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RESEARCH PAPER

Phenolic, Organic Acid and Sugar Content of Garlic (*Allium sativum* L.) Genotypes Grown in Different Regions of Türkiye

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Abstract

In this study, the biochemical profiles of five different genotypes of garlic (*Allium sativum* L.) harvested in different regions of Türkiye were investigated in detail, focusing on phenolic compounds, organic acids and sugar components. The analyses were carried out using high-performance liquid chromatography (HPLC) and showed that there were significant biochemical differences between the genotypes. A total of 18 phenolic compounds, 12 organic acids and 3 sugar components were determined in the samples. With the phenolic compounds and the antioxidant activity, while Genotype 4 had the highest chlorogenic acid (174.99 mg kg^{-1}), Genotype 5 had the highest catechin hydrate (158.77 mg kg^{-1}), gallic acid (22.35 mg kg^{-1}) and *o*-coumaric acid (12.78 mg kg^{-1}). The profile of organic acids was also presented, where Genotype 2 was the richest genotype for citric acid (7374.66 mg kg^{-1}). Other significant organic acids, succinic (12747.34 mg kg^{-1}) and isobutyric acid (149.54 mg kg^{-1}) which were identified the highest in Genotype 5. As far as sugar components are concerned, sucrose levels showed a significant variation between the genotypes, where Genotype 5 had 3197.79 mg kg^{-1} and Genotype 4 had 1950.93 mg kg^{-1}. There were statistically significant differences between the genotypes in terms of phenolic compounds, organic acids and sugar components ($p<0.05$), which are indicate that biochemical differences between genotypes are important in terms of agricultural and nutritional value. These data can be utilized by garlic breeders and garlic producers by regions.

1. Introduction

Allium sativum L., commonly known as garlic, has been used traditionally for both culinary and medicinal purposes (Londhe et al., 2011). Garlic first served as a protective food by construction workers in ancient Egypt and later valued as a functional food that imparts beneficial health effects to humans (Rivlin, 2001). Since this plant contains numerous chemical components including phenolic compounds, organic acids, and sugars, it thus holds great nutritional value. The health-enhancing constituents of these vegetables comprise antioxidant, antimicrobial, and cardiovascular protective properties (Papu et al., 2014). Chemical composition and biological activities of garlic may depend on the different genotypes, environmental conditions, and growing techniques (Asif, 2015; Besirli et al., 2022). It is very important to study these biochemical differences related to the effects of garlic on health (Rahman, 2003). Phenolic compounds, such as those in garlic, are chemical substances found in abundance in plants and used widely as antioxidants. These agents protect cells against oxidative stress by neutralizing free radicals; through that action, they may prevent the development of chronic diseases such as cardiovascular diseases, cancer, and aging

(Santhosha et al., 2013; Kallel et al., 2014). Garlic is considered high in phenolic and organic acid content in local and traditional genotypes. These genotypes have also developed resistance to environmental stress factors, and the studies on the chemical constituents have provided important data on the nutritive and health benefits of garlic. In addition, conservation of local genotypes is very important for sustainable farming practices too. The antimicrobial and autoinflammatory features of phenolic compounds in garlic are also remarkable. Studies show that these phenolic compounds in garlic are effective against bacteria and fungi and they reduce inflammation (Wilson and Demmig-Adams, 2007). These components, therefore, enable the potential medical uses of garlic. Organic acids meanwhile, are important in the plant cell metabolism and delimit the taste and aroma profiles of garlic.

Ascorbic acid (vitamin C) and malic acid are chemicals that are derived from living work and have been shown to be very helpful during the high-protein digestion process and to down-regulate the stomach's acidity. The main ones are acids that are important for regulating the pH ranges and the elimination of toxins in the body (Khan and Iqbal, 2016; Ma et al., 2021). Garlic is a product that can be used in medical treatments, as it is one of the leading foods with polyphenolic, organic acids and sugars. Antioxidants such as the ones that defend cells from free radical damage and as a result, inhibit the process of aging are the components that prove the benefits of garlic against chronic diseases like cardiovascular diseases, cancers, and neurodegenerative diseases. Antimicrobial properties stand as the most powerful mechanism for preventing and treating infections. The beneficial effects of digestion are those that speed up digestion and regulate the pH of the stomach (Aversa et al., 2016).

Garlic production areas grown in Balıkesir, Kırklareli, Kütahya and Gaziantep provinces, which are the most common garlic production areas in our country, were taken into consideration. In addition to regional differences in our country, different garlic genotypes are grown in each region. However, it was noted that there is not enough data on the nutritional properties of these genotypes. According to the regions, Iranian genotypes in Balıkesir and Chinese genotypes in Kırklareli stand out as important international genotypes in terms of yield and quality. Black type garlic (Kütahya) and Araban genotypes (Gaziantep) have been grown locally for a long time. Purple type garlic is also produced in Aksaray and is a popular garlic genotype.

This research was conducted with the aim of comparing the character of these five different genotypes of garlic, *Allium sativum* L., regarding their phenolic compounds, organic acids, and sugars. Through high-performance liquid chromatography (HPLC) analysis, each genotype was examined for 18 phenolics, 12 organic acids, and 3 sugar compounds. This study deepens our understanding of the biochemical changes that occur across different garlic genotypes and their contributions to the health benefits of these varieties. By highlighting the biochemical distinctions among various garlic types, the research underscores the importance of phenolic, organic acid, and sugar components in both agriculture and nutrition. The key findings from this investigation illustrate the crucial role these elements play in the agricultural and nutritional sectors. Additionally, these insights lay the groundwork for further exploration into the unique biochemical characteristics of garlic genotypes native to Türkiye.

2. Material and Methods

2.1. Materials

The study was carried out to examine the diversity of five garlic genotypes produced in the same areas between 2021-2022 in different regions of Türkiye. The observed genotypes were Persian (Genotype 1, Balıkesir), Chinese (Genotype 2, Kırklareli), Purple (Genotype 3, Aksaray), Black Garlic (Genotype 4, Kütahya), and Araban (Genotype 5, Gaziantep). Each local genotype was selected to show regional diversity and analyses were carried out on these genotypes.

The garlic genotypes were sown between August and September and reaped between June and July. In the whole season of growing this garlic, the foliar fertilization as well as irrigation was the main factor that getting the highest yield at the end was maintained, and the optimal conditions that were obtained were due to proper fertilization and irrigation. The precise location and elevation of the collection sites for the garlic genotypes are as follows: Aksaray (38°31’37” N, 33°50’00” E; 944 m), Balıkesir (39°30’40” N, 27°50’53” E; 231 m), Gaziantep/Araban (37°04’05” N, 37°23’35” E; 850 m), Kütahya/Şaphane (38°58’43” N, 29°16’17” E; 781 m), and Kırklareli/Babaeski (41°34’33” N, 27°50’43” E; 314 m).

At harvest, the bulbs of the garlic genotypes grown under these conditions were taken to the laboratory under cold chain conditions for the determination of phenolic compounds, organic acids and sugars. Each analysis was carried out under optimal laboratory conditions and a total of 15 outputs, the set containing three samples for each garlic genotype, were checked.

2.2. Extraction method for phenolic compounds and total antioxidant activity

At harvest, garlic samples (bulbs) were first cleaned to remove surface dirt. Approximately 25 g of each cleaned garlic bulb was subjected to extraction by adding 100 mL of methanol in sealed

round-bottomed flasks placed on magnetic stirrers at room temperature (Erol et al., 2024; Erol, 2024). The extraction process continued until the solvent was colorless and at least five times this process was repeated.

The resulting extracts were filtered through Whatman #1 filter paper and the filtrates were collected. The extracts were then concentrated by applying a Buchi R300 rotary evaporator at 60°C and 200 mm Hg. The residues that were still at the bottom of the flask were redissolved in the methanol in the different volumes of 200-300 µL, and the extracts were prepared for the analysis of total phenolic content and total antioxidant activity. This procedure has been optimized to efficiently extraction these plant-derived compounds from garlic samples.

2.3. Analysis of phenolic compounds by HPLC

The presence of phenolics in garlic extracts was established through ultra-high performance reversely-phase liquid chromatography (Agilent 1260 Infinity RP-HPLC, USA). The phenolic compounds were isolated utilizing C18 reversely-phase column (110 Å, 5 µm, 4.6 × 250 mm, ACE Generix). The injection volume was set at 10 µL and the mobile phases utilized were A (0.1% phosphoric acid-water solution) and B (100% acetonitrile). A gradient system was used, the temperature of the column oven was maintained at 30°C and measurements were made using a diode array detector (DAD).

Concentrations of chlorogenic acid, catechin hydrate, caffeic acid, 4-hydroxy benzoic acid, vanillin, rutin, trans-ferulic acid, hydroxycinnamic acid, naringin, *o*-coumaric acid, rosmarinic acid, salicylic acid, resveratrol, quercetin, trans-cinnamic acid, naringenin, chrysin and flavone components were determined by external standard method and characterized by retention times. The concentrations of phenolics were determined by the external standard method and characterized by retention times. The data obtained were reported in mg kg^{-1} wet weight (Uçan Türkmen et al., 2023; Uçan Türkmen et al,. 2024).

2.4. Antioxidant activity analysis method

The antioxidant capacity of the garlic extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical % inhibition method. The garlic extracts were first dissolved in methanol to prepare different concentrations for analysis. Meanwhile, 100 µL of the extracts dissolved in methanol were added to test tubes. 2.9 mL of the prepared 0.1 mM DPPH solution was added to each tube. The solutions were then kept in the dark for 30 min and the absorbance values were read against the solvent at a wavelength of 517 nm on a UV-Vis spectrophotometer (Shimadzu UV-1800). The % inhibition of DPPH radical was calculated from the absorbance values (Brand-Williams et al., 1995). Each sample was analyzed in triplicate.

2.5. Analysis of organic acids and sugars by HPLC

2.5.1. Extraction method

The analysis of organic acids and sugars in garlic samples was conducted using freshly harvested garlic bulbs, which were prepared for extraction. The samples were crushed and 2.5 g of samples were carefully placed in 50 mL Falcon tubes, which were then homogenized in 25 mL of a deionized water/methanol mixture (7/3, v/v) using a high-speed homogenizer (IKA model T18). The homogenized solution was incubated in a water bath at 40°C for 30 min. After this period, the centrifuge was run at 10,000 rpm for 10 min at 4°C to obtain the supernatant. This material was then filtered through a 0.45-micron syringe filter and stored at -20°C until analysis (Gallardo-Guerrero et al., 2010).

2.5.2. HPLC analysis conditions

Sugars and organic acids in garlic samples were analyzed by modifying the method of Korkmaz et al. (2020). Shimadzu Prominence Modular LC20A HPLC system was used for the analyses. For sugar analysis, a Rezex RCM-Monosaccharide Ca^{2+} (8%) LC Column (300 × 7.8 mm) was used. The column temperature for sucrose, glucose and fructose analysis was 80°C and isocratic mode, with a flow rate of 0.5 mL min^{-1}, using ultrapure water as mobile phase and completed in 15 min.

The results were calculated as mg g^{-1} fresh weight using calibration curves prepared with standard sugar solutions. All samples were analyzed in triplicate. Oxalic acid, citric acid, tartaric acid, malic acid, succinic acid, lactic acid, formic acid, acetic acid, fumaric acid, propionic acid, isobutyric acid and butyric acid analyses were conducted on the same HPLC system using a UV detector set at 210 nm, with the column temperature at 50°C, and a Rezex ROA-Organic Acid H^+ (8%) LC Column (300 × 7.8 mm) for organic acid separation. The analysis results were calculated using calibration curves generated from standard solutions and were expressed as mg kg^{-1} fresh weight.

2.6. Statistical analysis

Statistical analyses of the obtained data were performed using JMP 14 software. Each measurement was performed three times. Variance analysis and multiple comparisons were conducted using Tukey's HSD test. In the statistical analyses, results with a p-value below 0.05 for the variation parameters were considered statistically significant.

3. Results and Discussion

3.1. Phenolic compounds and antioxidant activity

In this study, the phenolic compound profiles and antioxidant activities of five different garlic genotypes were comprehensively evaluated. The amounts of chlorogenic acid varied widely among the genotypes, with the highest level found in Genotype 4 at 174.99 mg kg^{-1}, and the lowest in Genotype 2 at 34.86 mg kg^{-1}. Genotype 5 is the only genotype in which catechin hydrate was found and it had the highest level with 158.77 mg kg^{-1}. Caffeic acid was the highest in Genotype 5 with 0.95 mg kg^{-1} and the lowest in Genotype 1 with 0.36 mg kg^{-1}. In this study, important phenolic compounds such as gallic acid, o-coumaric acid and ferulic acid also differed among genotypes. Antioxidants were measured by DPPH% inhibition method and it was seen that antioxidant activity was linearly related to phenolic compounds. For example, DPPH% inhibition of Genotype 4 was determined as 53.99%, while that of Genotype 1 was 46.57% and that of Genotype 5 was 37.73%. Genotypes containing higher amounts of phenolic compounds exhibited higher antioxidant activity and these genotypes showed stronger antioxidant activity than the others.

Studies have established that the active compounds and, hence, antioxidant activity in garlic vary variable. For example, in the work of Fratianni et al. (2016), the content of chlorogenic acid in garlic was from 76.99 mg kg^{-1} to 93.95 mg kg^{-1}. In the work of Beato et al. (2011), the variability of caffeic acid was between 0.25 mg kg^{-1} and 13.74 mg kg^{-1}. The content of chlorogenic and caffeic acids in the studied genotypes was compared with amounts reported in the literature. According to Kim et al. (2013), the chlorogenic content in garlic ranged from 46.00 mg kg^{-1} to 134.00 mg kg^{-1} in different genotypes. The variation in the range of this value obtained by our study, and thus, it is very likely that because of the genotypes, there are differences in phenolic compounds, as in our case. The same findings were found by Šnirc et al. (2023), who mentioned that the amount of caffeic acid in garlic genotypes was between 11.43 mg kg^{-1} and 16.00 mg kg^{-1} on a dry weight basis, which is similar to this work.

Based on the antioxidant activity, the high catechin hydrate content in Genotype 5 indicates this genotype may be rich in antioxidants. A review of catechin hydrate's antioxidant and anti-inflammatory properties underline its health benefits (Pedro et al., 2020). The high chlorogenic acid content in Genotype 4 also means that this genotype may have significant antioxidant capacity.

We used principal component analysis (PCA) to try to understand the biochemical differences between the different genotypes (Figure 1). The PCA scores showed us how the first two principal components are related to each other, and together they account for 92.89% of the total variance (Component 1: 65.52%, Component 2: 27.37%). It means that the distinction between genotypes is to a great extent brought about by the phenolic content. Using catechin hydrate, 158.77 mg kg^{-1}, and chrysin, 36.44 mg kg^{-1}, as indicators, it can be inferred that such genotype as Genotype 5 is more liable to these two chemicals than any other genotypes. Moreover, Genotype 2 was found to be

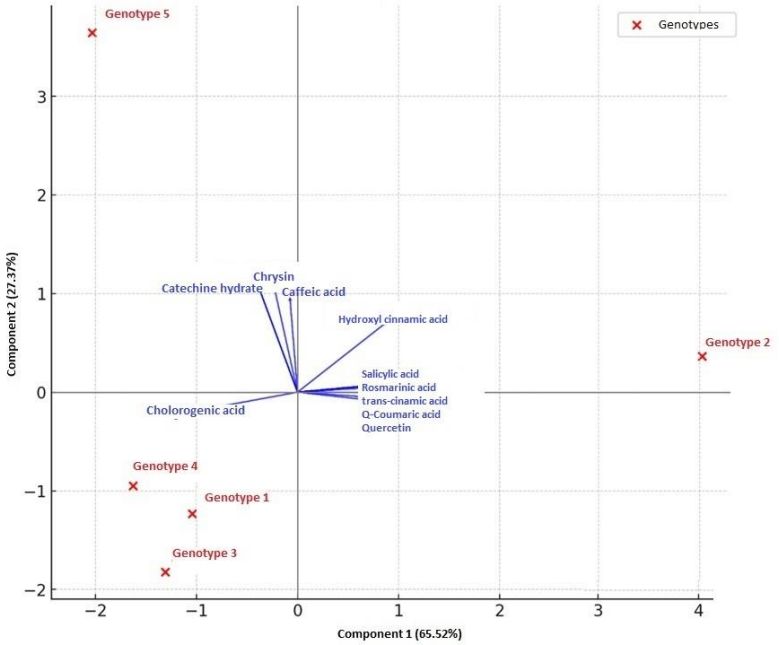

Figure 1. Principal component analysis (PCA) of phenolics.

the corelated to quercetin (4.14 mg kg^{-1}), salicylic acid (0.55 mg kg^{-1}), and rosmarinic acid (3.29 mg kg^{-1}). Genotypes 1, 3, and 4 showed the same phenolic profiles. It is evident from the literature that there is a considerable range of garlic phenolic compounds, which suggests that, genetic and environmental factors may play a role in influencing these profiles. This study therefore aims to highlight the potential health benefits of biochemical diversity among garlic genotypes, and to provide a valuable foundation for evaluating these genotypes as functional foods.

Interesting differences in the profile of phenolic compounds and in the antioxidant activities for different genotypes of garlic have been revealed within this study. The potential health benefits due to phenolic compounds present in these genotypes make them a great avenue for testing as functional foods. In addition, their phenolic profiles are of equal importance, since it forms the basis of breeding and cultivation within the garlic studies. This report should add to the literature that already exists on the biochemical diversity of garlic genotypes and its health impact.

3.2. Organic acid analysis

The aim of this study was to evaluate the organic acid profiles of five different garlic genotypes. The results indicate the potential for significant variations among the genotypes. The results clearly show that Genotype 5 had the highest levels of oxalic acid at 18.57 mg kg^{-1}, while Genotype 4 had the lowest levels (0.00 mg kg^{-1}). From the data available, it seems that Genotype 2 has the highest amount of citric acid, at 7374.66 mg kg^{-1}, while Genotype 4 has the lowest, at 2616.05 mg kg^{-1}. There appears to be considerable variation in tartaric acid levels, with the highest amount observed in Genotype 4 at 584.77 mg kg^{-1}. It is worth noting that some genotypes did not have any detectable levels. It would appear that malic acid is highest in Genotype 2 at 562.79 mg kg^{-1}, and that it is not detected in Genotypes 1 and 5. It would appear that succinic acid was present in the highest amount in Genotype 5 at 12747.34 mg kg^{-1}, although it was absent in Genotype 3. Similarly, acetic acid levels also varied among genotypes, with the highest level in Genotype 2 at 696.33 mg kg^{-1}, although it was not detected in some genotypes. Finally, formic acid was highest in Genotype 2 at 145.36 mg kg^{-1}.

The literature demonstrates that organic acids in garlic exhibit considerable variation. Şaşmaz et al. (2022), reported citric acid content in garlic ranging from 8.90 g kg^{-1} to 17.50 g kg^{-1} between genotypes. Citric acid levels in the present study were within this range, indicating that genetic and environmental factors may strongly determine the amount of citric acid in the genotype. Sangouni et al. (2021), reported variations in tartaric acid levels ranging from 50.00 mg kg^{-1} to 1200.00 mg kg^{-1} in garlic among genotypes studied. Tartaric acid values in the genotypes we studied were also within these above-mentioned ranges, representing a marked effect of genotypes on organic acid profiles in garlic. The great differences in concentrations between other organic acids, such as succinic acid and malic acid, are proof of the major functions they participate in during biochemical and physiological processes.

The principal component analysis (Figure 2) of organic acid profiles across all genotypes was determined. Two principal components accounted for 73.30% of the total variance: Component 1 = 52.53% and Component 2 = 20.77%. However, it is

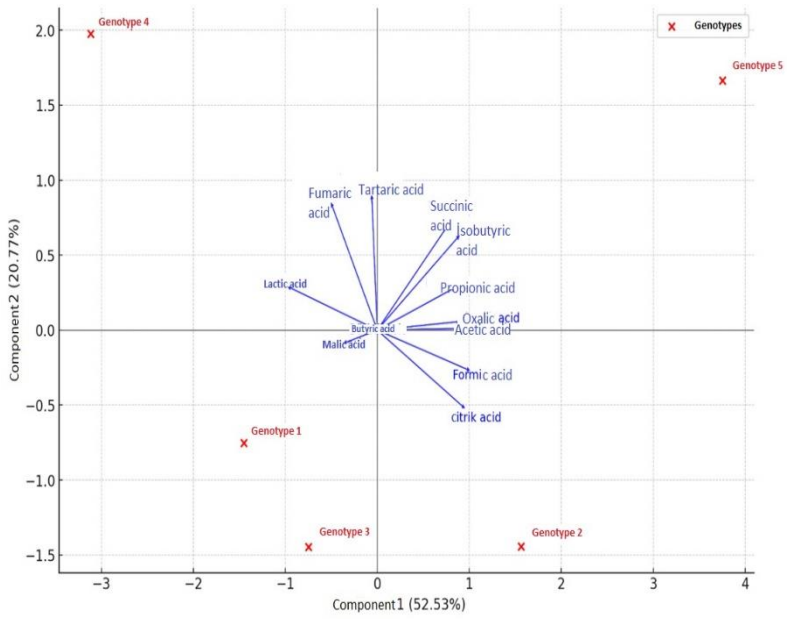

Figure 2. Principal component analysis (PCA) of organic acids.

essential to point out that Genotype 5 is separated from the others in the PCA plot since this genotype presents high succinic acid (12747.34 mg kg^{-1}) and isobutyric acid (149.54 mg kg^{-1}) content. This reveals that the genotype is rich in such compounds. Genotype 2 recorded high values for citric acid (7374.66 mg kg^{-1}) and acetic acid (696.33 mg kg^{-1}). Obviously, there was also an association with various other genotypes showing an association with certain organic acids, which reveals that this biochemical diversity might relate to organic acid profiles. These results imply that both genetic and environmental factors cause variation in the levels of organic acids between genotypes. It may thus be advantageous to include this variation in subsequent research on the breeding and growing of garlic. The present results restate that there is tremendous biochemical variation among different garlic genotypes concerning organic acids. The contents of organic acids make up very important parts of plant metabolism in connection with cellular respiration, energy production, and response to stresses. In this manner, differences express the genetic diversity and adaptability of the garlic plant toward environmental factors. Considering the potential health effects of organic acids provides an important basis for assessing these genotypes for nutritional and functional food purposes. The findings of the study, therefore, provide insights valuable for the biochemical diversity among genotypes of garlic and underline the health significance of such diversity.

3.3. Sugar analysis

A complete sugar profile of five garlic genotypes was intensively studied in the research. A sugar analysis was conducted that was confined to three central sugar components: fructose, glucose, and sucrose. Fructose and glucose were not identified in any of the genotypes, which, in turn, pointed to the substantial dissimilarities in the distribution of sugar components across the genotypes. Nevertheless, sucrose showed up in all genotypes, with quite significant variations between them. Particularly, Genotype 5 came out as the highest sucrose last at 3197.79 mg kg^{-1}, while Genotype 4 demonstrated the lowest at 1950.93 mg kg^{-1}.

Numerous studies in scientific literature have indicated that the amount of sugar components in different garlic plants can be quite divergent. Lisciani et al. (2017) are discussing the sugar content of garlic genotypes ranging from 100 mg kg^{-1} to 900 mg kg^{-1} of fructose and 300 mg kg^{-1} to 900 mg kg^{-1} of glucose. Our study had missed the fructose and glucose that the authors had mentioned to be the case in other studies. These results might be due to the fact that the levels of these components are influenced by environmental factors and genetic diversity. As for sucrose, the values are in line with literature that goes from 2420.00 mg kg^{-1} to 3080.00 mg kg^{-1} (Lisciani et al., 2017), and the value of 3197.79 mg kg^{-1} recorded in Genotype 5 in our study is close to the highest.

Our findings show that the garlic genotypes have notable biochemical differences in their sugar constituents. Sugars are the main source of energy for plants and are at the same time the key components that maintain the cellular energy balance (Patrick et al., 2013; Martins et al., 2016). One more thing, that the intake of the garlic genotypes can be an important tool for plant genetic uniqueness and its ability to adapt to the environment is quite clear from the differences in them. Furthermore, with the help of the potential health benefits of sugars, this is a significant groundwork not only for a healthy evaluation of these genotypes for food but also for their biological activity in functional food products.

The phenolic compounds, organic acids, antioxidant activity, and sugar values of the five different garlic genotypes were evaluated using a heatmap and clustering analysis (Figure 3). Genotype 1 and Genotype 4 were clustered among themselves by reason of their phenolic compound and organic acid profiles, respectively, especially displaying chlorogenic acid (88.35 mg kg^{-1} and 174.99 mg kg^{-1}) and lactic acid (1116.88 mg kg^{-1} and 1133.02 mg kg^{-1}) in great quantities. Genotype 5 was discovered to be quite unlike the rest, demonstrating high levels of succinic acid (1274.73 mg kg^{-1}) and no lactic acid, and also exhibiting high values for substances such as catechin hydrate (15.88 mg kg^{-1}) and chrysin (3.64 mg kg^{-1}). Genotype 2 has high levels of citric acid (737.47 mg kg^{-1}) and acetic acid (69.63 mg kg^{-1}), as well as quercetin (0.41 mg kg^{-1}) and rosmarinic acid (0.33 mg kg^{-1}) contents were its most striking components. In genotype 3, completely different phenolic compound and organic acid components were determined compared to other genotypes. These findings are important because they can serve as a guide for breeding well-adapted garlic varieties and subsequently releasing them for successful garlic cultivation.

4. Conclusion

In this study, the phenolic compounds, organic acids, and sugar components of five different garlic genotypes cultivated in various regions of Türkiye were examined. Encouragingly, the study found data showing biological differences between garlic populations. Notable variations were found in phenolic compounds and antioxidant activity. Genotype 4, for instance, was especially noticeable as it had the highest chlorogenic acid content, while Genotype 5, on the other hand, had the most catechin hydrate of any of the genotypes. With respect to the organic acid profiles, Genotype 2 was in the top position with its high citric acid content in

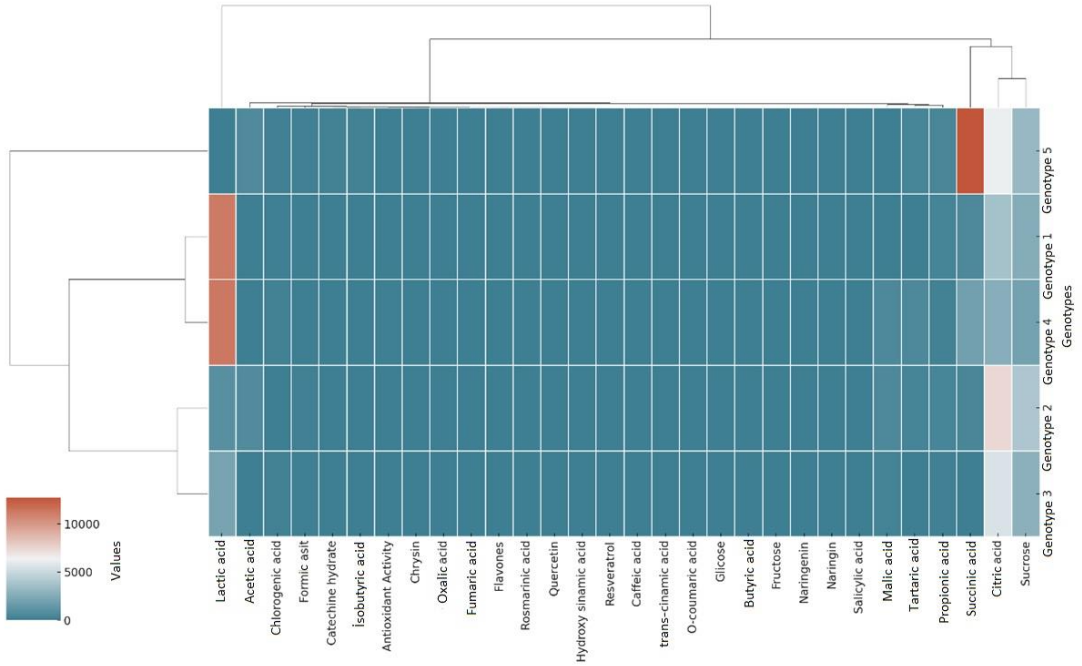

Figure 3. Heat map and cluster analysis of phenolics, organic acids, sugars and antioxidant activity contents.

the study. It was noted further that differences existed in the sucrose content of the genotypes. These measurements represent an important part of the study that would unveil the details on the nutritional characteristics and potentially derived health outcomes from the garlic and enable us to develop new varieties that are better adapted and more resistant. Among them is the identification of the biochemical diversity through phenolic compounds, organic acids, and sugar components, which is the necessary first stage in the process of understanding the genetic diversity attributes and adaptability to the environment of garlic.

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