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Research Article

Comparation of chicoric acid and rosmarinic acid in fresh and dry herbs of sweet and purple basil (*Ocimum basilicum* L.) populations

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ABSTRACT

Ocimum basilicum is used either as a fresh or dry herb. Most used species are characterized by sweet and purple leaves and are typically used for culinary purposes and in traditional medicine. Therefore, it is important to analyse the content of functional compounds, rosmarinic acid and chicoric acid, in dry and fresh form of the plant. It was determined that rosmarinic acid and chicoric acid content of dry and fresh form of sweet, purple, and purple flower colored of the plant by high pressure liquid chromatography equipped diode array detector (HPLC-DAD). According to obtained results, it was observed that the distribution of rosmarinic acid in fresh samples varied between 128.38 and 4072.20 mg kg⁻¹ DW, and in dry samples between 1792.20 and 8149.45 mg kg⁻¹ DW. It is clearly observed that rosmarinic acid content in dry samples higher than fresh samples. The concentration of chicoric acid in fresh samples varied between 51.53 and 2278.40 mg kg⁻¹ DW and it varied in dry samples between 38.55 and 555.85 mg kg⁻¹ DW. Chicoric acid levels in dry samples were observed to be lower than fresh samples. These changes were found statistically significant $(p<0.001)$.

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Keywords: Ocimum basilicum, Rosmarinic acid, Chicoric acid, HPLC-DAD.

1. INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) belong to the Lamiaceae family are known to be rich source of polyphenolic compounds, especially phenolic acids.**1,2** They produce medicinal and aromatic plants phytochemical contents in relatively complex mixtures. These chemical compounds are not distributed uniformly throughout the plant, but are generally found in the roots, leaves, flowers, fruits, or in aerial part. **3** Plant secondary metabolites that including polyphenols play an important role in the structure and function of the plant and are known potent antioxidant agents in the human diet.**⁴** It has been indicated that the plants which have rich polyphenol content, prevent development of certain diseases including cancer and cardiovascular diseases.**⁵**

The content of phytochemicals in plants particularly aromatic and medicinal plants is considerably influenced not only by the genetic of the respective species but also by the environment, the harvesting technique, drying, and storage also affect the content of the respective valuable components.**⁶** Sweet basil commercially used as a fresh and dry herb. Most used species are characterized by sweet and purple leaves and are typically used for culinary purposes, as ornaments gardens, and in traditional medicine.**⁷** The dry and fresh forms of the sweet and purple basil are used both as a

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spice in food and in traditional and complementary medicine. The water-soluble phenolic compounds primarily responsible for pigmentation in plants.**⁸**

Rosmarinic acid, a caffeic acid derivative is the most dominant phenolic acid in basil.**2,3** The compound has a wide spectrum of biological and pharmacological activities. Several *in vitro* studies have established rosmarinic and chichoric acids as the main polyphenols responsible for the plant's biological activities. According to previous studies performed on rosmarinic acid and its biological activity proved that the compound had antioxidant⁹, antibacterial¹⁰, antiviral¹¹, analgesic, anti-inflammatory**⁵** , antidiabetic**⁷** , hepatoprotective, anticancer, cardioprotective, neuroprotective effects.**¹²**

Chicoric acid was first purified in 1958 from *Cichorium intybus* L. and its structure was elucidated.**¹³** Chicoric acid is caffeic acid derivatives that also found in basil.**¹⁴** Chicoric acid exhibited effective inhibition of HIV integrase.**¹⁵** It has become popular due to its positive effects on health and desired diet rich by chicoric acid in foods. In recent years studies on chicoric acid have focused on its medicinal uses, its natural production, and its importance in food.⁸ Plants containing chicoric acid have been reported to protect themselves from insects and viruses, fungi, bacteria and nomadotes.**¹⁶** It is believed to be more beneficial for health in natural products form since its structure is damaged by both light and heat.¹⁴

Basil used as spice in food, in medicine and cosmetic industry for many years in the Anatolia and worldwide. Usually, aerial part of the plant is being used fresh and dried. It is well-known that the chemical ingredients of spices change during drying and storage period. However, according to our literature research, detailed study was not found on cichoric acid and rosmarinic acid changes during the drying process purple and sweet basil (*O. basilicum*). Therefore, in this study, it was aimed to evaluate the change of rosmarinic acid and cichoric acid in fresh and dry forms of sweet, purple, and purple flower colored *O. basilicum* populations.

2. MATERIALS and METHODS

2.1. Chemicals and reagents

All chemicals used in the study are HPLC grade. Rosmarinic acid and chicoric acid were purchased from the Sigma-Aldrich Company (Sternheim, Germany). Methanol, acetonitrile, formic acid, and all other chemicals used during the study and extraction processes were purchased from Merck Company (Istanbul, Turkey).

2.2. Plant Material

21 basil populations were used in the study. In accordance with the purpose of the study, 7 of these populations are basil populations similar to the sweet variety, and 7 are purple-colored populations. The remaining 7 populations are green leaves and purple flowers.

Populations of *Ocimum basilicum* were grown in the research plots of Gaziosmanpasa University, Faculty of Agriculture, Department of Field Crops and harvested during the vegetative period. Plants harvested from each population were divided into two groups. One of the groups was analyzed after drying at room conditions, while the other group was analyzed as fresh immediately.

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2.3. Preparation of Extracts for Chemical Analysis

The rosmarinic acid and chicoric acid contents of fresh and dry herbs of basil populations were to be compared in the study, therefore a part of the samples was freshly extracted immediately after harvesting. Other part was dried and then was extracted with the same solvent system. To compare the results accurately, the extraction yield of fresh samples was calculated as dry herb. For this reason, while preparing the fresh herb extract, an equal aliquot to the amount (2 gram) of fresh basil samples were also taken from each basil clone and was dried in the shade. Thus, a more accurate comparison could be made by giving the calculations of rosmarinic and chicoric acid in fresh and dried samples per gram dry plant.

The extraction process of fresh samples, 2 grams of each sample was taken and put into liquid nitrogen. Then 10 mL methanol/dichloromethane (3:1) mixture was added and vortexed. The extraction process of dry samples was applied in the same way as for fresh samples. Extraction yield for each sample was calculated after solvents were removed under low pressure and 35ºC temperature by rotary evaporator.

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The extracts dissolved in the mobile phase B (Acetonitrile) at 1000 mg/L concentration. When necessary, dilutions were made with mobile phases during injection.

2.4. Rosmarinic acid and Chicoric Acid Quantitative Analysis by HPLC

Quantitative analysis was carried out by High-Performance Liquid Chromatography combined with Diode Array Detector (HPLC-DAD) system coupled with an LC 20AT pump and CTO-20AC model column oven (Shimadzu, Japan). Reverse phase phenylhexyl, 4.6 x 150 mm, 3 μm (UP) (GL Sciences InterSustain, Japan) column was used for separation of analytes.

Table 1. Pump gradient program applied to HPLC.

As the mobile phase solvent A (1% formic acid/deionized water), and solvent B (Acetonitrile) applied pump program are given in Table 1. During the analysis process flow rate was set to 1 mL/min. Sample and the standards were injected to the column by autosampler as 10 µL injection volume. The temperature of the column was set to 25°C. Detection was performed by scanning between 190 and 800 nm and absorbances at 330 nm were used for quantitative analysis. Identification of rosmarinic acid and chicoric acid compounds was accomplished by comparing retention times and spectra with those of the original phenolic standards. Quantitative analyzes of rosmarinic acid and chicoric acid compounds were performed using calibration curves obtained for these compounds at different concentrations.

2.5. Data Evaluation and Statistical Analysis

All statistical analysis were performed using the GraphPad software program. Data are expressed as mean values and standard deviations (SD) of the triplicate results. Results were analyzed using two-way analysis of variance (ANOVA/Bonferroni posttests) for compare fresh and dried herb of every population.

3. RESULTS and DISCUSSION

In this study, rosmarinic acid and chicoric acid changes were evaluated in dry and fresh herbs of purple, sweet,

and sweet leaf with purple flower basil populations grown in Tokat region. Chicoric acid and rosmarinic acid were compared in both fresh and dry samples in the harvested basil populations. According to obtained results, it was observed that the distribution of rosmarinic acid in fresh samples varied between 128.38 and $4072.20 \text{ mg kg}^{-1}$ DW, and in dry samples between 1792.20 and 8149.45 mg kg^{-1} DW. The concentration of chicoric acid in fresh samples varied between 51.53 and 2278.40 mg kg^{-1} DW and in dry samples between 38.55 and $555.85 \text{ mg kg}^{-1}$ DW (Table 2).

3.1. Rosmarinic Acid Changes During Drying

3.1.1. Rosmarinic Acid Changes in Sweet Basil

The mean of rosmarinic acid concentration in sweet basil (green colour) populations was 476.36 mg kg^{-1} DW in fresh form but the mean of rosmarinic acid concentration in the same samples was determined as 3746.53 mg kg^{-1} DW in dried form. The average rosmarinic acid change during drying was approximately 7.9-fold (Figure 1). These changes were found to be statistically significant (*p*˂0.001). It has been reported in the previous reports that rosmarinic acid content varies between the species of the same genus, subspecies of the same species, samples collected time and region. 12 In accordance with findings from additional investigations, that the concentration of rosmarinic acid changes with drying temperature and that concentration decreases as temperature rises.**17,18** It is considered responsible for a wide spectrum of biological and pharmacological activities of plants containing rosmarinic acid. Therefore, concentration of rosmarinic acid in Lamiaceae family can be used as a quality parameter for estimating herbal drug. This value is in agreement with our results (Table 2).

Figure 1. Rosmarinic acid changes in fresh and dry sweet

3.1.2. Rosmarinic Acid Changes in Purple Basil

The mean of rosmarinic acid concentration in purple basil genotypes was 1920.42 mg kg^{-1} DW in fresh form but the mean of rosmarinic acid concentration in the same basil population was determined as 3938.34 mg $kg⁻¹$ DW in dried form. The purple basil genotype with the lowest rosmarinic acid concentration increased approximately 11-fold during drying from 324.58 mg kg^{-1} DW to 3580.1 mg kg^{-1} DW (Figure 2). The change was found to be statistically significant $(p<0.001)$. In purple basil populations, rosmarinic acid concentration increased with drying in R-16, Y-5, Y-9, and Y-31 populations, and decreased in R-43 and Y-29 populations. The change of rosmarinic acid value in Y-30 drying period was not observed as significant statistically. The reason for this change needs to be revealed by future research (Table 2). In previous studies, it has been reported that the amount of rosmarinic acid in basil is in a wide range. The results obtained in this study are in agreement with previous studies. 3,19–22

Figure 2. Rosmarinic acid changes in fresh and dry purple basil populations.

basil populations. Most of the purple basil cultivars had the highest concentration of rosmarinic acid, but rosmarinic acid concentrations differed according to the cultivar.

Rosmarinic acid levels ranged from 660 mg kg^{-1} DW to 1720 mg kg-1 DW in purple basil in a previous report.**⁸** The rosmarinic acid levels for the purple cultivars in this study are much higher than for the Iranian basil varieties studied by Javanmardi et al.**²** In some previous studies were reported to have rosmarinic acid concentration up to 100 g kg-1 DW.**⁸** The environment in which basil grows, variations in age, and the manner in which commercial samples are dried all have a significant impact on the potential of basil to create rosmarinic acid.**⁷**

3.1.3. Rosmarinic Acid Changes in Purple Flower Basil

The mean of rosmarinic acid concentration in purple flower basil population was 547.73 mg kg⁻¹ DW in fresh form but the mean of rosmarinic acid concentration in the same basil population was determined as 5742.25 mg kg-1 DW in dried form. The rosmarinic acid concentration increased after drying in all purple flowered basil populations analyzed in this study (Table 2). The highest increase was in the Y-20 population. In this population, rosmarinic acid was found to be 211.07 mg $kg⁻¹$ DW in the fresh sample, while this amount became 8149.45 mg kg^{-1} DW after drying (Figure 3). The results obtained for rosmarinic acid in the study are in agreement with some previous studies.**23–25** But our results was lower than a previous report (11285 mg kg⁻¹ DW).**²⁶**

Figure 3. Rosmarinic acid changes in fresh and dry purple flower basil populations.

In this study, the concentration of rosmarinic acid were found to be in agreement with those reported for *O. basilicum*. Many authors have reported a large variability in rosmarinic acid content in different species of *Ocimum*. Rosmarinic acid biosynthesis in basil is highly influenced by the environmental factors present during the growth period. The variations in its reported levels may be caused by different quantification

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techniques, growing environments, genotypic variations, age, and drying processes.⁸

According to the results of the study, it can be said that the increase in the amount of rosmarinic acid, a cinnamic acid derivative during drying is due to that the activities of enzymes involved in the synthesis pathway of rosmarinic acid continue.**²⁷** Another reason for the increase in the amount of rosmarinic acid is that the ongoing synthesis during drying may have perceived this situation as stress by the plant and increased the amount of rosmarinic acid as antioxidant. However, further studies are required to reveal the mechanism and details regarding this issue.

3.2. Chicoric acid

3.2.1. Chicoric Acid Changes in Sweet Basil

The average chicoric acid concentration was found as 379.64 mg kg-1 DW in fresh sweet basil, but it was decreased to 92.01 mg kg-1 DW after dried (Table 2). The change was statistically significant (*p*<0.001) except two population (Figure 4). The average chicoric acid change during drying was approximately 4-fold. The reason for this is thought to be due to the easily ethylene degradation of chicoric acid from light and oxygen. Because many studies have shown that chicoric acid is unstable and easily affected by light.**8,14,28**

Figure 4. Chicoric acid changes in fresh and dry sweet basil populations.

Chicoric acid is the dominant phenolic compound found in *Echinacea purpurea*. However, the compound is also found in many plants of the Lamiaceae family, especially in basil. In a previous study, it was reported that the amount of chicoric acid was found in 518 and 885 mg kg-1 leaf tissue in two different basil populations.**²⁹** This value is in agreement with our results (38.55-832.51 mg kg⁻¹ DW) (Table 2).

3.2.2. Chicoric Acid Changes in Purple Basil

The average chicoric acid concentration in purple basil populations was found as 1409.08 mg kg⁻¹ DW in fresh plant, but it was decreased to 101.19 mg kg⁻¹ DW after dried (Table 2). The change was statistically significant (*p*<0.001) except one population (Figure 5). The average chicoric acid change during drying was approximately 13-fold.

Purple Basil Populations

Figure 5. Chicoric acid changes in fresh and dry purple basil populations.

In a previous study, the amount of chicoric acid between 50 mg kg^{-1} DW and 400 mg kg^{-1} DW was reported in three types of dried purple basil cultivars.**⁸** It was found between 41.85 mg kg^{-1} DW and 122.15 mg kg^{-1} DW in dried samples of seven purple basil cultivars in our study (Table 2). It was reported that chicoric acid levels ranged from 30 mg/kg DW to 2780 mg kg^{-1} DW in fifteen basil varieties.**²⁵** Our results are in agreement with the results of previous studies.**29,30** Previous report on basil varieties also found chicoric acid levels to be significantly affected by cultivar. However, it was found that the basil cultivars with the highest change in this effect were purple-colored cultivars.**²⁵**

3.2.3. Chicoric Acid Changes in Purple Flower Basil

The mean of chicoric acid concentration in purple flower basil population was 379.04 mg kg⁻¹ DW in fresh form but the mean of chicoric acid concentration in the same basil population was determined as 182.43 mg kg-1 DW in dried form. While the chicoric acid at the lowest concentration 148.49 mg kg⁻¹ DW was found in Y-20 in the purple-flowered fresh basil populations, the highest concentration was found in the R-44 population at 860.82 mg kg^{-1} DW. (Table 2). After the samples were dried, the lowest chicoric acid (76.40 mg kg^{-1} DW) concentration was found in Y-20, while the highest (555.85 mg kg^{-1} DW) concentration was found in the R-44 population. The chicoric acid concentration in all populations decreased approximately 1.5-2-fold with

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drying. This decrease was found to be statistically significant $(p<0.001)$ (Figure 6). Chicoric acid has a wide range of advantageous biological qualities, making it a valuable natural substance of particular interest.**³¹**

Figure 6. Chicoric acid changes in fresh and dry purple flower basil populations.

4. CONCLUSION

It was observed that the rosmarinic acid concentration increased but the chicoric acid concentration decreased during drying in basil. Xing et al. analyzed the changes of essential oil components, total phenolic and rosmarinic acid with different drying techniques in purple basil. According to results in their study, it was observed that the amount of rosmarinic acid increased significantly at 50ºC in oven and freeze drying compared to the fresh form, while it was observed that rosmarinic acid decreased with sun drying technique.**³²**

Plant species belonging to Lamiaceae contain significant concentrations of phenolic compounds such as flavonoids and phenolic acids, which can act as a substrate for polyphenol oxidase (PPO) enzyme in the presence of molecular oxygen.**³³** Drying intends to inactivate the enzymes present in fresh leaves by removing water from the product and reducing water activity. **34**

In this study, the amounts of rosmarinic acid and chicoric acid were analyzed in both fresh and dried herbs of seven varieties of sweet, seven varieties of purple and seven varieties of purple-flowered basil samples. According to the results we obtained, it was observed that the amount of rosmarinic acid in basil varieties increased during the drying period, while the average amount of chicoric acid decreased. The rosmarinic acid concentration that increasing with drying was observed the most in the purple-flowered basil (approximately 10 fold) samples, while the lowest change (approximately 3.5 fold) was observed in the sweet variant. It was observed that the average amount of chicoric acid decreased with drying period in all cultivars. This decrease was highest (approximately 14 fold) in purple basil samples, while the lowest decrease

(approximately 2 fold) was found in purple flowered cultivars. The reason for these changes needs to be revealed by future research.

Based on the results obtained in this study, it can be said that if basil is to be evaluated for rosmarinic acid, it is recommended to use the dried form of the purple variety. If it is to be evaluated for chicoric acid, it is recommended to use the fresh form of the purple varieties.

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Conflict of Interest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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