content (CTC)

The method of Sun, Ricardo-da-Silva and Spranger (1998) was used to assess the total condensed tannin content (CTC). The vanillin-hydrochloric acid assay is a simple and widely used technique to quantify proanthocyanidins in plant material. In an acidic environment, monomeric or polymeric flavan-3-ols react with vanillin to form a red color complex detectable at 500 nm. The intensity of this red color is proportional to the amount of proanthocyanidins present in the sample (Mitsunaga, Doi, Kondo & Abe, 1998). An aliquot of 200 µl from each extract was combined with 500 µl of 1% vanillin in methanol and 500 µl of 9M hydrochloric acid in methanol. The mixture was then incubated at 30 °C for 20 minutes, and the absorbance was measured at 500 nm against a blank. Total CTC was determined using the linear equation from the catechin standard calibration curve, and results were expressed as milligrams of catechin equivalent per gram of dry weight (mg CE/g DW).

Total anthocyanin content (AC)

Total anthocyanin content in fresh and dried myrtle samples was determined using the pH differential method described by Wang and Xu (2007), employing two buffer systems: a potassium chloride buffer at pH 1.0 (0.025 M) and a sodium acetate buffer at pH 4.5 (0.4 M). Briefly, 1 ml of each sample was mixed with 4 ml of the corresponding buffer solution. The absorbance was measured at wavelengths of 510 nm and 700 nm. The following equation was used:

$$TA\ (mg\ CGE\ /g) = A \times MW \times DF \times 1000 \times V/\varepsilon \times l \times m \quad (1)$$

$$\text{Where } A = (A_{520nm} - A_{700nm})\ pH_{1.0} - (A_{520nm} - A_{700nm})\ pH_{4.5} \quad (2)$$

MW refers to the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, l is the path length of the cuvette (in cm), **ε** denotes the molar extinction coefficient of cyanidin-3-glucoside (26,900 $L \cdot mol^{-1} \cdot cm^{-1}$), 1000 is the conversion factor from grams to milligrams, V represents the total volume of extract (in mL), and m is the mass of the sample used (in g).

The anthocyanin content was expressed as milligrams of cyanidin-3-glucoside equivalents (mg CGE) per gram of sample (DW).

Determination of antioxidant capacity

To assess the antioxidant potential of the extracts, several antioxidant tests were used, including DPPH-radical scavenging activity (DPPH-RSA), ABTS-radical scavenging activity (ABTS-RSA), ferric reducing power (FRP), and phosphomolybdenum antioxidant activity (PAA) assay. These tests involve different mechanisms of action by which antioxidants counteract harmful free radicals.

DPPH-RSA assay

DPPH-RSA was determined using the method proposed by of Blois (1958). This approach relies on the ability of antioxidants to neutralize DPPH, resulting in the discoloration of the DPPH solution. Antioxidant activity is evaluated by measuring the reduction in absor-

bance at 517 nm. Briefly, 50 µl of each sample extract was mixed with 950 µl of DPPH solution (0.04% in methanol). The mixture was allowed to react in the dark at room temperature for 20 minutes, after which the change in absorbance was measured at 517 nm against a blank. DPPH scavenging activity was expressed as milligrams of Trolox equivalent per gram of dry weight (mg TE/g DW), based on the linear equation from the Trolox standard calibration curve.

ABTS-RSA assay

The method used in this assay was described by Re et al. (1999) and is based on the reduction of the $ABTS^{\bullet+}$ radical generated by oxidizing ABTS with potassium persulfate. This reduction occurs in the presence of antioxidants, leading to proportional decolorization of the solution that correlates with antioxidant concentration. Briefly, a concentrated ABTS solution of 7 mM was prepared by dissolving it in 10 ml of distilled water. This solution was mixed in equal volume with a 2.45 mM potassium persulfate solution to generate the $ABTS^{\bullet+}$ radical, incubating in the dark at room temperature for 16 hours. The stock solution was then diluted with ethanol to achieve an absorbance of 0.700 ± 0.02 at 734 nm. Next, 1.950 ml of this diluted solution was added to 50 µl of the extract. The mixture was incubated in the dark for 6 minutes at room temperature, and the absorbance was measured at 734 nm against a blank. Antioxidant activity was expressed as milligrams of trolox equivalent per gram of dry weight (mg TE/g DW), using the linear equation from the Trolox standard calibration curve.

Ferric reducing power (FRP) assay

The FRP assay for fresh and dried myrtle fruit extracts was conducted following the method of Oyaizu (1986). Compounds exhibiting reduction potential react with potassium ferricyanide, leading to the formation of potassium ferrocyanide. This compound then interacts with ferric chloride to generate a colored ferric-ferrous complex, characterized by peak absorption at 700 nm (Gupta, Karmakar, Sasmal, Chowdhury & Biswas, 2016). To prepare the solution, 1 ml of each extract was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated in a water bath at 50 °C for 20 minutes. Following this, 2.5 ml of 10% (w/v) trichloroacetic acid was added. Then, 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) $FeCl_3$ were added to 2.5 ml of the resulting mixture. After vortexing, the a-bsorbance of the colored complex was measured at 700 nm against a blank. Antioxidant activity was expressed as milligrams of ascorbic acid equivalent per gram of dry weight (mg AAE/g DW), using the linear equation from the ascorbic acid standard calibration curve.

Phosphomolybdenum antioxidant activity (PAA) assay

The method validated by Prieto, Pineda and Aguilar (1999) was employed to estimate the phosphomolybdenum antioxidant activity of the extracts. This assay relies on the reduction of Mo (VI) to Mo (V) by the antioxidants present in the sample, resulting in the formation of a green phosphate/Mo (V) complex under acidic conditions. Briefly, 0.1 ml of each extract was combined with 1 ml of reagent solution (composed of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a test tube. The tube was vortexed, sealed, and placed in a water bath at 95 °C for 90 minutes. After cooling to room temperature, the absorbance of each extract was measured at 695 nm against a blank. PAA was expressed as milligrams of Trolox equivalent per gram of dry weight (mg TE/g DW), using the linear equation from the trolox standard calibration curve.

Statistical analysis

All measurements were conducted in triplicate. Statistical analyses were performed using Statistica 12 software, with results reported as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was employed to compare the effects of various drying conditions and methods. Statistical significance was determined at $p < 0.05$. Additionally, principal component analysis (PCA) was conducted using the HJ-biplot method included in JMP Pro Version 14.0 software (SAS, USA).

RESULTS AND DISCUSSION

Total phenolic content

The TPC of fresh myrtle was measured at 252.38 mg GAE/g DW. The TPC of dried samples varied significantly depending on the

drying method employed. The freeze-dried (FD) sample recorded the highest value at 88.12 mg GAE/g DW, followed by sun drying (SD) at 51.30 mg GAE/g DW, oven drying (OD) at 52.74 mg GAE/g DW, and microwave drying (MWD) at 53.22 mg GAE/g DW. No significant differences were observed among the latter three methods (Fig. 1A).

Previous studies have reported that drying methods significantly affect the TPC in plant materials (Bassey et al., 2024; Pashazadeh, Redha & Koca, 2024; Yue et al., 2021). The notable rise in TPC values in dried samples likely stems from two factors. First, the drying process concentrates the sample molecules by removing water. Second, the heating during drying may break down large biomolecules, disrupting covalent bonds and releasing phytochemical compounds, thereby enhancing the levels of TPC in dried myrtle fruits.

These results are further supported by Alkaltham et al. (2021), who studied the effects of various drying methods (conventional oven, microwave, and room temperature drying) on antioxidant activity, total phenolic content, and individual phenolic compounds in myrtle *(M. communis* L.*)* fruits.

Our findings align with those reported in previous studies. In the research conducted by Bakar, Çakmak, Özer, Karataş & Saydam (2021), it was found that the biochemical content of dried myrtle fruits was higher with freeze drying (FD) compared to sun and microwave (MW) drying methods. Saifullah et al. (2019) confirmed that the FD method yielded the highest values for total phenolic content (TPC), total flavonoid content (TFC), proanthocyanidins, gallic acid, hesperetin, and antioxidant activity compared to other drying methods, including hot air drying, vacuum drying, microwave drying, sun drying, and shade drying, in lemon myrtle (*Backhousia citriodora*) leaves.

Yue et al. (2021) studied the effects of hot air drying (HD), microwave drying (MD), vacuum drying (VD), vacuum microwave drying (VMD), and vacuum freeze-drying (VFD) on purple cabbage, reporting that VFD resulted in the greatest retention of TPC, TFC, anthocyanins, and antioxidant capacity**.** Bi et al. (2024) also reported that FD and pulsed vacuum drying (PVD) were effective strategies for minimizing the degradation of rape bee pollen quality during the drying process.

It is important to note that precision in selecting drying conditions is crucial for achieving reliable comparisons and accurate results, as variations in these conditions can significantly impact outcomes. Some studies have confirmed that prolonged drying processes and high temperatures negatively affect TPC values, leading to decreased levels in dried samples compared to fresh samples (Alean, Chejne & Rojano, 2016; Kayacan et al., 2020; Snoussi et al., 2022).

Total flavonoid content (TFC)

As with polyphenols, the flavonoid content is also influenced by the drying method applied (Fig. 1B). The highest value was observed in the sample dried using the FD method, which recorded 12.05 mg QE/g of DW. This was followed by the SD sample at 11.68 mg QE/g of DW, the OD sample at 11.27 mg QE/g of DW, and the MWD sample at 7.15 mg QE/g of DW. The TFC of fresh myrtle was measured at 28.98 mg QE/g DW.

Our results corroborate previous studies reported in the literature. Hamrouni-Sellami et al. (2013) found that different drying methods significantly affected the flavonoid content of sage (*Salvia officinalis* L.). Periche, Castelló, Heredia and Esriche (2016) noted that most flavonoids exhibited higher concentrations when freeze drying was applied to stevia leaves, which aligns with our findings. However, Mohd Zainol et al. (2009) reported that drying methods led to flavonoid degradation, with air-oven treatment resulting in the highest total flavonoid degradation, while freeze drying resulted in the lowest degradation. This degradation is likely related to the extended drying times, which can compromise the stability of the compounds. The low degradation observed in the freeze-drying process may also be attributed to the lower temperature used compared to other drying methods. Catechin and rutin were identified as the most stable flavonoids.

Total flavonol content

Flavonols are a subgroup within the extensive family of flavonoids, exhibiting a wide range of chemical structures and characteristics (Aherne & O'Brien, 2002). Analysis by HPLC-UV at 280 nm identified flavonol gly-

colsides as the predominant phenolics in myrtle fruit extracts, accounting for 58% of the total quantified polyphenols (Barboni, Cannac, Massi, Perez-Ramirez & Chiaramonti, 2010).

Similar to total phenolic content (TPC) and total flavonoid content (TFC), the flavonol content also varied depending on the drying method used (Fig. 1(C)). The highest value was observed in the freeze-dried sample at 29.99 mg RE/g of DW, followed by the oven-dried sample at 19.20 mg RE/g of DW and the sun-dried sample at 18.05 mg RE/g of DW, which did not show significant differences from each other. The microwave-dried sample exhibited the lowest flavonol content at 14.10 mg RE/g of DW. The flavonol content of fresh myrtle fruit was measured at 59.28 mg RE/g DW.

Total condensed tannins content (CTC)

Similarly to the TPC, TFC and flavonols, the content of CTC varied depending on the drying method used (Fig. 1(D)). The FD sample exhibitted the highest value at 75.40 mg GAE/g of DW, while the MWD sample showed the lowest value at 15.40 mg GAE/g of DW. Both the OD sample (58.18 mg GAE/g of DW) and the SD sample (51.49 mg GAE/g of DW) were significantly lower than the FD sample, with $p < 0.05$ for all comparisons. The CTC of the fresh sample was measured at 220.56 mg GAE/g DW.

Our findings align with those reported by Turkiewicz,Wojdyło, Lech, Tkacz and Nowicka (2019), who studied the influence of different drying methods on the quality of Japanese quince fruit. They concluded that the MWD method caused a significant reduction in flavan-3-ol content by 30% compared to freeze drying. In contrast, drying with convective-vacuum-microwave at 70°C best preserved both flavan-3-ols and polymeric proanthocyanidins, showing levels closest to those found in freeze-dried Japanese quince fruit. Additionally, Bouaoudia-Madi et al. (2022) investigated the impact of ultrasound as a pre-treatment for microwave drying (MD) on the dehydration of myrtle *(M. communis)* fruits, their phytochemical content, and antioxidant activity. They demonstrated that microwave drying alone could lead to the degradation of phenolic compounds due to high temperatures and extended drying times. Therefore, combining microwave drying with a pretreatment such as ultrasonic treatment is a promising approach to minimize quality degradation.

Total anthocyanins content (AC)

Anthocyanins are natural pigments found in myrtle fruits (Maldini et al., 2016; Scorrano et al., 2017). Similar to TPC, TFC, flavonols, and CTC, the concentration of anthocyanins also varied depending on the specific drying method employed (Fig. 1(E)). Among the drying methods, the FD sample exhibited the highest AC at 4.96 mg CGE/g of DW. Conversely, the MWD sample displayed the lowest AC, with a value of 0.65 mg CGE/g of DW. The two remaining methods, SD and OD, showed intermediate AC values of 3.82 mg CGE/g of DW and 4.00 mg CGE/g of DW, respectively, with no significant differences between them ($p > 0.05$), both lower than the FD sample. The AC of the fresh sample was measured at 5.23 mg CGE/g DW.

Our results are consistent with those reported in the literature. Wu, Frei, Kennedy and Zhao (2010) assessed the effects of refrigerated storage and processing technologies on the bioactive compounds and antioxidant capacities of Marion and Evergreen blackberries, noting that freeze-dried Evergreen had a higher anthocyanin content. Similarly, Nemzer, Vargas, Xia, Sintara and Feng (2018) demonstrated that freeze-dried blueberries, tart cherries, and strawberries retained significantly more anthocyanins compared to those subjected to convection and refractance window drying methods. The low anthocyanin content observed in the MWD samples is likely due to two mechanisms. First, the high temperatures and pro-longed irradiation times during microwaving can directly degrade anthocyanins (De la Fuente-Blanco, De Sarabia, Acosta-Aparici, Blanco-Blanco & Gallego-Juárez, 2006). Second, furfural compounds generated by the thermal degradation of sugars during MWD may further promote anthocyanin breakdown (Sun, Zhang, Xu & Zheng, 2020). Additionally, Charmongkolpradit, Somboon, Phatchana, Sang-aroon & Tanwanichkul (2021) indicated that high temperatures significantly reduce anthocyanin levels, particularly at 80 °C, suggesting that lower temperatures may be more suitable for preserving these compounds.

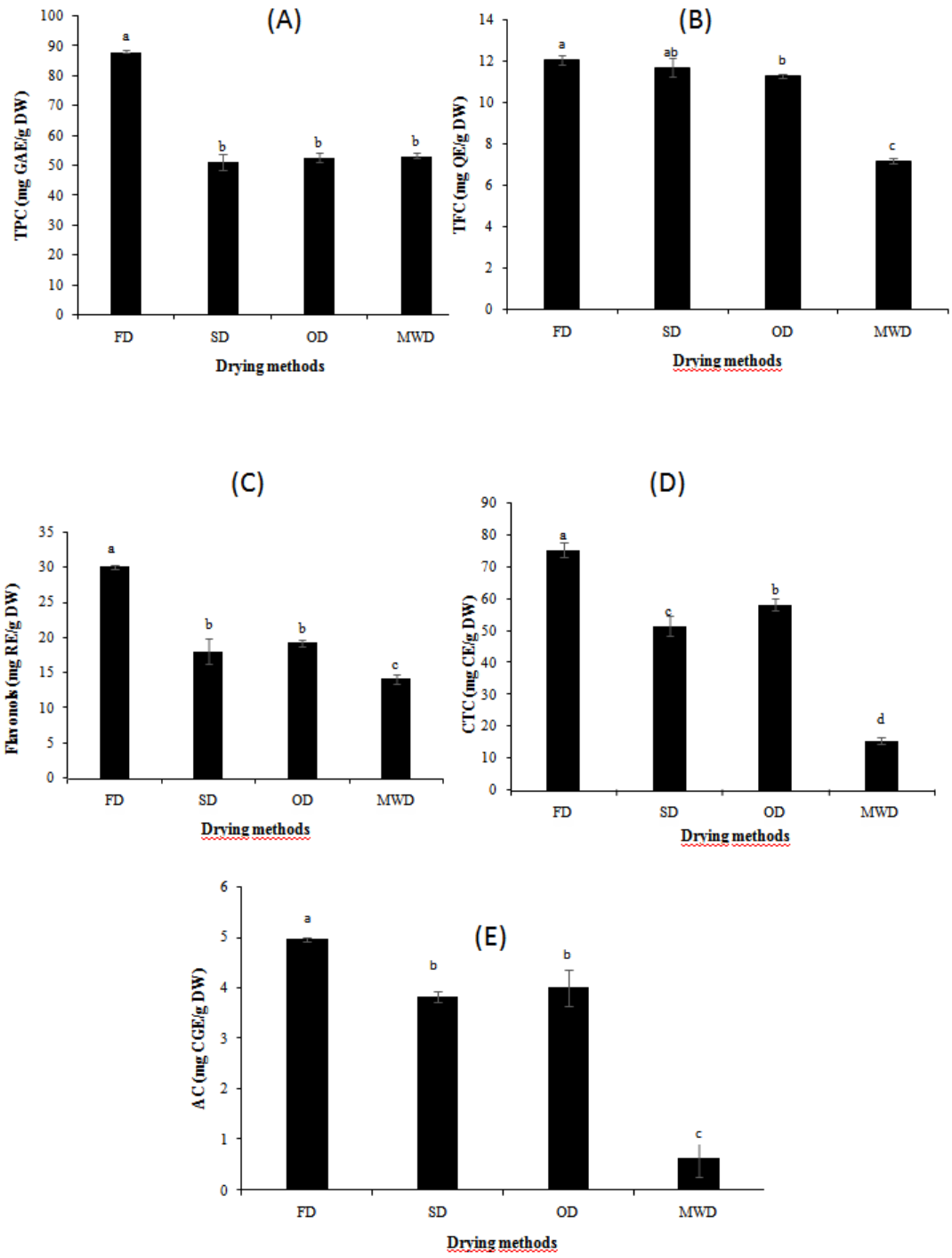

Figure 1. Influence of drying method (FD: freeze drying, SD: sun drying, OD: oven drying and MWD: microwave drying) on the extraction of total phenolic content (A), total flavonoid content (B), flavonols (C), condensed tannins content (D), and anthocyanins content (E) from myrtle (*Myrtus communis* L.) fruit (n=2)*. Values are presented as means ± SD of six measurements. Values identified by different lowercase letters (a-d) show significant differences ($p<0.05$). *Repeat extractions

Antioxidant activity

The DPPH-RSA recorded the highest antioxidant activity in the FD sample, with a value of 143.37 mg TE/g of DW. This was followed by the OD sample at 125.30 mg TE/g of DW the MWD sample at 124.01 mg TE/g of DW, and the SD sample at 122.57 mg TE/g of DW. No significant differences were observed among the latter three drying methods (Fig. 2A). The DPPH-RSA of fresh sample was 383.36 mg TE/g DW.

Similar results were obtained for the ABTS-RSA, where the highest value was also recorded in the FD sample at 154.31 mg TE/g of DW. The OD and MWD samples displayed intermediate values of 100.88 mg TE/g of DW and 99.05 mg TE/g of DW, respectively, with no significant difference between them **(**Fig. 2B). The SD sample exhibited the lowest antioxidant activity at 89.24 mg TE/g of DW. The ABTS-RSA of fresh myrtle fruits was 310.91 mg TE/g DW.

Consistent with the DPPH-RSA and ABTS-RSA, the FRP also varied with the drying method employed. The FD sample exhibited the highest value at 89.25 mg AAE/g of DW. The MWD, OD, and SD samples showed intermediate values of 49.96 mg AAE/g of DW, 47.11 mg AAE/g of DW, and 41.51 mg AAE/g of DW, respectively (Fig. 2C). The FRP of fresh myrtle fruits was 230.58 mg AAE/g DW.

For the PAA, a similar trend was observed; the amounts varied depending on the drying method used (Fig. 2D). The FD sample again exhibited the highest PAA at 354.58 ± 5.47 mg TE/g of DW, followed by the OD sample at 262.21 mg TE/g of DW, the SD sample at 235.65 mg TE/g of DW, and the MWD sample at 152.21 mg TE/g of DW (Fig. 2D). The PAA of fresh myrtle fruits was 441.06 mg TE/g DW.

Our findings align with those reported in previous studies of various plants. Das, Raychaudhuri and Chakraborty (2012), studied the effects of freeze drying and oven drying on the antioxidant properties of fresh wheatgrass and concluded that FD yielded the highest values in both DPPH-RSA and FRP assay. Valadez-Carmona et al. (2017) investigated the impact of microwave, hot air (HAD), and freeze-drying on the phenolic compounds, antioxidant capacity, enzyme activity, and microstructure of cacao pod husks *(Theobroma cacao* L.*).*

They found that FD resulted in the greatest increase in antioxidant capacity, followed by MWD and HAD methods. The ABTS assay showed a two-fold increase after HAD, a three-fold increase after MWD, and a four-fold increase after FD. Similarly, the DPPH assay demonstrated increases of two, four, and five-fold after HAD, MWD, and FD, respectively.

The observed increase in antioxidant capacity may be attributed to enhanced extraction of phenolic compounds during the drying processes.

Principal component analysis

Figure 3 illustrates the results of the principal component analysis (PCA) examining the effects of different drying methods (freeze drying (FD), microwave drying (MWD), oven drying (OD), and sun drying (SD)) on the phenolic compounds and antioxidant capacity of myrtle fruits. Two principal components (PCs) characterized the total phenolic content (TPC), total flavonoid content (TFC), flavonols, condensed tannins (CTC), and antioxidant capacities (DPPH-RSA, ABTS-RSA, FRP, and PAA) of myrtle fruits. The first principal component (PC1) had the highest eigenvalue of 7.43 and accounted for 82.6% of the variability in the dataset. The second principal component (PC2) had an eigenvalue of 0.95 and accounted for 10.6% of the variance. Together, these components explained a total of 93.2% of the variability in the plotted data.

In the biplot, the angles between the parameter vectors indicate their correlations: acute angles (less than 90 °) represent positive correlations, obtuse angles (greater than 90 °) or straight angles (180 °) indicate negative correlations, and right angles (90 °) suggest no correlation (Özcan et al., 2020). The biplot (Fig. 3) shows acute angles between the vectors of bioactive compounds (TPC, TFC, flavonols, CTC, and antioxidant capacities) and the various antioxidant assays (DPPH-RSA, ABTS-RSA, FRAP, and PAA), suggesting a strong positive correlation.

Furthermore, the freeze-dried (FD) samples are positioned on the far right, close to the variable arrows, indicating their high effectiveness in preserving these compounds. In contrast, sun-dried (SD) and oven-dried (OD) samples are positioned to the right, near the origin, reflecting moderate effectiveness in retaining antioxidants. Microwave-dried (MWD) samples, located on the left, suggest negative correlations with bioactive compounds and antioxidant activity.

These findings indicate that both the levels of antioxidants and their interactions with other plant constituents can significantly impact the antioxidant capacity of plant extracts. This aligns with observations by Terpinc, Čeh, Ulrih and Abramovič (2012), who noted that samples with similar total phenolic concen-

trations can exhibit substantial differences in antioxidant activity due to synergistic and antagonistic interactions among antioxidants.

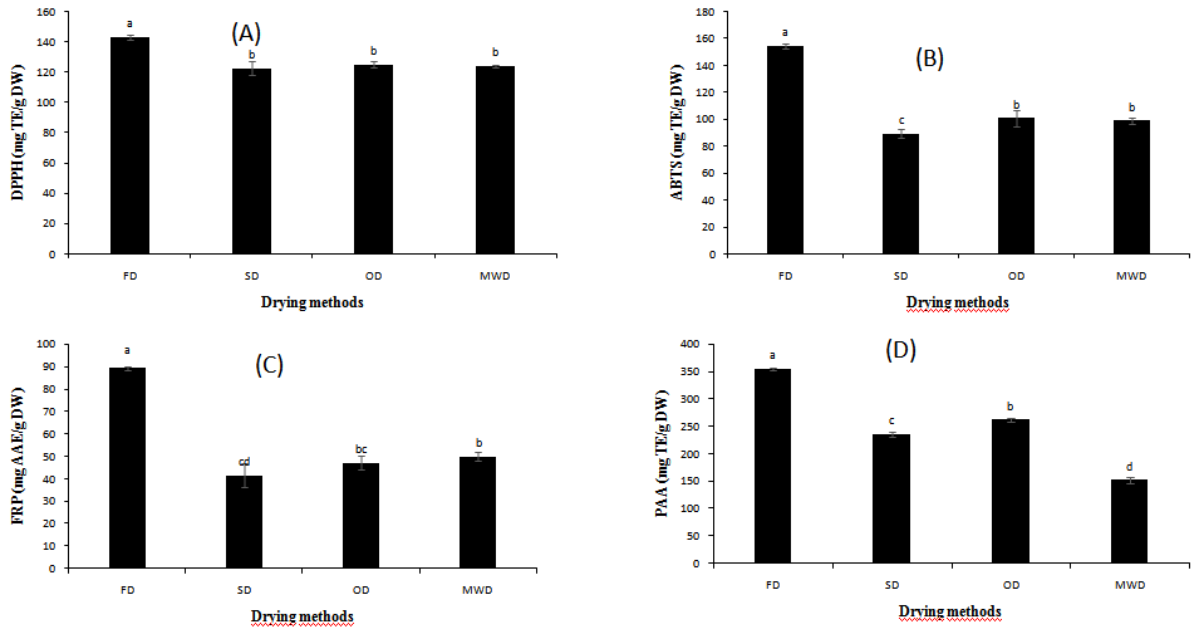

Figure 2. Influence of drying method (FD: freeze drying, SD: sun drying, OD: oven drying and MWD: microwave drying) on the antioxidant activity (DPPH (A), ABTS (B), ferric reducing power (C) and phosphomolybdenum antioxidant activity (D)) from myrtle (*Myrtus communis* L.) fruit (n=2)*. Values are presented as means ± SD of six measurements. Values identified by different lowercase letters (a-d) show significant differences ($p<0.05$). *Repeat extractions

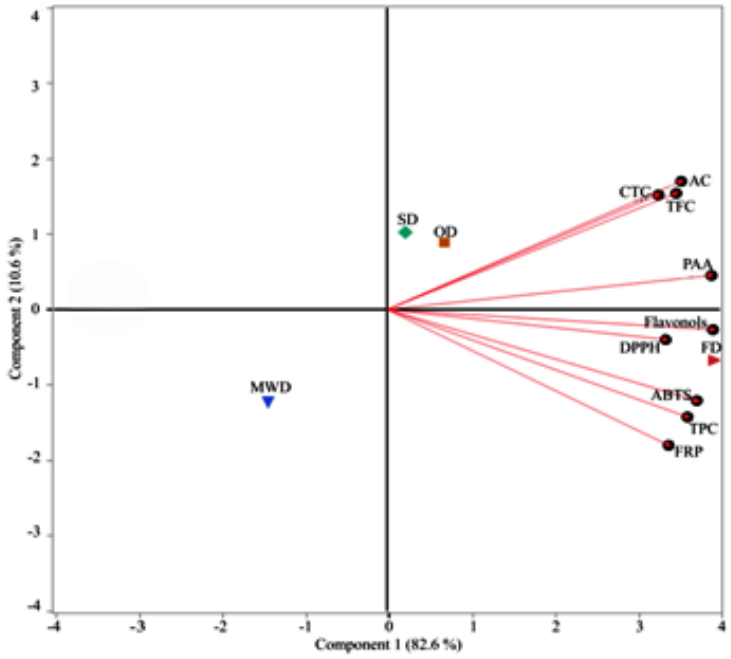

Figure 3. Principal component analysis biplot of phenolic compounds (TPC: total phenolic content, TFC: total flavonoids content, flavonols, CTC: total condensed tannins content, AC: total anthocyanins content) and antioxidant capacity (DPPH, ABTS, FRP: ferric reducing power and PAA: phosphomolybdenum antioxidant activity) in fresh and dried myrtle fruit samples. (FD: freeze drying, SD: sun drying, OD: oven drying and MWD: microwave drying)

CONCLUSIONS

Myrtus communis L. fruits are an important source of bioactive compounds but are prone to spoilage due to their high moisture content. Drying offers an effective solution for presserving these valuable fruits. In this study, we investigated the influence of different drying methods, including FD, SD, OD, and MWD on the content of phenolic compounds (TPC, TFC, flavonols, CTC, and AC) as well as antioxidant capacity (DPPH-RSA, ABTS-RSA, FRP, and PAA) in myrtle fruits growing wild in Bejaia province, Algeria. Among the four drying methods assessed, freeze drying emerged as the most effective for presserving both phytochemicals and antioxidant properties of myrtle fruits.

However, while freeze drying offers superior retention of bioactive compounds, it also has significant disadvantages. This method is typically more costly and energy-intensive compared to other drying techniques.

Additionally, the complex equipment required for freeze drying may not be accessible in all settings, limiting its practicality for large-scale applications. In contrast, sun drying, oven drying, and microwave drying generally preserve good levels of most phenolic compounds and antioxidant capacity in myrtle fruits.

However, microwave drying resulted in lower levels of condensed tannins and anthocyanins, likely due to the degradation of these bioactive molecules. Additionally, the correlation test and Principal Component Analysis (PCA) confirmed the effectiveness of FD method in preserving the bio-active compounds and antioxidant activities of myrtle fruits.

These findings indicate that myrtle fruit is an excellent source for recovering bioactive compounds with beneficial functional properties, making it suitable for the development of functional foods and nutraceuticals. Ultimately, while freeze drying is effecttive for conserving healthy bioactive components, its practical limitations should be considered when selecting a drying method for commercial applications.

AUTHOR CONTRIBUTIONS

Conceptualization, M.T., Y.A. and S.W.; Methodology, L. B. and K.M.; Investigation, formal analysis, validation, writing-original draft preparation, T.A. and M.A.; Writing-review and editing, K.A. and F.G.; Supervision, M.A.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. This work was supported by the Algerian Ministry of Higher Education and Scientific Research and did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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UTICAJ TERMIČKE I ALTERNATIVNE OBRADE NA FITOHEMIJSKI KVALITET I ANTIOKSIDATIVNU AKTIVNOST PLODOVA ALŽIRSKE MIRTE (*MYRTUS COMMUNIS* L.)

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Sažetak: Plodovi mirte (*Myrtus communis* L.) predstavljaju ključni rezervoar biološki aktivnih jedinjenja korisnih po zdravlje. Ovi plodovi su sve više u centru pažnje zbog svog potencijala da promovišu ljudsko zdravlje zahvaljujući raznovrsnim bioaktivnim fitojedinjenjima. Sušenje, uobičajena posleberbena obrada, može značajno uticati na sadržaj i biološku efikasnost ovih jedinjenja. Cilj ove studije bio je da istraži sadržaj fenolnih jedinjenja i antioksidativni kapacitet plodova mirte *(Myrtus communis* L.*)* pod uticajem četiri različite metode sušenja: FD (sublimaciono sušenje), SD (sušenje na suncu), OD (sušenje u rerni) i MWD (mikrotalasno sušenje). Kvantifikovani su sadržaji različitih bioaktivnih jedinjenja, uključujući ukupne fenole (TPC), ukupne flavonoide (TFC), ukupne flavonole, ukupne kondenzovane tanine (CTC) i antocijanine (AC). Antioksidativni kapacitet procenjen je pomoću četiri različita testa: DPPH test a (DPPH-RSA), ABTS test (ABTS-RSA), test redukcije gvožđa (FRP) i test fosfomolibdenske antioksidativne aktivnosti (PAA). Rezultati su pokazali da proces sušenja značajno utiče na fitokemijsku sastav i antioksidativni kapacitet plodova mirte. Konkretno, metoda sublimacionog sušenja (FD) dala je najveće vrednosti TPC, TFC, flavonola, CTC i AC, sa vrednostima od 88,12 mg GAE/g DW, 12,05 mg QE/g DW, 29,99 mg RE/g DW, 75,40 mg CE/g DW i 4,96 mg CGE/g DW. Pored toga, FD je bila povezana sa najjačom antioksidativnom aktivnošću, pokazujući DPPH-RSA od 143,37 mg TE/g DW, ABTS-RSA od 154,31 mg TE/g DW, FRP od 89,25 AAE/g DW i PAA od 354,58 TE/g DW, svi nadmašujući druge metode sušenja. Nasuprot tome, sušenje na suncu (SD) i sušenje u rerni (OD) imale su umeren uticaj na fitohemijski sastav i antioksidativni kapacitet, dok je mikrotalasno sušenje (MWD) rezultiralo najnižim nivoima fitohemikalija i relativno niskim antioksidativnim kapacitetom. Pored toga, test korelacije i analiza glavnih komponenti (PCA) potvrdili su efikasnost FD metode u očuvanju bioaktivnih jedinjenja i antioksidativnih aktivnosti plodova mirte. Ove nalaze sugerišu da je FD najefikasnija metoda za očuvanje i unapređenje bioaktivnih svojstava plodova mirte.

Ključne reči: *plodovi mirte, tretmani sušenja, sublimaciono sušenje, sušenje na suncu, sušenje u rerni, mikrotalasno sušenje, fenolna jedinjenja, antioksidativni kapacitet*

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