

Innovative strategies for reducing astringency in Mandilaria wines

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Abstract. Mandilaria, a red grape variety native to the Aegean Islands, is recognized for its robust tannins and notable astringency, which can affect the appeal and marketability of its wines. This study aimed to reduce the astringency in wines made exclusively from Mandilaria grapes by employing dehydration techniques and specific winery practices. Three distinct dehydration techniques were tested in an experimental vineyard in Paros Island: sun exposure, air dehydration under controlled conditions and extended ripening on the vine. Additionally, mechanical removal of 20% and 30% of seeds was implemented during maceration to reduce the extraction of astringent phenolics. According to the results, air and sun dehydration treatments significantly increased phenolic content, tannin concentration, and antioxidant activity. These treatments also resulted in wines with improved phenolic ripeness and reduced harsh astringency. The findings indicate that integrated use of specific dehydration practices and selective winery interventions can effectively enhance the sensory qualities and consumer appeal of Mandilaria wines.

1. Introduction

Historically, Greece has played a crucial role in viticulture and wine production, maintaining a strong winemaking tradition from ancient times to the present. Traditionally, Greek grape growers cultivated local grape varieties, as there is a genetically rich grapevine population with over 300 indigenous *Vitis vinifera* varieties [1-3]. However, the globalization of the wine market reduced genetic diversity significantly, as many local varieties were replaced by a few well-known ones known for their phenolic and aromatic properties, which are crucial for vinification [4-5]. Recently guidelines have changed vineyard planting from international (established) varieties to native stocks. This new direction has also derived from a strong interest in preserving rare native varieties that are at risk of extinction [5-7]. Although a lot of work has been done in this direction, many of these native varieties remain unexploited [8-9].

Wine polyphenols come from grape berries, owned a significant role in plant metabolism [10]. Their composition in grapes is influenced by genetic differences, environmental conditions, and viticultural practices [11-12]. From a sensory perspective, flavanols are critical as they polymerize to form tannins [13]. These condensed tannins, or proanthocyanidins, are vital for red wine

quality, causing astringency by precipitating proteins [14]. Tannin contribution to astringency depends on their size and subunit composition, which are affected by winemaking practices, especially during fermentation and pressing, although their content may stabilize or decrease with extended maceration [12,15]. They also stabilize red wine color through interactions with anthocyanins [16].

Astringency in wine is a tactile sensation characterized by drying, roughening, and shrinking in the mouth [17]. It is considered pleasant when balanced with other factors like alcohol and sugar content. However, when tannins and acids are present in higher concentrations relative to sugar, the wine can become overly astringent, often described as 'harsh,' 'unripe,' or 'green' [18]. Astringency significantly influences the quality of red wine, making it crucial for winemakers to understand the structures of astringent compounds in the wine matrix and their impact on sensory properties [19-20]. Various approaches during the red wine production process can influence the concentration and composition of tannins, and by extension the sensation of astringency.

Extensive research has been conducted on the mechanisms underlying wine astringency perception, the factors that influence astringency in wine, and the structures of certain contributing tannins. The outcomes of many studies have provided the correlation of the reduced

perceived astringency in wines with their tannin content, pH values, polyphenolic content and alcoholic degree [8,11,13,21]. Conversely, new methods for mitigating the undesirable perception of phenolic astringency are becoming more common, but comprehensive results of these approaches remain limited [22].

The aim of this study was the reduction of the astringency in wines made exclusively from Mandilaria grapes, which is an indigenous variety grown on the islands of the Aegean, specifically under the environmental conditions of the Paros Island. To achieve this goal, dehydration techniques and tannin reduction methods were used. Grape dehydration is a pre-fermentation technique that extends the ripening process, though it differs from physiological maturity. This method varies globally, depending on the style of wine, geographic region, grape variety, and viticultural practices [23-24]. On the other hand, reduce of tannins is a fermentation technique which has the potential to reduce the sensation of astringency.

2. Materials and Methods

2.1. Samples

Grapes (*Vitis vinifera* L. cv Mandilaria) from Paros Island of Greece were harvested in 2023 when the soluble solids content reached $18.8 \pm 1^\circ\text{Brix}$. The dehydration of grapes was carried out using three distinct methods: sun-drying (D-SUN), dehydration in a closed chamber with shaded air circulation (D-AIR), similar to the Amarone technique, and extended ripening on the vine through pedicel crushing (D-VIN). To monitor the chemical changes during the dehydration process, grape samples were also collected at harvest to serve as control (CTRL). The dehydration process was carefully monitored through daily measurements of sugar content and berry weight. Samples of 100 berries were collected for the grape maturity analysis of each treatment in accordance with the OIV standard methods for wine analysis [25]. Additionally, 100 berries were weighed, and the skins and seeds were manually separated to determine the average berry mass and quantify the distribution of berry mass components across the different dehydration methods.

The dehydration technique was studied both in grapes and wine samples. The grapes were destemmed and crushed in the Agricultural Cooperative of Paros and then were vinified according to the traditional winemaking method for Mandilaria. After the completion of alcoholic fermentation, the wines were stored at -20°C until the analyses.

Simultaneously, during the winery phase, two interventions were tested: the mechanical removal of 20% (SE20) and 30% (SE30) of seeds during the early stages of maceration. Seed removal was conducted to minimize the extraction of seed-bound phenolics, which are primarily responsible for astringency.

2.2. Chemical analysis

The analysis of phenolic compounds in grapes and wines was conducted using the assays detailed below.

2.2.1. Polyphenolic content, wine color intensity and hue

Before analysis of total polyphenolic index (TPI), all the grape and wine samples were filtered and then diluted with distilled water at a 1:100 ratio. The absorbance measurements were recorded at 280 nm using a UV-VIS spectrophotometer (Jasco, Victoria, BC, Canada).

Color intensity (CI) and hue measurements were also conducted. Wine samples were filtered, transferred into UV-VIS cuvettes, and placed in a spectrophotometer to record absorbances at 420, 520, and 620 nm.

2.2.2. Total phenols and antioxidant activity

Total polyphenol concentration of wines was measured using the Folin-Ciocalteu assay, with the slight modifications as described previously [26]. The results were reported in mg/L gallic acid equivalents (GAE).

The antioxidant activity of the wine samples was estimated using the DPPH method and it was expressed as Trolox equivalent antioxidant capacity (TEAC). Absorbance measurements were recorded at 515 nm on a UV/VIS spectrophotometer.

2.2.3. Analysis of anthocyanins

Total anthocyanin content of grape berries was measured according to Iland method [27], while the Glories method was applied for the quantitative evolution of extractable anthocyanins of the grapes [28]. Furthermore, the analyses of grapes and wines were conducted by high-performance liquid chromatography according to previous methods [3,29]. Identification was achieved comparing the retention times of the detected peaks with those of the original compounds. All peaks were quantified as malvidin-3-O-glucose (Mlv) equivalents.

2.2.4. Tannin determination with Bovine Serrum Albumin and Methyl Cellulose Precipitation

The tannin content in wine samples was measured using the protein precipitation method outlined by Harbertson et al. [30]. Absorbance measurements at 510 nm were taken using a UV/VIS spectrophotometer and the tannin levels were quantified using a standard curve of catechin and expressed as milligrams of catechin equivalents per liter.

The assay, developed and validated by Sarneckis et al. [31], determined tannin concentration by comparing a control sample with a methylcellulose-precipitated sample at 280 nm. Tannins were quantified using a catechin standard curve and expressed as milligrams of catechin equivalents per liter.

2.2.5. Sensory evaluation

The astringency of the wine samples was evaluated using sensory analysis. Each sample consisted of 30 mL of wine presented at room temperature (20°C) was served in tulip-shaped wine glass coded with three digits and

presented in a random sequence. Participants were instructed to rate the intensity of astringency and bitterness using a scale from 0 (not perceived) to 7 (very intense) [8].

2.3 Statistical analysis

Statistical analyses were conducted using Statistica v.7 (Statsoft InC., Tulsa, OK, USA) program. The significance of the results was assessed using an unpaired t-test or one-way ANOVA followed by Tukey's HSD test for comparing mean values when significant differences were found ($p < 0.05$). All analyses were performed in triplicate.

3. Results and discussion

3.1. Berry features of dehydrated grapes

The variations observed in the classical analysis data of the berries, as well as their physical properties across different dehydration methods, indicate the differing time requirements for complete moisture removal. Grapes exposed to sunlight (D-SUN) achieved the highest sugar concentration, reaching 27.6 °Brix, along with a higher pH (3.71). In contrast, dehydration in shade and air (D-AIR) resulted in a more moderate sugar increase (23.5 °Brix), a significant reduction in pH (3.25), and the highest titratable acidity (7.8 g tartaric acid·L⁻¹). Extended ripening on the vine (D-VIN) produced intermediate values for both °Brix and acidity (Table 1).

The size of the berry is a crucial factor in determining yield. Mandilaria is a particularly tannic variety, robust with very dense grape clusters. Through the different techniques of dehydration, an effort was made to manage and improve the intense density of the grapes.

As expected, all the techniques significantly reduced the berry weight (Table 1). However, the distribution of grape components (skins, seeds and flesh) in dehydrated berries varied considerably between the techniques. Specifically, a significant increase in the weight of the skins was observed in sun drying grapes, while the percentage of seeds increased relatively similarly across all dehydration treatments. The ratio of skin to flesh in berries has a crucial role in determining the quality and sensory characteristics of wine. Skins are abundant in phenolic and aromatic compounds. During the vinification process, these compounds are extracted into the must, and their impact

on the wine's organoleptic properties is more pronounced as the skin-to-flesh ratio increases [5]. As shown in the Table 1, the D-SUN grapes were characterized by the higher skin/flesh (0.270), while the other methods, D-VIN (0.081) and D-AIR (0.129) resulted in lower ratios.

The required time for the grapes to dehydrate differed depending on the dehydration method, affecting the characteristics of the berries. As part of monitoring the dehydration process, the weight and sugar content were measured every two days. The weight loss and Brix curves in relation to dehydration time showed a linear decline (Fig. 1a), with higher determination coefficients of $R^2 = 0.9404$ for the D-SUN method. Regarding weight loss (Fig. 1b), the dehydration process using the D-VIN method showed the most linear decline, with a strong correlation ($R^2 = 0.924$).

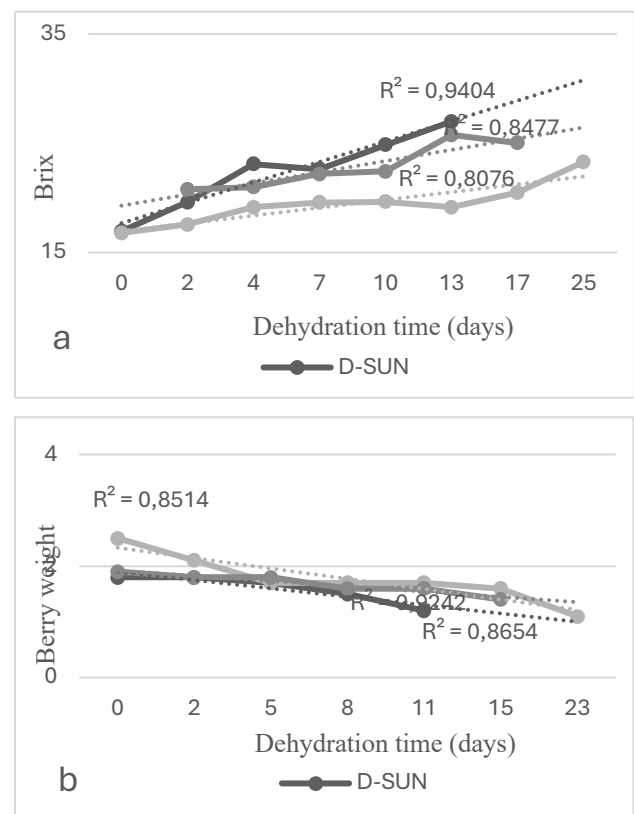


Figure 1. Evolution of sugars (a) and weight (b) in the dehydration experiment.

Table 1. Grape characteristics of dehydrated grapes.

	°Brix	pH	Total acidity	Weight of 50 grapes (g)	% skins/berry	% seeds/berry	% flesh/berry	skin/flesh	Harvest day
CTRL	18.8 ± 0.3 d	3.86 ± 0.0 c	2.6 ± 0.2 cd	114.2 ± 0.4 c	7.6 ± 0.2 de	3.7 ± 0.2 d	88.7 ± 0.7 c	0.085 ± 0.1 e	22/9/23
D-VIN	24.4 ± 0.5 c	3.53 ± 0.0 e	4.9 ± 0.3 b	65.8 ± 0.5 d	7.1 ± 0.5 de	5.3 ± 0.2 c	87.6 ± 0.4 c	0.081 ± 0.0 e	9/10/23
D-SUN	27.6 ± 0.6 a	3.71 ± 0.1 d	5.7 ± 0.2 a	43.5 ± 0.4 e	19.6 ± 0.3 a	7.8 ± 0.6 a	72.6 ± 0.2 e	0.270 ± 0.1 b	5/10/23
D-AIR	23.5 ± 0.5 c	3.25 ± 0.0 f	7.8 ± 0.4 a	48.8 ± 0.3 e	10.7 ± 0.7 c	6.3 ± 0.4 b	83.0 ± 0.5 d	0.129 ± 0.3 d	17/10/23

3.2. Phenolic composition of grapes and wines

The determination of phenolic compounds in wines produced using different dehydration methods, along with the effects of mechanical seed removal, are summarized as mean values with their respective standard deviations and presented in Table 2.

Table 2. Phenolic composition of wines.

*	MCP	BSA	DPPH	TPI	TP
CTRL	2416.0± 304 de	580.11 ± 10.11 d	16.09 ± 0.73 c	90.15 ± 0.03 e	3790.91 ± 240.91 cd
SE20	1984.0± 8 ef	427.98 ± 9.04 e	12.86 ± 0.62 d	84.53 ± 0.13 g	3531.82 ± 9.09 cd
SE30	1512.0 ± 304 f	370.00 ± 0.00 f	12.70 ± 0.08 d	77.92 ± 0.05 h	3372.73 ± 40.91 d
D-VIN	5114.7 ± 341.79 c	1400.64 ± 24.47 c	25.63 ± 0.95 b	155.70 ± 0.30 c	6518.18 ± 109.09 b
D-SUN	7568.0 ± 57.69 b	2086.38 ± 36.17 b	28.61 ± 0.05 a	187.60 ± 0.60 b	9018.18 ± 336.36 a
D-AIR	8405.3 ± 473.10 a	2224.68 ± 0.00 a	29.64 ± 0.62 a	236.20 ± 0.00 a	9281.82 ± 345.45 a

* Tannins measured with MCP and BSA methods are expressed as (mg/L), DPPH as TEAC (mM) and total phenolics as (mg GAE/L)

3.2.1. Tannin Content (Tannins MCP and Tannins BSA): Correlation with observed astringency

The tannin content values of wines obtained from both assays (MCP and HA) demonstrate a direct correlation with observed astringency. According to Table 2, D-AIR treatment showed the highest tannin content using both the MCP and BSA methods, indicating a significant increase in tannin concentration due to air dehydration. It was observed that D-SUN followed closely, also showing high tannin levels. Additionally, it's worth noting that despite the low tannin content in D-VIN treatment, they were perceived as astringent as other dehydrated wines. This could be due to the high proportion of seed tannins relative to skin tannins, which has been previously reported [32], as seed tannins are generally considered more astringent than skin tannins [11].

In the present study, the technique of seed removal during fermentative maceration led to a reduction in tannin concentrations, as anticipated. Consequently, the wines were perceived as having the lowest levels of astringency. However, this result has not been consistently validated across all studies [33].

Figure 2 shows the correlation between astringency and tannin content using either the MCP or BSA method. The data obtained from both methods demonstrated a strong correlation, with R² values of 0.794 for the MCP method and 0.813 for the BSA method. A different study [11]

demonstrated that the linear correlation between astringency and tannin content reaches a saturation point. This suggests that beyond a certain tannin concentration, an increase in tannins does not correspond to a proportional increase in the astringency scores assigned by the judges. The intensity of astringency in the samples studied is categorized as high (>4.5) in the dehydrated treatments, low (<2.5) in the seed removal treatments, and intermediate for the control samples.

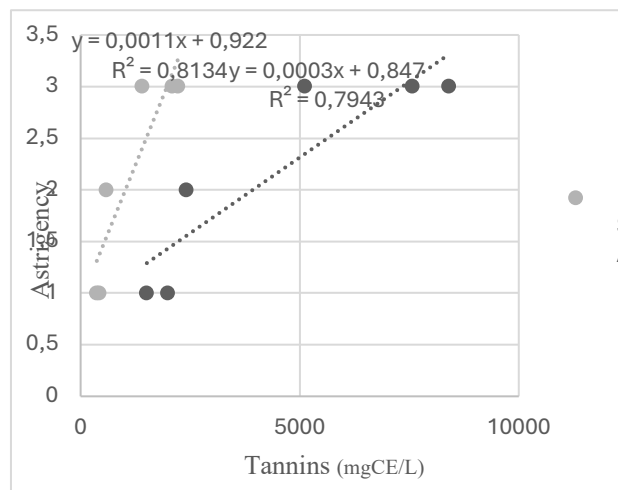


Figure 2. Correlation between astringency and tannin content calculated using both MCP and BSA methods.

3.2.2. Total phenolic content and antioxidant capacity in wines

Table 2 presents the total phenolic content and the total phenolic index of the wine samples. The results indicate that wines from dehydrated grapes were characterized by higher levels of total polyphenols and total phenolic index compared to the control sample. Specifically, the D-AIR treatment showed the highest TPI and total phenolics, indicating a substantial increase in phenolic content, which is crucial for the wine's body and aging potential. D-SUN followed closely, with slightly lower values than D-AIR. D-VIN exhibited a significant increase compared to the control and seed removal treatments but remained lower than D-AIR and D-SUN. SE20 and SE30 had the lowest TPI and total phenolic content, as it was expected. The elevated polyphenol content in wines produced from dehydrated grapes is attributed to the concentration effect caused by water loss [34].

The antioxidant activity of phenolic compounds is strongly correlated with the individual and total phenolic contents [35]. This is also evident in our results as the D-AIR and D-SUN treatments exhibited the highest antioxidant activities, with D-AIR being slightly higher, suggesting that these dehydration methods enhance the concentration of antioxidant compounds. D-VIN also demonstrated a notable increase in antioxidant activity compared to the control and seed removal treatments. In contrast, SE20 and SE30 wines showed lower antioxidant activities, as predicted in proportion to their lower phenolic content.

3.2.3. Anthocyanin composition of grapes and wines and chromatic parameters

The grapes dehydrated under the sun were characterized by the lowest total anthocyanin content and exhibited reduced extractability compared to the other two dehydration methods. Specifically, the D-SUN grapes exhibited particularly low total anthocyanin levels (0.61 mg/berry) and extractability values (14.20) (Table 3).

Table 3. Anthocyanin content of dehydrated grapes.

	anthocyanins (mg/berry)	extractability (AE%)	anthocyanins (mg/g fresh skin)
CTRL	1.41 ± 0.04 c	45.26 ± 2.17 cd	12.20 ± 0.20 d
D-SUN	0.61 ± 0.07 e	14.20 ± 1.25 f	3.10 ± 0.10 g
D-AIR	0.69 ± 0.05 e	18.58 ± 2.00 e	10.60 ± 0.30 e
D-VIN	1.07 ± 0.05 d	17.31 ± 1.43 e	9.70 ± 0.40 e

Similar trends were observed for the individual anthocyanin content in grape skins. Specifically, anthocyanin levels significantly decreased during sun dehydration, while no major differences were observed between air dehydration and vine over-ripening, except for peonidin (Pn = 0.61 mg/g fresh skin for D-AIR and 0.38 mg/g fresh skin for D-VIN) (Table 4). However, this finding was not consistently validated across all studies [36] as Moreno et al. (2008) reported an increase in anthocyanin levels during postharvest dehydration. Nevertheless, most researchers agree that greater exposure to light, and consequently higher temperatures, intensifies the rupture of grape skins, potentially leading to more severe oxidative degradation of anthocyanins [37, 38].

Table 4. Anthocyanin concentration in dehydrated grapes (mg/g fresh skin).

*	Dlp	Cyn	Pt	Pn	Mlv
CTRL	0.41 ± 0.03 d	0.12 ± 0.01 d	0.39 ± 0.04 f	0.97 ± 0.07 e	6.79 ± 0.12 f
D-SUN	0.08 ± 0.01 f	0.03 ± 0.00 f	0.09 ± 0.01 j	0.26 ± 0.02 i	1.53 ± 0.09 i
D-AIR	0.17 ± 0.04 e	0.04 ± 0.00 f	0.22 ± 0.05 g	0.61 ± 0.03 f	5.86 ± 0.14 g
D-VIN	0.17 ± 0.02 e	0.03 ± 0.00 f	0.22 ± 0.03 g	0.38 ± 0.06 h	5.09 ± 0.16 g

* Dlp: delphinidin-3-O-glucoside; Cyn: cyanidin-3-O-glucoside; Pt: petunidin-3-O-glucoside; Pn: peonidin-3-O-glucoside; Mlv: malvidin-3-O-glucoside.

As illustrated in Figure 3, wines produced from dehydration treatments exhibited low anthocyanin content. Specifically, the wine produced from grapes subjected to sun dehydration contained considerably lower amounts of anthocyanins (2.07 mg/L). In contrast, dehydration on the vine resulted in higher anthocyanin levels (101.80 mg/L), which, although still relatively low, represented the highest value among all the dehydration methods tested. Additionally, in wines where seeds were removed during winemaking, no significant impact on the anthocyanin profile was observed, consistent with previous findings [33]. A slight, non-significant reduction in total anthocyanin content was noted in the SE20 wine. Overall,

under the same maceration conditions, the extraction of polyphenols from skins and seeds occurred independently, and seed removal did not affect anthocyanin extraction from the skins [33].

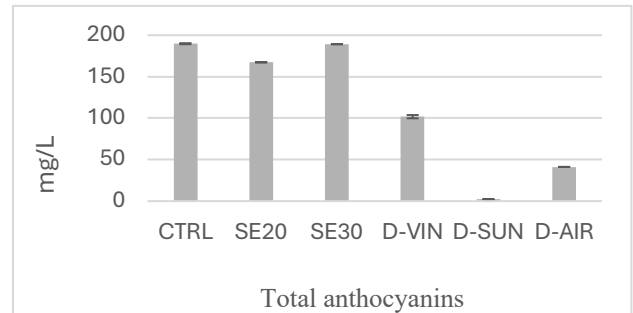


Figure 3. Total anthocyanins in wines of dehydration treatments and the removal of seeds.

No significant differences were observed between seed removal treatments and the control sample concerning color parameters, including intensity and hue (Figure 4). In contrast, among the dehydration treatments, D-VIN wines displayed the highest color intensity (1.85 AU) and the lowest hue values (0.61 AU), indicating a significant shift towards a deeper or more intense color. D-SUN wines exhibited slightly lower color intensity (1.79 AU) along with the highest hue value (0.84 AU), reflecting a different color shift compared to D-VIN. D-AIR wines demonstrated moderate color intensity and a hue value comparable to that of the seed removal treatments (0.69 AU), suggesting a more balanced color development. These findings are consistent with those of Panceri et al. [39], who reported that wines produced from dried grapes exhibited a higher content of polymeric anthocyanins, resulting in increased color intensity and hue values.

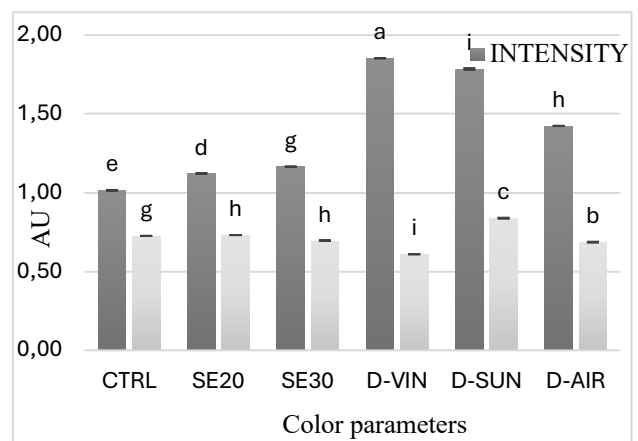


Figure 4. Color parameters in wines of dehydration treatments and the removal of seeds.

4. Conclusions

This study demonstrates that post-harvest dehydration techniques, particularly air and sun dehydration, significantly influence the quality indicators of Mandilaria wines, enhancing phenolic content, tannin levels, and antioxidant activity, while also improving phenolic ripeness and reducing the harsh tannic profile typically

associated with this native Greek variety. Air dehydration emerged as particularly promising, and the removal of seeds during early maceration further reduced astringent compounds without compromising the wine's structural integrity. The integrated approach combining specific dehydration practices with selective winery interventions proves to be an effective strategy for enhancing both the sensory appeal and consumer acceptance of Mandilaria wines, thereby contributing to the preservation and appreciation of Greece's rich viticultural heritage.

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