proceedings

MDPI

Proceeding Paper

Qualitative and Quantitative Characteristics of the Grapes of Different Biotypes of Grapevine Cultivar Assyrtiko in Santorini †

Katerina Biniari *, Dimitris Klimis, Ioannis Daskalakis, Despoina Bouza and Maritina Stavrakaki

Laboratory of Viticulture, Department of Crop Science, School of Plant Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece; dimklms2000@gmail.com (D.K.); john-daskalakis@hotmail.com (I.D.); desph@aua.gr (D.B.); maritina@aua.gr (M.S.)

* Correspondence: kbiniari@aua.gr

† Presented at the 11th International Conference on Information and Communication Technologies in Agriculture, Food & Environment, Samos, Greece, 17–20 October 2024.

Abstract: The aim of the present study was to assess the qualitative and quantitative characteristics of the grapes and berries of seven different biotypes (A1–A7) of grapevine cultivar Assyrtiko, cultivated in Santorini. All biotypes of grapevine cultivar Assyrtiko are cultivated in the same soil and climatic conditions in the area of Akrotiri. Grapes from the different biotypes were collected during technological maturity. In the must of all samples, the following measurements were carried out: total soluble solids concentration, active acidity (pH), and total titratable acidity. Also, mechanical analyses of the grapes and berries of all the biotypes under study were performed. Moreover, using a spectrophotometer, the content of grape's skin in total phenolics, condensed tannins, total ortho-diphenols, total flavonoids, total flavanols, total flavonols and flavones, and their antioxidant capacity with the use of FRAP and DPPH methods were quantified, while high-performance liquid chromatography (HPLC) identified the most important individual acids and individual sugars. The same measurements were also carried out for the grape seeds. The results of the present study revealed the effect of the biotype both in the qualitative and the quantitative characteristics of grape cultivar Assyrtiko, with statistically significant differences being observed among the different biotypes.

Keywords: grapevine; indigenous varieties; Assyrtiko; biotypes; phenolics

Academic Editors: Thomas Bournaris and Athanasios Ragkos

Published: 9 May 2025

Citation: Biniari, K.; Klimis, D.; Daskalakis, I.; Bouza, D.; Stavrakaki, M. Qualitative and Quantitative Characteristics of the Grapes of Different Biotypes of Grapevine Cultivar Assyrtiko in Santorini. *Proceedings* **2025**, *117*, 25. https://doi.org/10.3390/proceedings2025117025

1. Introduction

Greece exhibits a remarkable history when it comes to viticulture and winemaking. Although the surface area cultivated with vines is relatively small compared to other wine-producing countries, the Greek vineyard is characterized by varietal richness. As a result, many Greek grapevine cultivars are indeed single or multiple hybrids [1], which also results in their polyclonality. The polyclonal nature of Greek varieties can be even in small regions, as is the island of Santorini. Santorini is characterized by a very peculiar terroir, both in terms of its microclimate and its soil properties. In Santorini, indigenous varieties have been trained with the traditional training system 'kouloura' and 'kladeftiko', and as a result, high-quality PDO wines are produced [2]. Assyrtiko is considered one of the most well-known white grape cultivars of the Greek vineyard, with its cultivation being spread worldwide (Australia, United States, Italy, and others). Its main cultivation center is the island of Santorini, where 50–55% of the total cultivated area of Assyrtiko is found, where also many biotypes have been identified, but not yet certified as being clones. The biotype-possible clones of a single variety might be different both in yield

potential but also in their organoleptic properties [1]. The aim of the present study was to assess, with the use of spectrophotometry and HPLC, the berry skin and seed content in polyphenols and antioxidant properties of seven biotypes of Assyrtiko (*Vitis vinifera* L.), with the aim of recommending the clones best suited for production and exploitation of high-quality wines.

2. Materials and Methods

2.1. Plant Material

Seven (7) biotypes of grapevine variety Assyrtiko (*Vitis vinifera* L.) were selected in order to assess their phenolic potential. All biotypes of grapevine cultivar Assyrtiko are cultivated in the same soil and climatic conditions in the area of Akrotiri, in the island of Santorini and they have been identified with the use of the ampelographic description and molecular methods. The vines are own rooted and they are trained with the traditional training system of Santorini 'kouloura' where the vines are cane-pruned to 4–6 canes of 8–10 nodes at 2 m × 2 m intervals.

2.2. Mechanical Properties of Grapes and Berries and Characteristics of Must

The mechanical properties of the grapes and berries (length, width, weight), which were collected from the selected vines, were assessed as described in [3]. The characteristics of the must, namely total soluble solids, total titratable acidity, and pH, were determined as described in [4]. The ratio of ripening was calculated by dividing the total soluble solids to the total titratable acidity.

2.3. Determination of Different Polyphenolic Compounds

For sample preparation for spectrophotometric and HPLC analyses for all biotypes and in order to separate the seeds and skins from their respective berries, the samples were prepared according to [5]. Total phenols were measured as described in [5]. The total flavanol content was estimated using the p-dimethylaminocinnamaldehyde (DMACA) method [6]. The total flavonoid content was determined using a colorimetric method as described by [7]. The total flavone and flavonol contents were determined using the method described by [8], as described in [4]. The determination of total (condensed) tannins was carried out following the method of methyl cellulose, as described in [9], with some modifications. The determination of total orthodiphenols was carried out following the method described in [10].

The antioxidant activity was assessed and quantified through two different methods, namely the free radical scavenging activity of DPPH and the Ferric Reducing Antioxidant Power (FRAP) as described by [11] and by [12], respectively.

The HPLC method was used to separate and determine the organic acids of grape musts, following the processes described in [4]. For the separation of individual sugars (glucose, fructose), grape musts were diluted at a ratio of 1:20 in water (HPLC grade). Next, the solution was membrane-filtered (0.45 μm). HPLC analysis was performed by employing a 250 × 4.6 mm ID, 5 μm, Waters SPHERISORB NH2 column operating at 20 °C, under isocratic conditions (mobile phase flow rate: 1 mL min^{-1}). The mobile phase was acidified with 0.1 *v/v* formic acid in a water solution. Extracted sugars were detected with a Reflactive Index (RI) detector. For the quantification of sugars, calibration curves were constructed using standard solutions for glucose and fructose, respectively.

2.4. Data Analysis

Descriptive statistics for considered characteristics were presented as mean ± SE (Standard Error) of the three (3) replications out of three (3) samples/bunches (i.e., the

three bunches were considered as one replication). All determinations were analyzed in triplicate. Data were analyzed by analysis of variance (ANOVA). Following the ANOVA, Tukey's range test at ≤ 0.05 was used to determine statistically significant groups. All statistical analyses and correlations were performed using JMP v. 10 statistical software (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

The qualitative and quantitative characteristics of the grapes and berries of the studied biotypes of grape cultivar Assyrtiko are presented in Table 1.

Table 1. Qualitative and quantitative characteristics of grapes and berries from biotypes studied.

Treatments	A1	A2	A3	A4	A5	A6	A7
Berry length(mm)	14.62 ± 0.08 c	17.47 ± 0.1 a	14.81 ± 0.29 bc	16.13 ± 0.06 ab	16.27 ± 0.55 a	16.45 ± 0.41 a	17.20 ± 0.13 a
Berry width (mm)	13.59 ± 0.02 c	16.72 ± 0.31 a	14.30 ± 0.27 c	15.60 ± 0.12 b	15.72 ± 0.5 ab	15.66 ± 0.37 ab	15.91 ± 0.19 ab
Weight of 30 berries (g)	46.67 ± 1.45 c	78.33 ± 9.53 a	49.33 ± 1.86 c	63.67 ± 2.33 b	65.00 ± 3.51 b	63.00 ± 6.08 b	70.00 ± 1.15 b
Total soluble solids (Brix°)	14.50 ± 0.00 c	16.07 ± 0.03 b	13.73 ± 0.07 e	14.23 ± 0.03 d	16.80 ± 0.0 a	16.67 ± 0.07 a	16.00 ± 0.0 b
pH	2.86 ± 0.00 b	2.97 ± 0.00 a	2.76 ± 0.00 c	2.77 ± 0.00 c	2.81 ± 0.00 c	2.77 ± 0.00 c	2.86 ± 0.00 b
Total acidity (g tartaric/ L must)	6.40 ± 0.13 f	9.10 ± 0.23 c	12.15 ± 0.09 a	10.20 ± 0.09 b	8.35 ± 0.05 d	7.45 ± 0.05 e	8.15 ± 0.13 d
Maturity index	2.28 ± 0.05 a	1.77 ± 0.03 c	1.13 ± 0.01 e	1.39 ± 0.01 d	2.01 ± 0.01 b	2.24 ± 0.01 a	1.96 ± 0.03 b
Total phenolics skins (mg gallic acid/g FW)	3.85 ± 0.29 b	3.79 ± 0.25 b	3.77 ± 0.23 b	3.86 ± 0.20 b	6.05 ± 0.10 a	4.74 ± 0.36 ab	5.46 ± 0.38 a
Total phenolics seeds (mg gallic acid/g FW)	41.07 ± 1.69 b	39.48 ± 1.82 b	42.14 ± 3.69 b	42.23 ± 1.77 b	58.26 ± 1.01 a	44.07 ± 2.89 b	49.79 ± 3.52 ab
Total flavanols skins (mg catechin/g FW)	2.98 ± 0.06 c	3.12 ± 0.11 c	3.46 ± 0.09 bc	3.75 ± 0.21 bc	5.38 ± 0.07 a	4.44 ± 0.13 ab	5.13 ± 0.55 a
Total flavanols seeds (mg catechin/g FW)	48.99 ± 3.31 d	50.90 ± 1.03 cd	58.53 ± 3.74 abc	53.80 ± 2.88 bcd	67.72 ± 3.68 a	62.63 ± 1.10 ab	63.90 ± 1.02 ab
Total flavonoids skins (mg catechin/g FW)	15.99 ± 0.68 c	15.92 ± 0.72 c	16.80 ± 0.89 c	13.65 ± 0.03 c	29.60 ± 0.72 a	23.31 ± 0.42 b	23.52 ± 2.32 b
Total flavonoids seeds (mg catechin/g FW)	298.85 ± 6.83 b	236.88 ± 0.57 c	234.59 ± 4.88 c	181.86 ± 3.15 d	221.34 ± 3.56 c	212.17 ± 7.56 cd	342.72 ± 16.11 a
Total flavones and flavonols skins (mg rutin/g FW)	0.50 ± 0.08 b	0.39 ± 0.01 b	0.35 ± 0.04 bc	0.33 ± 0.01 c	0.50 ± 0.00 a	0.38 ± 0.00 bc	0.26 ± 0.02 d
Total flavones and flavonols seeds (mg rutin/g FW)	0.45 ± 0.01 a	0.43 ± 0.02 a	0.35 ± 0.02 a	0.39 ± 0.01 a	0.44 ± 0.05 a	0.22 ± 0.02 b	0.13 ± 0.01 b
Total Ortho diphenols skins (mg caffeic acid/g FW)	0.31 ± 0.00 d	0.33 ± 0.01 bcd	0.32 ± 0.00 cd	0.37 ± 0.01 bc	0.50 ± 0.01 a	0.38 ± 0.01 b	0.31 ± 0.02 d
Total Ortho diphenols seeds (mg caffeic acid/g FW)	2.43 ± 013 a	2.06 ± 0.13 a	2.30 ± 0.08 a	2.04 ± 0.25 a	2.04 ± 0.14 a	2.66 ± 0.22 a	2.77 ± 0.05 a
Total tannins skins (mg catechin/g FW)	13.27 ± 0.48 cde	10.80 ± 0.27 e	12.10 ± 0.55 de	13.63 ± 0.66 cd	17.95 ± 0.57 b	26.40 ± 1.75 a	15.58 ± 0.38 bc
Total tannins seeds (mg catechin/g FW)	96.03 ± 0.26 c	134.21 ± 5.03 b	83.24 ± 1.35 cd	127.13 ± 3.31 b	137.78 ± 1.58 ab	73.37 ± 0.33 d	149.79 ± 3.95 a
Frap skins (mg trolox/g FW)	44.39 ± 0.15 bc	46.00 ± 0.15 ab	48.42 ± 0.59 a	41.16 ± 1.27 cd	22.56 ± 0.07 e	40.08 ± 1.40 d	39.19 ± 0.73 d
Frap seeds 9mg trolox/g FW)	242.93 ± 1.46 ab	197.39 ± 0.23 c	218.70 ± 4.64 bc	253.90 ± 2.84 a	254.82 ± 11.75 a	266.14 ± 9.16 a	260.26 ± 4.85 a
DPPH skins (mg trolox/g FW)	139.95 ± 2.99 a	71.63 ± 0.86 cd	84.47 ± 0.59 b	64.97 ± 0.38 d	55.88 ± 1.30 e	73.22 ± 1.78 cd	74.16 ± 2.33 c
DPPH seeds (mg trolox/g FW)	413.81 ± 5.61 a	395.28 ± 2.12 a	241.93 ± 10.00 c	167.43 ± 1.37 d	242.87 ± 7.43 c	352.70 ± 8.03 b	353.57 ± 9.20 b
Tartaric acid (mg tartaric acid/mL must)	17.304 ± 0.225 c	15.752 ± -0.367 d	17.882 ± 0.054 c	22.081 ± 0.120 a	17.033 ± 0.255 c	20.837 ± 0.113 b	20.122 ± 0.128 b
Malic acid (mg malic acid/mL must)	10.059 ± 0.191 c	12.269 ± 0.147 a	11.225 ± 0.172 b	5.439 ± 0.292 d	3.894 ± 0.097 e	4.680 ± 0.068 de	4.989 ± 0.087 d
Ascorbic acid (µg ascorbic acid/mL must)	278.19 ± 5.16 c	354.16 ± 2.40 b	395.17 ± 4.64 a	235.09 ± 15.57 d	199.77 ±0.77 e	218.55 ± 2.95 de	243.85 ± 6.38 d
Succinic acid (µg succinic acid/mL must)	10.13 ± 0.05 cd	12.25 ± 0.44 c	9.10 ± 0.33 cd	6.90 ± 0.14 d	19.69 ± 1.47 b	18.51 ± 0.34 b	26.91 ± 1.49 a
Fumaric acid (µg fumaric acid/mL must)	436.33 ± 1.82 a	291.86 ± 1.38 bc	300.29 ± 22.32 bc	54.41 ± 2.21 d	314.63 ± 20.05 bc	265.38 ± 11.22 c	339.05 ± 15.33 b
Fructose (g/L)	141.80 ± 0.97 b	168.84 ± 0.87 a	108.61 ± 3.29 c	117.41 ± 0.07 c	154.07 ± 4.06 ab	159.07 ± 3.67 a	142.81 ± 5.53 b
Glucose (g/L)	150.41 ± 1.01 bc	180.79 ± 2.12 a	115.63 ± 3.89 d	125.79 ± 0.13 d	159.86 ± 2.57 bc	163.49 ± 3.79 b	148.07 ± 5.35 c

The values are the means of the triplicates. In each cell, the results are presented as the mean ± Standard Error. The values in the same row carrying a different letter (a–f) are significantly different at significance level $p \leq 0.05$, according to Tukey's test.

The results show that biotype A2 exhibits the largest berries (length 17.47 mm and width 16.72 mm) and the greatest berry weight (78.33 g). In contrast, A1 records the smallest

berries and the lowest berry weight (46.67 g). Biotypes A5 and A6 exhibit the highest Brix values (16.80 and 16.67, respectively), indicating a higher sugar content. A2 exhibits the highest pH (2.97), while A3 has the highest acidity (12.15 g tartaric acid per liter of must), making it more acidic. The ratio of ripening is highest in the berries of biotypes A1 and A6, suggesting that these have the best balance between sugars and acids, which is essential for wine production. Overall, the results provide a clear view of the qualitative differences among grape biotypes, highlighting the advantages and variations that may arise from different cultivation conditions and uses.

Biotypes A5 and A7 stand out, presenting the highest levels of total phenolic compounds in skins (6.05 mg/g and 5.46 mg/g, respectively) and the highest tannin concentrations in seeds. A7 has the maximum tannin concentration in seeds (149.79 mg/g), while A5 shows high flavonoid concentrations in seeds. Flavanols and flavones also show significant variations, with A5 and A1 exhibiting higher concentrations. The high levels of phenolic compounds, especially in the grape skins of clones A5 and A7, indicate antioxidant properties, which are essential for the quality of the wine produced.

Overall, the results show significant differences among the different biotypes, offering valuable insights for optimizing the quality of the wines produced.

The antioxidant capacity, as determined with the FRAP method, is highest in the skins of biotype A3 (48.42 mg trolox/g), while the seeds of biotype A6 display the maximum antioxidant capacity (266.14 mg trolox/g). With the use of the DPPH method, the skins of biotype A1 have the highest value (139.95 mg trolox/g), with A1 seeds also leading among other biotypes at 413.81 mg trolox/g. Among the individual organic acids, A4 has the highest concentration of tartaric acid (22.081 mg/mL), while the skins of biotype A3 excel in malic and ascorbic acid. The skins of biotype A7 have the highest concentration of succinic acid (26.91 μg/mL). Regarding sugar concentration (fructose and glucose), A2 stands out with 168.84 g/L fructose and 180.79 g/L glucose, indicating high sweetness, which may be significant for specific types of wine. The notable differences among biotypes offer valuable insights for identifying ideal biotypes—potential clones—according to their grape use.

4. Conclusions

The results of the present study show differences among the seven biotypes of grapevine Assyrtiko in morphology, phenolic compounds, and antioxidant capacity. These data provide valuable insights for selecting appropriate biotypes depending on the winemaking purpose, as each biotype exhibits unique characteristics that can aid in optimizing wine quality. Overall, the study underscores the importance of understanding the physicochemical differences among biotype-potential clones for producing high-quality wines from grapevine cultivar Assyrtiko.

Author Contributions: Conceptualization, K.B., M.S. and D.B.; methodology, K.B. and I.D.; validation, M.S., I.D. and D.B.; formal analysis, I.D. and D.K.; investigation, K.B., M.S., I.D. and D.K.; data curation, I.D. and D.K.; writing—original draft preparation, M.S, D.B., I.D., D.K.; writing—review and editing, K.B and M.S.; supervision, K.B. and M.S.; funding acquisition, K.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financed by the European Union—NextGenerationEU in the context of the National Recovery and Resilience Plan (Greece 2.0) under the call 16971: RESEARCH—CREATE—INNOVATE (Project code: TAEDK-06183, NATIVINE).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

HPLC	High-performance liquid chromatography
PDO	Protected designation of origin
DMACA	p-dimethylaminocinnamaldehyde
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
ANOVA	Analysis of variance

References

1. Stavrakakis, M.N. *Ampelography*; Embryo Puplications: Athens, Greece, 2021.
2. Xyrafis, E.G.; Deloire, A.; Petoumenou, D.; Paraskevopoulos, I.; Biniari, K. The unique and extreme vineyards of Santorini Island (Cyclades). *IVES Tech. Rev. Vine Wine* **2021**. [CrossRef]
3. Stavrakaki, M.; Biniari, K.; Daskalakis, I.; Bouza, D. Polyphenol content and antioxidant capacity of the skin extracts of berries from seven biotypes of the Greek grapevine cultivar Korinthiaki Staphis (*Vitis vinifera* L.). *Aust. J. Crop Sci.* **2018**, *12*, 1927–1936. [CrossRef]
4. Biniari, K.; Xenaki, M.; Daskalakis, I.; Rusjan, D.; Bouza, D.; Stavrakaki, M. Polyphenolic compounds and antioxidants of skin and berry grapes of Greek Vitis vinifera cultivars in relation to climate conditions. *Food Chem.* **2020**, *307*, 125518. [CrossRef]
5. Biniari, K.; Gerogiannis, O.; Daskalakis, I.; Bouza, D.; Stavrakaki, M. Study of some qualitative and quantitative characters of the grapes of indigenous Greek grapevine varieties (*Vitis vinifera* L.) using HPLC and spectrophotometric analyses. *Not. Bot. Horti Agrobot.* **2018**, *46*, 97–106. [CrossRef]
6. Vivas, N.; Glories, Y.; Lagune, L.; Saucier, C.; Augustin, M. Estimation du degré de polymérisation des procyanidins du raisin et du vin par la méthode au p-dimethylaminocinnamaldéhyde. *J. Int. Des Sci. De La Vigne Et Du Vin* **1994**, *28*, 319–336. [CrossRef]
7. Dewanto, V.; Wu, X.; Adom, K.K. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010–3014. [CrossRef]
8. Bonvehi, J.S.; Coll, F.V.; Jorda, R.E. The composition, active components and bacteriostatic activity of propolis in dietetics. *J. Am. Oil Chem. Soc.* **1994**, *71*, 529–532. [CrossRef]
9. Sarneckis, C.J.; Dambergs, R.G.; Jones, P.; Mercurio, M.; Herderich, M.J.; Smith, P.A. Quantification of condensed tannins by precipitation with methyl cellulose: Development and validation of an optimized tool for grape and wine analysis. *Aust. J. Grape Wine Res.* **2006**, *12*, 39–49. [CrossRef]
10. Roussos, P.A.; Pontikis, C.A. Phenolic Compounds in Olive Explants and their Contribution to Browning During the Establishment Stage in vitro. *Gartenbauwissenschaft* **2001**, *66*, 298–303. [CrossRef]
11. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25–30. [CrossRef]
12. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef] [PubMed]