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DETERMINATION OF QUALITY CHARACTERISTICS IN MATURE PARSLEY (*Petroselinum hortense*) PLANTS, PARSLEY MICROGREENS, AND PRIMED PARSLEY MICROGREENS

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Abstract: The term "microgreen" describes tiny seedlings of edible plants that have cotyledon leaves that form in the first 1-2 weeks after planting. Microgreen is a new topic in vegetable growing and has the potential to provide significant profits in a short time if marketing opportunities are found. For this reason, it is an important issue to compare microgreens with their mature forms and evaluate them in terms of their contribution to our health. In this study, mature parsley plants, parsley microgreens and primed parsley micro greens were compared in terms of yield and some biochemical properties. The study was carried out in greenhouse conditions at Çanakkale Onsekiz Mart University, Faculty of Agriculture, Dardanos Farm in spring and summer of 2023. Seeds of a standard parsley variety (*Petroselinum hortense* cv. Toros) were used as plant material in the experiment. In the study, ascorbic acid (mg/100g), pH, Titratable Acidity (TEA), water-soluble dry matter (WSDM), apigenin amount and yield (g/m^2) parameters were examined. At the first harvest, parsley microgreens had more yield in a shorter time compared to mature parsley plants. The yield has increased especially with the priming application. The amount of ascorbic acid was found to be statistically ($P<0.05$) less in parsley micro greens than in mature parsley plants. The highest amount of apigenin was obtained from primed parsley microgreens.

Keywords: Apigenin, Ascorbic acid, Microgreens, *Petroselinum hortense*, Yield

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1. Introduction

The microgreens market is expected to reach $3.4 billion by 2030, showing a bright future for the industry. While North America dominates the market, the Asia Pacific region is experiencing rapid growth (Anonymous. 2024). Microgreens, which include various vegetables, medicinal plants, and herbaceous plants characterized by thin structures, cotyledon leaves, and early harvesting, are highly diverse in terms of color, structure, and flavor (Bhatt and Sharma, 2018). These plants possess delicate tissues and can be incorporated into a variety of dishes such as salads, soups, and sandwiches (Choe et al., 2018). The cultivation of microgreens can be a profitable business opportunity when a market demand exists. Although microgreen cultivation is not widely recognized in the Çanakkale region and current production is limited, there is a growing potential for marketing these products, particularly within local restaurants, as awareness of microgreens increases.

Priming is a process that involves soaking seeds under controlled conditions (imbibition) to improve germination and promote the early onset of germination events, followed by drying the seeds to their initial moisture content (Varier et al., 2010). Microgreens are closely related to seed priming, as their production involves the sowing of various seeds, and priming is known to enhance seed germination potential. In a study conducted by Tok and Kurt (2019), parsley seeds were collected from the Arsuz and Samandağ districts in Hatay province, and it was reported that 80% of the healthy, non-infected parsley seeds successfully germinated. In another study (Khan et al., 2023) on curly-type parsley seeds, germination was tested at 25±2°C and the control group showed a germination rate of approximately 70-80%, while seeds subjected to 24-hour hydropriming exhibited a germination rate of 78.7%. In a study by Dimita et al. (2022), it was observed that the total phenolic contents in the microgreen stage of Chinese red basil (*P. frutescens* var. *crispa*) and Chinese green basil (*P. frutescens* var. *frutescens*) varieties were lower than in their mature stages. However, the amount of volatile organic compounds during the microgreen stage of Chinese red basil (*P. frutescens* var. *crispa*) was approximately twice as high (236.83 ng/g FW) compared to the mature stage (127.16 ng/g FW) (Dimita et al., 2022). In lettuce microgreens (*L. sativa* L. var. *capitata*), the contents (g kg^{-1}) of several minerals (Ca, Fe, Mn, Zn, Mo, Se) were found to be statistically higher than in their

mature stages (Pinto et al., 2015).

Farzaei et al. (2013) mentioned in their studies that parsley has been used in the treatment of diseases such as hypertension, heart disease, and diabetes, and that flavonoid compounds, especially apigenin, apiin, and 6"-Acetilapiin have been detected in parsley. Abid et al. (2022) described apigenin as a flavonoid that has long been recognized as a traditional immune-stimulating agent, with health-promoting properties against various cancers, cardiovascular diseases, and other ailments. Considering the study by Farzaei et al. (2013), it is important to investigate the changes in apigenin content in parsley, which is particularly rich in flavonoids such as apigenin, apiin, and 6"-acetylapiin as a result of various treatments. At the same time, it is important to evaluate the topic of microgreens through comparisons made with the mature form of the plants.

The aim of the study is to compare the yield and certain quality parameters of both microgreens and microgreens derived from seeds subjected to priming treatment, with those of mature parsley plants (*Petroselinum hortense* cv. Toros).

2. Materials and Methods

The study was carried out in greenhouse conditions at Çanakkale Onsekiz Mart University, Faculty of Agriculture, Dardanos Farm in Spring and Summer of 2023. In the study, parsley plants, parsley microgreens and primed parsley microgreens were determined as the subjects and these three were compared in terms of yield and some biochemical properties. In the experiment, the seeds of a standard parsley variety *(Petroselinum hortense* cv. Toros) were used as the plant material.

The priming treatment applied to parsley microgreens was conducted by soaking parsley seeds (*Petroselinum hortense*) in water at 10°C for 12 hours, which was found to be the most effective treatment for germination rate among the priming methods for parsley (Dursun and Ekinci, 2010).

The seeds were weighed before priming, and after priming, they were spread on filter papers in a 40×40 cm tray in a shaded and ventilated environment and dried to their initial weight (Varier et al., 2010).

The germination capacity of the vegetable seeds was determined according to Ellis and Roberts (1980). The germination rate of seeds without priming was determined to be 70%, while the germination rate of primed seeds was found to be 80%.

The growing medium in the experiment was prepared by mixing 2/3 peat with 1/3 perlite to retain the applied water.

Rectangular containers with dimensions of 36×27×7 cm were used in the experiment. Two holes were made at the bottom of each container using a soldering iron to allow for excess water drainage.

The containers have a surface area of 36×27 = 972 cm^2. The mature vegetables were planted in each container with 6 plants spaced evenly, providing each plant with a surface area of 13.5×12 = 162 cm^2. The seeds of the vegetables, considered to be mature, were first sown in the compartments of the seedling trays. Once germination occurred, the seedlings were transferred to containers containing the same growing medium, with the root zone remaining undisturbed, along with all the growing medium from the seedling tray. Prior to planting, an equal amount of growing medium, equivalent to the growing medium volume in the seedling trays, was added to the containers where the microgreens would be grown. This ensured that an equal amount of growing medium was used in each container. During the microgreens cultivation stage, parsley seeds were sown in containers with a surface area of 972 cm^2 at a seeding density of 5 seeds per cm^2 (Carillo et al., 2022). Initially, the required number of seeds per container was calculated as 5 × (972 cm^2) = 4860. The amount of seeds to be sown was determined based on their germination capacity. Additional seeds were added in proportion to the amount of seeds that do not germinate. The added seeds are assumed to be selected from those with a germination rate of less than 100%, and the calculations were made accordingly.

The germination capacity of the seeds used was determined to be 70%, and the thousand seeds weight was 2.3 g. As a result, for microgreens cultivation, 15.96 g of seeds were used per container (with a surface area of 972 cm^2). 4860 (972 x5) seeds were sown in a container with the calculation of 5 seeds per cm^2. The same amount of seed (15.96 g) was used for the subject to which priming was applied, and then priming was applied. It was observed that 164.2 g of seeds should be used for 1 m^2 area. The primed and non-primed seeds were evenly spread in the container filled with growing medium, and their surfaces were covered with a 0.5 cm layer of growing medium. To enhance uniform distribution, the surface area of the container and the seed quantity were divided into eight sections, with the seeds being spread separately in each section.

Daily, in the evening, water was applied to the containers until water emerged from the evenly distributed holes at the bottom of the containers. To promote the elongation of microgreens and prevent excessive thickening of the hypocotyls, a 40% shaded mesh material was used 20 days after sowing. Microgreens can be harvested when the cotyledon leaves are fully grown or when the plant has its first two true leaves (Gerovac et al., 2016; Waterland et al., 2017; Li et al., 2021). Di Gioia et al. (2023) defined the commercial harvesting stage of microgreens as the time when the cotyledons are fully developed and the first true leaves begin to grow. In our study, microgreens were harvested at the stage when the cotyledons were fully grown and the first true leaves began to grow (Di Gioia et al., 2023).

2.1. Measurements and analyses performed in the study

Ascorbic Acid Content (mg/100g): The ascorbic acid (vitamin C) content of the microgreens was analyzed

according to the Pearson and Churchill (1970) method. For each sample, 175 ml of 0.4% Oxalic Acid was added to 25 g of sample. L1 value was determined by reading of Oxalic acid/2.6 Diclorophenol indophenol: 1/10 solution in response to Oxalic acid/Pure water: 1/10 solution at 520 transmittance value. L2 value was determined by reading of filtered sample/2.6 Diclorophenol indophenol: 1/10: solution in response to Oxalic acid/Pure water: 1/10 solution at 520 transmittance value. In this way, ascorbic acid content was calculated by using the formulation.

Water-Soluble Dry Matter (%): It was found by direct reading as a percentage value with a hand refractometer, with 3 readings in each repetition. pH Value and Titratable Acidity (TETA) (g/100g): It was determined according to the titration method using 0.1 N NaOH in the samples. The titratable total acidity (g/100g) was calculated by formula in terms of citric acid (Jadczak et al., 2019) by finding the NaOH value detected when the pH value was 8.1 with the help of burette and digital desktop pH meter (WTW, Bavaria, Germany) (Anonymous, 1968).

Calculation of Apigenin Content (mg/kg): HPLC Method: System Used: Shimadzu Prominence Brand HPLC, CBM: 20ACBM, Detector: DAD (SPD-M20A), Column Oven: CTO-10ASVp, Pump: LC20 AT, Autosampler: SIL 20ACHT, Computer Program: LC Sotution; Mobile Phase: A: 3% Formic acid B: Methanol (The method of Gomes et al. (1999) was modified and used in HPLC analysis).

Sample preparation: 2 g sample was taken. 10 ml of 96% ethanol was added to it. It was mixed in the homogenizer for 2 minutes. It was kept in a water bath at 45°C for 1 night. At the end of this period, it was centrifuged at 4000 rpm for 5 minutes. The supernatant was taken and evaporated in a rotary evaporator at 45°C until it was completely dry. The extracts were then dissolved in 1 ml of methanol and used in phenolic compound analyzes (Kiselev et al., 2007).

Yield at First Harvest (g/m^2): The yield for the first harvest has been determined, along with the time required to achieve it.

All analyses and measurements in microgreens were conducted at harvest maturity (Di Gioia et al., 2023). In parsley, all analyses and measurements were performed on leafy and stemmed portions (up to the first point of leaf-stem attachment, excluding thick stems not suitable for consumption) for the first harvest.

The experiment was designed according to a randomized complete block design (Table 1) with three replications, each replication consisting of one container (each container containing 6 parsley plants or microgreens). Statistical analyses were performed using the SAS 9.0 software package, with analysis of variance (ANOVA) conducted, and differences between means were compared using the LSD test (P<0.05). Biplot analysis was used to interpret data for different quality parameters, and the data were also evaluated graphically.

Table 1. Trial design of the study.

Replication I	Replication II	Replication III
Mature Parsley	Parsley Microgreens	Primed Parsley Microgreens
Parsley Microgreens	Primed Parsley Microgreens	Mature Parsley
Primed Parsley Microgreens	Mature Parsley	Parsley Microgreens

3. Results and Discussion

Microgreens reached sufficient maturity in 25 days, while mature parsley reached maturity in 50 days. Yield values were given in Table 2. The yield at the first harvest was highest to lowest in the following order: primed parsley microgreens, parsley microgreens, and mature parsley (P<0.05).

Table 2. Yield values (g/m^2) at the first harvest of parsley at maturity stage, microgreens and primed microgreens*

Subjects	Yield Values (g/m^2) at the First Harvest
Mature Parsley	667.7 C
Parsley Microgreens	765.6 B
Primed Parsley Microgreens	886.4 A
LSD (P<0.05):	60.278

*Means followed by the same letter within a column are significantly different according to the least significant difference (LSD) test.

In a study (Carillo et al., 2022), parsley microgreens were grown in a peat-based growth medium with Hoagland solution under different light-emitting lighting systems in a climate chamber (5 seeds cm^{-2}), and the yield ranged from 1 to 2 kg m^{-2} (fresh weight). In another study (Thuong and Minh, 2020), the highest yield in red radish (*Raphanus sativus*) microgreens was not obtained from the highest seed planting density. This suggests that a gradual increase in seed planting density may not necessarily result in a corresponding gradual increase in yield.

It has been determined that the priming application in parsley microgreens increased the yield compared to non-primed microgreens (P<0.05) in the current study. Dursun and Ekinci (2010) conducted priming treatments on parsley (*Petroselinum crispum* L.) seeds using different materials at various temperatures and durations. In their study, the germination rate was determined to be 49.25% at 25°C, while the highest germination rate (90%) was achieved with a 12-hour hydropriming treatment at 10°C. In our study, it was observed that subjecting parsley seeds to a 12-hour priming treatment at 10°C had a positive effect on the yield of parsley microgreens. In a study conducted by Tamindzic et al. (2023), hydropriming (10 hours at room

temperature) was applied to pea varieties (*Pisum sativum* L. cv. E-244, Dukat, Partner), and it was found that hydropriming increased germination (%) and fresh shoot weight (g) in the Dukat pea variety. Additionally, hydropriming treatment on curly-type parsley seeds led to an increase in germination percentage (Khan et al., 2023).

In the current study, the lowest ascorbic acid content was obtained from the microgreens that did not undergo priming. It was observed that the microgreens subjected to priming and the mature parsley plants had statistically (P<0.05) the same ascorbic acid content. However, the highest ascorbic acid content was obtained from the mature plants (Table 3).

Table 3. Ascorbic acid contents (mg/100g) at the first harvest of parsley at maturity stage, microgreens and primed microgreens*

Subjects	Ascorbic Acid Contents (mg/100g)
Mature Parsley	234.94 A
Parsley Microgreens	176.15 B
Primed Parsley Microgreens	214.07 A
LSD (P<0.05):	28.231

*Means followed by the same letter within a column are significantly different according to the least significant difference (LSD) test.

In a study conducted by Karkleliene et al. (2014), the ascorbic acid content in five different parsley varieties (*Petroselinum crispum* cv. Moss Curled, Astra, Festival, Gigant D'Italia, and Average) ranged from 138.4 to 162.8 mg/100g. In another study conducted by Jadczak et al. (2019), the ascorbic acid content in the leaves of seven parsley varieties varied between 132.41 and 210.52 mg/100g (fresh weight).

Parsley (*Petroselinum crispum* cv. Comune) microgreens were grown in a peat-based growth medium with Hoagland solution, using different light-emitting lighting systems in a climate chamber. The ascorbic acid content ranged from 12.9±0.7 to 37.27±0.24 mg/100g fresh weight (Carillo et al., 2022).

Parsley is known to be a vegetable rich in ascorbic acid (vitamin C), as demonstrated in the studies mentioned above (Karkleliene et al., 2014; Jadczak et al., 2019; Carillo et al., 2022). The ascorbic acid values closest to those observed in our study (176.15 – 234.94 mg/100g) were reported in the study conducted by Jadczak et al. (2019), where values ranged from 132.41 to 210.52 mg/100g. When examining studies that used either mature plants or microgreens of parsley (Karkleliene et al., 2014; Jadczak et al., 2019; Carillo et al., 2022), it was observed that ascorbic acid levels in mature parsley plants were generally higher than in microgreens (Carillo et al., 2022). However, it is also known that the ascorbic acid content can vary depending on the variety, cultivation environment, and other external factors.

The titratable acidity (TEA), water-soluble dry matter (WSDM), and pH walues were given in Table 4). The highest TEA value was determined in mature parsley plants. The TEA values of parsley micro greens and primed parsley micro greens were in statistically similar groups (P < 0.05). The same result was observed for the water-soluble dry matter (WSDM) parameter too. The study showed that parsley microgreens had the highest pH value, while mature parsley plants had the lowest pH value (P<0.05).

Table 4. Titratable acidity (g/100g), water-soluble dry matter (%), and pH values at the first harvest of parsley at maturity stage, microgreens and primed microgreens*

Subjects	WSDM (%)	TA (g/100g)	pH
Mature Parsley	7.8 A	0.22 A	6.06 C
Parsley Microgreens	4 B	0.16 B	6.58 A
Primed Parsley Microgreens	5 B	0.16 B	6.29 B
LSD (P<0.05):	1.854	0.0305	0.1653

*Means followed by the same letter within a column are significantly different according to the least significant difference (LSD) test. WSDM= water-soluble dry matter, T= titratable acidity.

In the study performed by Can (2022), parsley seeds were planted in a mixture of peat and perlite, then transferred to hydroponic culture in trays, where different applications of fulvic acid, amino acids, and chitosan were tested. In the second experiment of the study, soluble solids content (%), pH and acidity (%) were determined. The control group had soluble solids content (%), pH and acidity (%) values of 8.9, 6.2, and 0.57 respectively. In another study on parsley (*Petroselinum crispum* (Mill.) Nyman ex A. W. Hill), where different plant growth regulators were applied (Gonzales, 2021), the control group showed soluble solids content values ranging from 0 to 1 °Brix, with pH values between 6 and 7. In another study on parsley conducted under greenhouse conditions, different vermicompost applications were tested (Peyvast et al., 2008), and the control group showed a soluble solids content of 2.2%. When evaluating the aforementioned studies on parsley (Peyvast et al., 2008; Gonzales, 2021; Can, 2022), the values closest to our study were obtained in Can (2022). In our study, as the plants matured, both The TEA and WSDM increased.

The apigenin contents at the first harvest of parsley at maturity stage, microgreens and primed microgreens were given in Table 5. The highest apigenin content in the study was obtained from the priming-treated parsley microgreens (P<0.05). Both mature parsley and parsley microgreens showed statistically similar values (P<0.05).

Tablo 5. Apigenin contents at the first harvest of parsley at maturity stage, microgreens and primed microgreens (mg kg^{-1}) *

Subjects	Apigenin (mg kg^{-1})
Mature Parsley	18.534 B
Parsley Microgreens	23.657 B
Primed Parsley Microgreens	97.776 A
LSD (P<0.05):	5.5948

*Means followed by the same letter within a column are significantly different according to the least significant difference (LSD) test.

In a study (Carillo et al., 2022) which different light systems were used for growing parsley microgreens, apigenin levels ranged from 2.12 to 4.6 mg kg^{-1} (dry weight). However, no priming treatment was applied in the study. In another study (Cao et al., 2010), flavonoid contents were determined in various vegetables and fruits in which the apigenin content in parsley recorded as 4.4±0.1 mg kg^{-1} (fresh weight). The apigenin levels observed in our study were found to be higher than those reported in the studies (Cao et al., 2010; Carillo et al., 2022).

It has been observed that the priming treatment significantly improved parsley germination in the current study. Similar to our findings, Dursun and Ekinci (2010) reporthed that among the priming treatments applied to parsley (*Petroselinum crispum* L.), the highest germination rate was observed when parsley seeds were soaked in water for 12 hours at 10°C. This could have positively influenced the synthesis of the apigenin flavonoid in the microgreens. However, it can also be noted that further studies are needed on this topic.

Bi-plot presenting the correlation between the tested parameters of parsley plants was given in Figure 1. The Component 1 and Component 2 components explained 100% of the variance for the three different topics and five parameters in our study. However, the biplot was used because it provided a visual opportunity to evaluate all the topics together.

The ascorbic acid, Water-Soluble Dry Matter and titratable acidity parameters, along with the mature parsley subject (OMA), are positioned in the positive direction of PC1 (opposite direction of the other subjects) in the figure, indicating that these parameters have higher values in mature parsley. The priming-treated (PMIP) and non-treated (MIP) microgreen subjects are located in the same direction as the yield in first harvest and pH parameters and in the opposite direction of the mature parsley subject (PC1<0), which suggests that the yield and pH parameters in the microgreens are higher compared to those of mature parsley plants. The priming-treated microgreen subject (PMIP) is the only subject aligned along the same axis as the yield in the first harvest parameter (PC1<0; PC2>0), indicating that the highest yield value was obtained from this subject. The Apigenin parameter is found in the opposite direction of the mature parsley subject (MAP) and in the same direction as the other subjects (MIP, PMIP) (PC1<0), showing that the lowest apigenin content was obtained from mature parsley. The priming-treated parsley microgreen subject (PMIP) being positioned along the same axis as the apigenin parameter (PC1<0; PC2>0) indicates that this subject has the highest apigenin content (Figure 1).

4. Conclusion

Under the conditions of the study, it was possible to obtain higher yields from parsley microgreens in half the time compared to mature parsley plants during the first harvest. This yield was further increased with the application of priming. The ascorbic acid content in the mature parsley microgreens was found to be higher than that in the parsley microgreens. In terms of the ascorbic acid parameter, the priming-treated parsley microgreens were statistically grouped with the mature parsley plants. The mature parsley plants showed the highest statistical values for Titratable Acidity and Water-Soluble Dry Matter parameters. No significant effect of priming on the Titratable Acidity and Water-Soluble Dry Matter values was observed in the microgreens. In the study, the apigenin content was similar in the mature parsley and parsley microgreens subjects, but it increased significantly in the priming-treated parsley microgreens subject, highlighting the importance of priming when growing parsley microgreens.

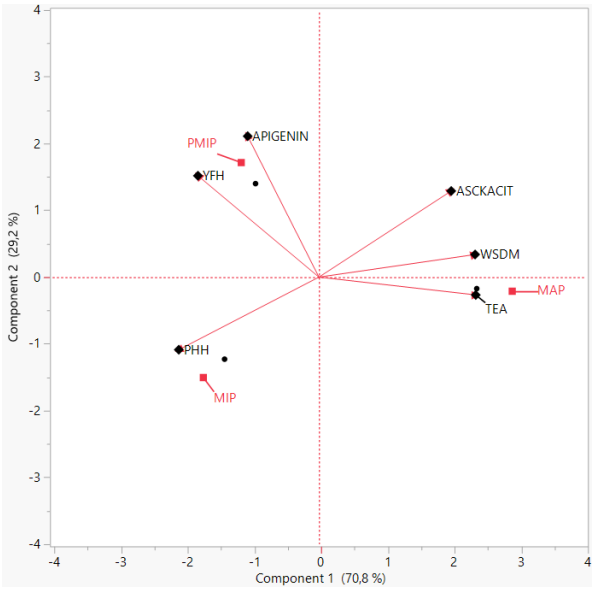

Figure 1. Bi-plot presenting the correlation between the tested parameters of parsley plants. MAP= mature parsley, MIP= Parsley Microgreen, PMIP= primed parsley microgreen, ASCKACIT= ascorbic acid, WSDM= water-soluble dry matter, TEA= titratable acidity, PHH= pH, YFH= yield in first harvest, APIGENIN= apigenin

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	A.Ç.	T.S.	N.Ö.
C		100	
D	30	50	20
S			100
DCP	90	10	
DAI	40	50	10
L		100	
W		70	30
CR			100
SR		70	30
PM	90	10	
FA	90	10	

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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