

Article

Influence of Vineyard Location on Physicochemical Properties, Phenolic Content, and Antioxidant Capacity of ‘Touriga Nacional’ Grapes Cultivated in Brazil and Portugal

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Abstract

The use of new grape cultivars is essential for the continued development of Brazilian viticulture. Thus, this study aimed to determine the general physicochemical parameters, global phenolic composition, and antioxidant capacity of grape musts from the Portuguese red variety ‘Touriga Nacional’ during ripening in two Brazilian vineyards (states of Rio Grande do Sul and Santa Catarina). The results were compared with data obtained from the same variety cultivated in a vineyard located in northern Portugal, which is the region of origin of this variety. This research was conducted over three consecutive vintages (2022–2024). Overall, the results indicated that soil and climate conditions at each location were associated with differences in the composition of ‘Touriga Nacional’ grape musts. Grapes from both Brazilian vineyards showed significantly higher berry weight, must volume, and yield compared with grapes collected from the Portuguese vineyard. On the other hand, grapes collected from the vineyard located in the state of Santa Catarina exhibited the highest values of total titratable acidity, malic acid, total phenols, flavonoids, total anthocyanins, and chromatic characteristics. Nevertheless, grapes collected from the Portuguese vineyard showed higher values of total tannins. The results suggest that the ‘Touriga Nacional’ variety shows better adaptation to the conditions of the Santa Catarina vineyard than to those of the Rio Grande do Sul vineyard. These findings help winegrowers, particularly in southern Brazil, to have more options for introducing different grape varieties, thereby contributing to the production of wines with distinctive characteristics, while consumers will have access to a greater diversity of wines available on the market.



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1. Introduction

Grape production represents an important economic activity in Brazil. In 2025, grape production reached 2,209,104 tons, cultivated over an area of 86,711 ha [1]. However, according to the most recent official reports from the 2023 vintage, grapes destined for wine and juice production accounted for 1,064,763 tons, representing 59.2% of total grape production [2].

Viticulture in Brazil extends from the state of Rio Grande do Sul (31° S) to the state of Rio Grande do Norte (05° S), covering nearly 5000 km from south to north. Consequently, Brazil exhibits high diversity in soil and climatic conditions, resulting in a wide range of grapevine varieties, management systems, and, ultimately, grapes and different types of wine with distinct characteristics [3].

Soil and climatic conditions are recognized as major modulators of grape berry composition [4]. Specifically, the diversity and concentration of phenolic compounds in grapes are determined by multiple biotic and abiotic factors, all of which are associated with the *terroir* concept. These compounds in grapes are intrinsically linked to chemical and enzymatic reactions, which depend on the berry's physiological status and, primarily, on the ecophysiological conditions of the vines [5]. In this context, *terroir* has gained prominence as a framework describing the interactions between natural and human factors that define the typicality of grapes and wines [6–9]. Among the main biotic and abiotic elements comprising *terroir* and determining grape phenolic composition are climate (precipitation, solar radiation, humidity, and temperature), soil, altitude, vine nutritional status, grape variety, and viticultural practices [10–16].

Phenolic compounds are secondary metabolites that exhibit several nutraceutical properties and have been associated with protective effects against conditions such as hypertension, diabetes, and cardiovascular diseases [17]. Moreover, phenolic compounds have been reported to present anti-inflammatory and anticancer activities and to modulate human immune responses [18]. Furthermore, phenolic compounds are essential in enology, contributing to key wine characteristics such as color, bitterness, and astringency [19,20].

Recent studies have demonstrated the effect of environmental conditions on the physicochemical composition of grapes. For example, Hamie et al. [21] demonstrated that, for several table grapes, sugar concentrations and fruit firmness decreased with altitude. Similarly, Canturk et al. [6] reported for 'Kalecik Karası' (*Vitis vinifera* L.) grapes cultivated in vineyards located at higher altitudes (1.180 m), high concentrations of total anthocyanins and lower tannin content. Meanwhile, Mansour et al. [22] demonstrated that higher UV-B radiation at higher elevations provided higher concentrations of anthocyanins in the berries. Mateus et al. [23] described that 'Touriga Nacional' grapes cultivated under different altitudes in the Douro wine region (Portugal) showed an increase in the phenolic content in grapes cultivated in the higher altitude areas of the vineyards, but a reduction in the alcohol content, berry volume and yield per plant. Instead, Satisha et al. [24] observed that high temperatures led to greater degradation of organic acids and a reduction in the concentration of anthocyanins.

Water availability is also a key factor that affects the development of vines and the physicochemical composition of grape berries. Water scarcity can be associated with terminal stress, leading to a reduction in total acidity [25]. In contrast, excess water promotes the dilution of different grape berry constituents, such as organic acids and sugars [26]. In addition, according to several authors, the ripening stage of the grapes is also decisive for their composition [27–29]. However, Costa et al. [30] studied the adaptability of several Portuguese red grape varieties and reported that 'Touriga Nacional' was one of the grape varieties that showed the highest anthocyanin concentration, regardless of the ripening level.

'Touriga Nacional' is one of the most important Portuguese red grape cultivars [31]. In 2023, this grape cultivar was the third most cultivated variety in Portugal, with a planted area of 12,567 hectares, corresponding to 7.1% of the national wine-growing area [32]. This variety is an autochthonous Portuguese variety originating from the Dão Wine region (northern Portugal). In general, this cultivar adapts well to a wide range of soil types and shows no particular susceptibility to a majority of common diseases and pests [33]. Their

grapes are characterized by small berries and a tendency to present high concentrations of sugars, phenols, and varietal aromatic precursors [34,35]. Furthermore, this variety is well adapted to different soils and high temperature conditions [36], due to its high capacity to dissipate heat through the evaporative cooling mechanism [4,37]. Previous works reported the cultivation of this variety outside Portugal, such as in France [38] and the United States (California) [39]. In Brazil, 'Touriga Nacional' is a common grape variety in the semi-arid region of the northeastern part of the country, particularly in the S. Francisco Valley [36,40,41]. However, for the southern regions of Brazil, data on the adaptability of the 'Touriga Nacional' variety are still very scarce.

Therefore, this study intended to compare the physicochemical composition, general phenolic profile, and antioxidant capacity of the grape musts from the 'Touriga Nacional' variety during the ripening over three harvest seasons (2022–2024) in two different vineyard locations from southern Brazil (states of Santa Catarina and Rio Grande do Sul). In addition, the results were compared with the data obtained from 'Touriga Nacional' grape musts collected from a vineyard located in the region of origin of this variety (Viseu, northern Portugal). This research contributes in an innovative way to assessing the plasticity of the 'Touriga Nacional' variety to gain more knowledge of its oenological potential in different environmental conditions, such as in southern Brazil.

2. Materials and Methods

2.1. Vineyard Locations and Grape Sampling

'Touriga Nacional' grapes (VIVC: 12594) were sampled weekly from veraison to harvest during three consecutive seasons (2022–2024) in two vineyards located in southern Brazil: Santa Catarina ($28^{\circ}12'41.0''$ S $50^{\circ}06'08.0''$ W) and Rio Grande do Sul ($28^{\circ}15'58.0''$ S $51^{\circ}15'23.0''$ W) states. In addition, grape samples from the same variety were collected during ripening from another vineyard located in the Dão wine region, northern Portugal ($40^{\circ}38'18.0''$ N $7^{\circ}54'56.0''$ W) (Figure 1).

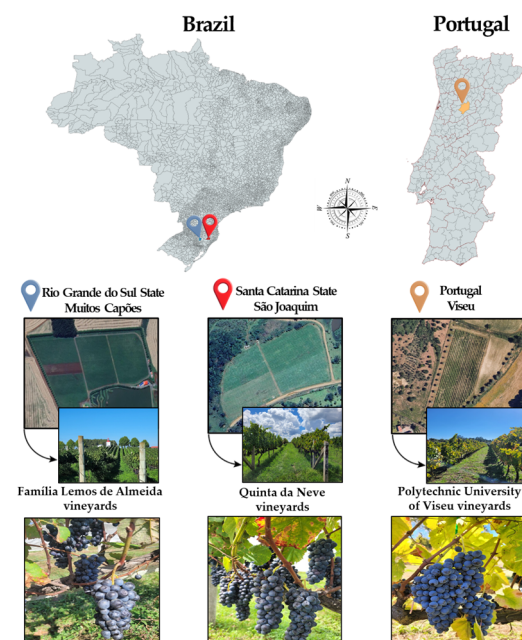


Figure 1. Locations and general appearance of the different vineyards studied. (photos: authors).

Veraison was defined when 50% of the berries per bunch showed red coloration. On the other hand, none of the vineyards studied had irrigation. Specific locations and other general characteristics of the vineyards studied are described in Table 1.

Table 1. General characteristics of the ‘Touriga Nacional’ vineyards used in this research.

Vineyard Location	Altitude (m) ¹	Soil Type	Plants Age (years) ⁴	Rootstocks	Plant Spacing (m)	Row Spacing (m)	Training System	Production Yield (kg/plant) ⁵	Vineyard Row Orientation
Portugal	452	Cambisol ²	12	R110	1.3	2.5	Guyot	1.9	Northeast/Southeast
Santa Catarina	1.200	Cambisol ³	9	Paulsen 1103	1.5	3.2	Guyot	1.4	North/South
Rio Grande Sul	843	Latosol ³	10	101-14 Mgt	1.0	2.5	Guyot	1.6	Northeast/Southeast

Portugal—Viseu; state of Santa Catarina—São Joaquim, Brazil; state of Rio Grande do Sul—Muitos Capões, Brazil. ¹ Above sea level; ² classification according to National Soil Maps (EUDASM)—Soil Map of Portugal [42]; ³ classification according to the Brazilian Soil Classification System [43]; ⁴ age at the beginning of the study; ⁵ average values for the three vintages studied.

In the vineyards, short winter pruning was carried out using a spur-pruned cordon system, maintaining 15 to 20 spurs per plant. Leaf removal was carried out after flowering and the beginning of maturation, while shoot topping was performed when the canopy reached the maximum height of the training system. Regarding soil management, the ground cover was established between rows using ryegrass (*Lolium multiflorum* L.) and clover (*Trifolium repens* L.), with monthly mowing. No herbicides were used in any of the vineyards studied throughout the three years of study.

Grape samples consisted of three independent biological replicates, each replicate comprising 100 berries pooled from 200 vines. The grapes were randomly selected from the selected plants and picked using a standardized scheme: 2 berries per bunch position (top, middle and bottom) and canopy side. For each growing season, grapes were sampled weekly from the second week after veraison until technological maturity, defined by explicit maturity criteria (estimated alcohol degree between 11.0 and 12.5% *v/v*), which corresponded to 53–56 days after veraison. After each collection, the samples were transported to the laboratory at a low temperature in a thermal bag and then immediately frozen and stored at −20 °C until being analyzed. All samples had an average storage period in the freezer of between 20 and 22 days until they were used in the evaluation of all the parameters studied.

2.2. Characterization of Climatic Conditions

The vineyards in southern Brazil are located in regions characterized by a humid mesothermal climate (Cfb) according to the Köppen classification [44]. The vineyard in Viseu (Portugal) is located in a region with a Mediterranean climate (Csb) according to the Köppen classification [45], which is characterized by hot summers and low rainfall. It is important to note that this research was conducted in vineyards located in two different hemispheres, meaning that the grape ripening periods corresponded to different months. In the vineyard located in Portugal (Northern Hemisphere), the grape ripening period occurs between June and September, while in the vineyards located in Brazil (Southern Hemisphere), the grape ripening period corresponds to the period between January and April.

The meteorological data were obtained from stations very close to the vineyards studied. For the vineyard located in Santa Catarina (Brazil), the data were provided by the Agricultural Research and Rural Extension Corporation of the State of Santa Catarina using the Epagri/Ciram database. On the other hand, meteorological data for the vineyard located in Rio Grande do Sul (Brazil) were provided by the Brazilian National Institute of Meteorology. Finally, meteorological data for the vineyard in Viseu, Portugal, were provided by the Portuguese Institute for Sea and Atmosphere. The comparative data of the monthly average climatic conditions for the 2021, 2022, 2023, and 2024 vintages in the three vineyard locations are summarized in Figure 2.

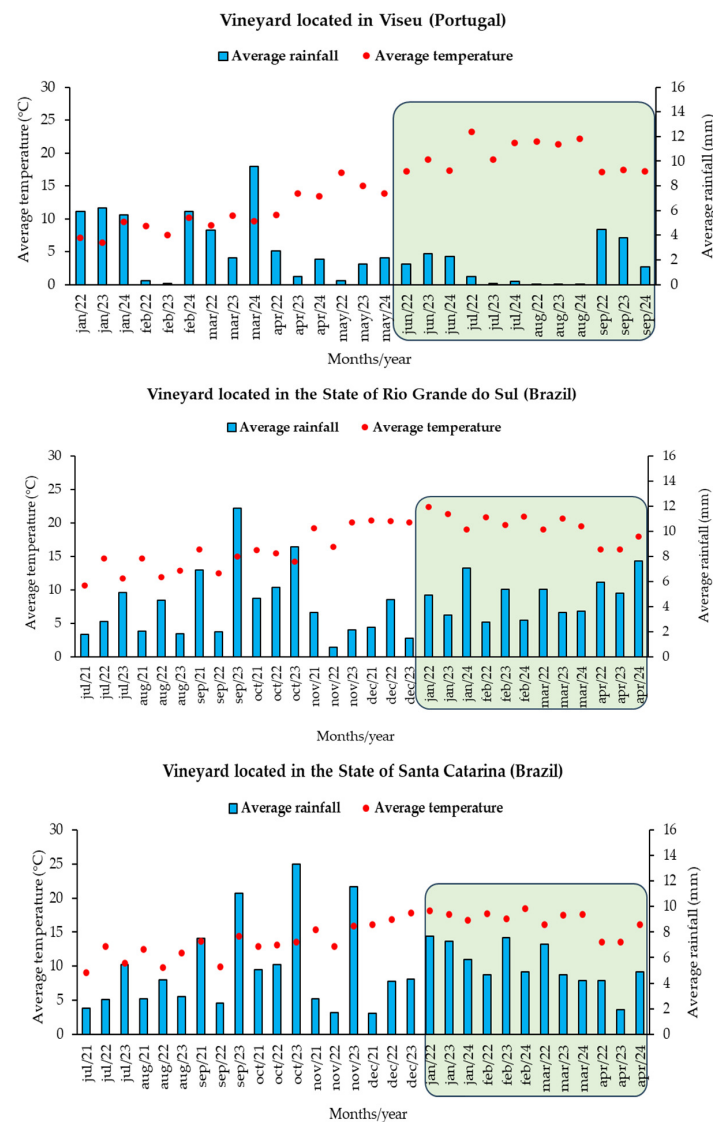


Figure 2. Comparative monthly mean climatic conditions during the 2021, 2022, 2023 and 2024 vintages in the three different vineyard locations studied.

In the vineyard located in Viseu (Portugal), the average monthly temperature during grape ripening (June to September) ranged from 17.0 to 23.2 °C, and the average monthly rainfall ranged from 0.0 to 3.8 mm. The accumulated monthly rainfall varied between 0.0 and 134.5 mm. In 2022, during grape ripening (June to September), the accumulated monthly rainfall was 6.8 mm, while for the 2023 and 2024 vintages, the values ranged from 6.4 to 3.9 mm, respectively. For the vineyard located in São Joaquim (state of Santa Catarina, Brazil), during grape ripening (January to April), the average monthly temperature ranged from 13.5 to 18.4 °C, and the average monthly rainfall ranged from 1.9 to 7.7 mm. The accumulated monthly rainfall varied between 59.4 and 238.0 mm. In 2022, during grape ripening (January to April), the accumulated monthly rainfall was 23.6 mm, while for the 2023 and 2024 vintages, the values ranged from 21.4 to 19.8 mm, respectively.

Finally, for the vineyard located in Muitos Capões (state of Rio Grande do Sul, Brazil), the monthly average temperature ranged from 16.0 to 22.4 °C, whereby the average monthly rainfall varied between 2.8 and 7.7 mm. The accumulated monthly rainfall ranged from 77.6 to 229.4 mm. In 2022, during grape ripening (January to April), the accumulated monthly rainfall was 18.9 mm, while for the 2023 and 2024 vintages, the values ranged from 17.3 to 21.2 mm, respectively.

2.3. Preparation of Grape Samples

Physicochemical parameters were measured directly from must (containing seeds and skins) obtained after manual pressing (for 3–4 min). Phenolic composition, chromatic parameters, and antioxidant activity were quantified from an extract previously obtained following the procedure described by Carbonneau and Champagnol [46]. Succinctly, grape extracts were obtained by macerating the crushed grapes at 25 °C for 24 h in a pH 3.27 buffer solution (containing tartaric acid 0.033 M in distilled water) at a ratio of 1:1 (1 mL of grape must per 1 mL of buffer solution) and ethanol 96% (in a ratio corresponding to the weight of 100 berries divided by eight), with a subsequent clarification of the suspension by centrifugation (10 min at $1.372 \times g$ and RCF of 10 cm). After centrifugation, aliquots of each phenolic extract obtained were filtered using cellulose filters (Whatman-Cytiva, Velisy-Villacoublay, France) with a pore diameter of 0.45 μm and frozen at $-20\text{ }^{\circ}\text{C}$ until analysis. Centrifugation was performed using a centrifuge model 260 R (MPW Med. Instruments, Warsaw, Poland). In general, each phenolic extract was frozen for approximately 20–25 days before being analyzed.

2.4. General Physicochemical Grape Analysis

General physicochemical analysis, such as volume and weight of 100 berries, must yield, estimated alcohol degree (OIV-MA-AS2-02), pH (OIV-MA-AS313-15), and titratable acidity (OIV-MA-AS313-01) were evaluated according to the methods established by the OIV [47]. The pH was quantified using a pH meter (model K39-1420, Kasvi, Weissópolis, Pinhais, PR, Brazil), while the estimated alcohol degree was determined by converting the concentration of total soluble solids into probable alcohol ($1.70\text{ }^{\circ}\text{Brix} = 1\%$ alcohol, adjusted to a temperature of $20\text{ }^{\circ}\text{C}$), using a digital refractometer (model PAL-1, Atago, Tokyo, Japan). Following the OIV analytical methods, titratable acidity was determined by titration with 0.1 N NaOH and an endpoint of pH 7.0 in the presence of an indicator (bromothymol blue). Results were expressed in g tartaric acid equivalents per liter of must ($\text{g}\cdot\text{L}^{-1}$), and then converted into mg tartaric acid equiv. g^{-1} of berry. Malic and tartaric acids were determined using a stated colorimetric protocol from Zoecklein et al. [48], including wavelengths and calibration standards.

2.5. General Phenolic Composition and Chromatic Parameters

Total phenolic compounds were determined initially based on the measurement of absorbance at 280 nm [49], while non-flavonoid and flavonoid phenols were quantified according to the procedure proposed by Kramling and Singleton [50]. The results for total phenols, non-flavonoid phenols and flavonoids were expressed as mg gallic acid equivalents per g^{-1} of berry and based on the gallic acid calibration curve containing different concentrations of gallic acid (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Total anthocyanins were determined using the SO_2 bleaching method with specified reagent concentrations, wavelengths, and calibration details as described by Ribéreau-Gayon and Stonestreet [51]. Results were expressed as mg malvidin-3-monoglucoside equivalents per g^{-1} of berry and based on the calibration curve containing different concentrations of malvidin-3-monoglucoside (Extrasynthese, Genay, France).

Total tannins were quantified according to the method established by Ribéreau-Gayon and Stonestreet [52]. For analysis, the diluted extract (2:100) was reacted with a standard solution prepared by dissolving 75 mg of $\text{Fe}_2(\text{SO}_4)_3$ in 500 mL of a 1:1 mixture of n-butanol ($\text{C}_4\text{H}_9\text{OH}$, 95%) and hydrochloric acid (HCl, 37%). The mixture was divided into two aliquots of 10 mL, one of which was incubated in a boiling water bath at $100\text{ }^{\circ}\text{C}$ for 30 min, while the other was kept protected from light for the same period. After this period, absorbances were measured at 540 nm. The results were expressed in

(+)-catechin equivalents, based on the calibration curve obtained with different concentrations of (+)-catechin (Extrasynthese, Genay, France).

Color intensity and hue were estimated by the use of the OIV method [47]. Briefly, the color intensity was the sum of the absorbances found at wavelengths of 420, 520 and 620 nm, while the color hue was expressed as the ratio between the absorbance at 420 nm and the absorbance at 520 nm. Furthermore, the a^* coordinate (redness) was obtained by the CIELAB system using VISIONlite™ Software, version 5.0 (Thermo Scientific™, USA).

All parameters were performed using a Genesys 50 UV-Vis spectrophotometer (Thermo Scientific, Ilkirch-Graffenstaden, France) coupled to VISIONlite Wine Analysis software, version 5.0 (Thermo Fisher Scientific, Waltham, MA, USA). All analyzed extracts consisted of 3 samples per vineyard, per date, and vintage.

2.6. Antioxidant Capacity Determination

To evaluate antioxidant capacity using the DPPH assay, analyses were performed according to the methodology proposed by Brand-Williams et al. [53]. In summary, 0.1 mL aliquots of different sample concentrations were mixed with 3.9 mL of a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) at 25 mg L⁻¹. Tested concentrations ranged from 0 to 100 µg mL⁻¹, and the criterion used to compute antioxidant capacity was IC50 values (concentration of the extract required to achieve 50% of radical inhibition). DPPH solutions were prepared daily, maintained under light-protected conditions, and absorbance was measured at 515 nm after a 30-min reaction period at 20 °C, using methanol as the blank reference. For the antioxidant capacity using the ABTS^{•+} method, the conditions described previously by Re et al. [54] were used. This methodology is based on the discoloration that occurs when the ABTS radical cation (ABTS^{•+}) is converted to its non-radical form, ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). The radical species was produced by reacting an aqueous 7 mM ABTS^{•+} solution with 2.45 mM potassium persulfate in a 1:1 (v/v) ratio, followed by incubation in the dark at room temperature. The analysis itself consisted of mixing 980 µL of the ABTS^{•+} solution with 20 µL of the previously diluted sample (1:50, v/v) with water. After 15 min of reaction, the absorbance was measured at 734 nm. The antioxidant capacity quantified by the ABTS^{•+} and DPPH methods was expressed in Trolox equivalents, using calibration curves generated daily ($R^2 \geq 0.995$), with Trolox concentrations between 0.015 and 0.42 mM. Spectrophotometric readings for both methods were performed on a Genesys 50 UV-Vis spectrophotometer (Thermo Scientific, Ilkirch-Graffenstaden, France).

2.7. Statistical Analysis

The results were presented as the mean $\pm$ standard deviation. The data were analyzed for normal distribution using the Shapiro–Wilk test, and then the homogeneity of variances was checked using Levene's test. The experimental unit consisted of the vineyard $\times$ vintage $\times$ sampling date (days after veraison). For each vintage separately (2022, 2023, and 2024), the data were subjected to an analysis of variance (ANOVA), considering a factorial design with fixed effects of the vineyard (Portugal, Rio Grande do Sul, and Santa Catarina) and the sampling data (days after veraison), as well as the interaction of these factors (vineyard $\times$ sampling data). When significant interactions were observed, these were analyzed in greater detail, comparing the vineyards within each sampling date (days after the start of grape ripening), and each vineyard was evaluated separately across all sampling dates (days after the start of grape ripening). The means were compared using Tukey's test, with a significance level of 5% ($p \leq 0.05$).

The principal component analysis (PCA) and cluster analysis (UPGMA) were performed to verify the relationships between the vines and maturation components, using the results obtained from the harvest. For the cluster analysis, data standardization was performed using the z-score method. This cluster analysis used the Euclidean distance, and the linkage method used for group formation was UPGMA (Unweighted Pairwise Clustering Method with Arithmetic Mean). The dataset used in the cluster analysis (UPGMA) and principal component analysis (PCA) consisted of 9 observations, corresponding to the averages of the three independent biological replicates of each vineyard (Portugal, Rio Grande do Sul, and Santa Catarina) at harvest for each crop year (2022, 2023, and 2024). Before conducting the principal component analysis, the data were subjected to Bartlett's test of sphericity and the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy. Both tests, as well as the principal component analysis (PCA), were performed using SPSS software, version 22.0 (SPSS Inc., headquartered in Chicago, IL, USA), considering only variables with high communality values (≥ 0.7). Meanwhile, the clustering of samples using the UPGMA approach was performed using the STATISTICA program, version 8.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. General Physicochemical Composition

The physicochemical composition of 'Touriga Nacional' grapes during ripening across different vineyard locations and three consecutive vintages (2022–2024) is summarized in Tables 2 and 3.

After a comparative analysis of 100-berries weight (Table 2), in general, grapes from the vineyards located in the two Brazilian states showed significantly higher values during ripening than grapes collected in the Portuguese vineyard. This trend was observed during the three consecutive vintages. At harvest, grapes collected in the vineyard located in the state of Santa Catarina showed an average value between 192.98 and 219.92 g, while grapes collected in the Portuguese vineyard showed average values between 148.09 and 174.99 g. Intermediate values (between 164.83 and 195.11 g) were obtained for the grapes collected in the vineyard located in the state of Rio Grande do Sul.

Regarding the volume of must from 100 berries, a progressive increase was observed during grape ripening (Table 2). This trend was observed across all vineyard locations. Grapes collected in the Santa Catarina state vineyard showed significantly higher must volume at harvest in the 2023 and 2024 vintages, with an average value of 136 and 140 mL, respectively. On the contrary, grapes from the Portuguese vineyard showed the significantly lowest must volume at harvest throughout all the vintages, with average values ranging from 67 to 118 mL.

Table 2. General physicochemical composition of 'Touriga Nacional' grape berries during ripening (2022 to 2024) from vineyards located in Portugal and Brazil.

Parameters	Vintages	Vineyard Location	Days After Veraison				
			14	28	42	49	56 *
Grape berry weight (g) ¹	2022	Portugal	157.66 ± 0.05 Cc	164.75 ± 0.05 Bbc	179.17 ± 0.05 Aa	164.56 ± 0.05 Bbc	174.99 ± 0.05 Bab
		R. Grande Sul	182.29 ± 4.29 Ab	184.54 ± 4.54 Aab	170.54 ± 3.54 Ac	176.62 ± 5.62 Abc	195.11 ± 2.89 Aa
		Santa Catarina	171.91 ± 0.09 Bb	176.46 ± 4.46 Ab	177.92 ± 3.08 Ab	187.60 ± 1.48 Aa	192.98 ± 1.98 Aa
	2023	Portugal	144.84 ± 0.05 Cbc	139.28 ± 0.00 Bc	165.10 ± 0.00 Ba	162.04 ± 0.00 Ca	148.09 ± 0.05 Cb
		R. Grande Sul	190.05 ± 1.61 Ab	186.85 ± 2.41 Abc	201.79 ± 2.79 Aa	183.18 ± 0.52 Ac	164.83 ± 1.32 Bd
		Santa Catarina	177.72 ± 6.46 Bbc	181.69 ± 0.62 Ab	167.74 ± 1.11 Bc	176.23 ± 3.10 Bbc	218.47 ± 6.39 Aa

Table 2. Cont.

Parameters	Vintages	Vineyard Location	Days After Veraison				
			14	28	42	49	56 *
Grape berry weight (g) ¹	2024	Portugal	102.20 ± 0.05 Ce	118.80 ± 0.03 Bd	164.40 ± 0.08 Cb	195.20 ± 0.06 Aa	162.80 ± 0.05 Bc
		R. Grande Sul	200.34 ± 0.74 Aa	175.99 ± 2.59 Acd	184.43 ± 0.28 Ab	181.96 ± 6.16 Abc	172.04 ± 0.30 Bd
		Santa Catarina	153.02 ± 3.68 Bc	173.53 ± 2.65 Ab	177.18 ± 2.62 Bb	178.90 ± 10.25 Ab	219.92 ± 9.96 Aa
Must volume (mL) ²	2022	Portugal	102.00 ± 0.05 Bb	109.00 ± 0.01 Aab	116.00 ± 0.04 Aa	110.00 ± 0.00 Bab	118.00 ± 0.05 Ba
		R. Grande Sul	112.50 ± 2.50 Ab	115.50 ± 4.50 Ab	114.50 ± 4.50 Ab	121.00 ± 2.00 Ab	157.50 ± 2.50 Aa
		Santa Catarina	89.00 ± 1.00 Cc	92.00 ± 2.00 Bbc	100.50 ± 3.50 Bb	113.50 ± 5.50 ABa	115.00 ± 5.00 Ba
	2023	Portugal	84.00 ± 0.00 Ca	75.00 ± 1.00 Cb	85.00 ± 1.05 Ca	65.00 ± 0.00 Bc	67.00 ± 0.05 Cbc
		R. Grande Sul	126.50 ± 2.50 Ab	121.50 ± 2.50 Abc	132.00 ± 2.00 Aa	116.00 ± 2.00 Ac	97.00 ± 1.00 Bd
		Santa Catarina	108.00 ± 6.00 Bb	112.00 ± 2.00 Bb	106.50 ± 1.50 Bb	109.00 ± 5.00 Ab	136.00 ± 1.00 Aa
	2024	Portugal	52.00 ± 0.04 Ce	68.00 ± 0.05 Cd	96.00 ± 0.08 Cb	115.98 ± 0.03 Aa	83.98 ± 0.04 Cc
		R. Grande Sul	129.5 ± 1.50 Aa	123.00 ± 5.00 Aa	129.25 ± 5.25 Aa	110.50 ± 1.50 Bb	99.50 ± 0.50 Bc
		Santa Catarina	91.00 ± 5.00 Bd	97.00 ± 3.00 Bcd	105.00 ± 1.00 Bbc	106.50 ± 4.50 Bb	140.00 ± 0.02 Aa
Must yield (%) ³	2022	Portugal	74.92 ± 0.02 Ac	75.02 ± 0.03 Ab	74.01 ± 0.02 Ad	76.72 ± 0.03 Aa	74.99 ± 0.03 Ab
		R. Grande Sul	71.74 ± 1.29 Aa	69.47 ± 0.59 Bab	73.05 ± 1.19 Aa	69.47 ± 2.22 Bab	66.42 ± 1.27 Bb
		Santa Catarina	61.87 ± 3.25 Bb	62.18 ± 2.36 Cb	64.33 ± 0.86 Bab	66.64 ± 1.03 Bab	68.17 ± 0.10 Ba
	2023	Portugal	58.00 ± 0.80 Ca	53.85 ± 2.07 Bb	51.50 ± 0.83 Cb	40.11 ± 1.17 Bd	45.24 ± 2.03 Bc
		R. Grande Sul	80.37 ± 0.93 Aa	80.77 ± 1.35 Aa	80.22 ± 0.51 Aa	78.97 ± 1.34 Aa	75.63 ± 1.68 Ab
		Santa Catarina	77.16 ± 0.05 Ba	78.20 ± 1.18 Aa	78.06 ± 0.93 Ba	78.01 ± 0.86 Aa	78.53 ± 1.84 Aa
	2024	Portugal	50.90 ± 2.32 Cb	57.26 ± 1.34 Ca	58.40 ± 0.51 Ca	59.40 ± 1.08 Ca	51.60 ± 1.06 Cb
		R. Grande Sul	84.86 ± 2.04 Aa	81.39 ± 0.71 Aa	82.02 ± 0.83 Aa	75.85 ± 1.73 Bb	75.19 ± 0.61 Bb
		Santa Catarina	73.36 ± 2.60 Bc	75.28 ± 1.96 Bc	73.87 ± 0.19 Bc	86.17 ± 0.43 Aa	79.78 ± 1.50 Ab
Estimated alcohol degree (% <i>v/v</i>)	2022	Portugal	10.29 ± 0.00 Ce	11.06 ± 0.00 Bd	11.97 ± 0.04 Bc	12.65 ± 0.00 Aa	12.29 ± 0.00 Bb
		R. Grande Sul	11.26 ± 0.03 Ad	11.97 ± 0.03 Ab	11.65 ± 0.12 Cc	12.26 ± 0.03 Ca	11.59 ± 0.00 Cc
		Santa Catarina	10.62 ± 0.03 Be	12.00 ± 0.00 Ad	12.38 ± 0.03 Ac	12.47 ± 0.00 Bb	12.85 ± 0.03 Aa
	2023	Portugal	6.71 ± 0.00 Cd	8.18 ± 0.00 Cc	10.35 ± 0.00 Cb	11.47 ± 0.00 Ca	11.59 ± 0.00 Ca
		R. Grande Sul	12.54 ± 0.17 Ab	12.33 ± 0.14 Ab	12.59 ± 0.14 Ab	13.16 ± 0.07 Aa	12.50 ± 0.08 Ab
		Santa Catarina	9.75 ± 0.09 Bd	10.81 ± 0.12 Bc	11.53 ± 0.00 Bb	12.10 ± 0.17 Ba	11.90 ± 0.05 Ba
	2024	Portugal	4.26 ± 0.04 Ce	6.74 ± 0.04 Cd	10.21 ± 0.04 Bc	11.24 ± 0.00 Ca	11.12 ± 0.00 Cb
		R. Grande Sul	11.08 ± 0.03 Ac	11.00 ± 0.00 Ac	11.98 ± 0.03 Ab	12.04 ± 0.03 Ab	12.33 ± 0.03 Aa
		Santa Catarina	8.71 ± 0.00 Be	9.55 ± 0.03 Bd	10.24 ± 0.00 Bc	11.47 ± 0.00 Bb	12.06 ± 0.00 Ba

¹ Grape berry weight (100 berries); ² must volume (100 berries); ³ must yield (100 berries); * harvested between 53 and 56 days after veraison. All data express the average of 3 replicates ± standard deviation. Average values with different uppercase letters (column) comparing different vineyard locations for the same vintage and for each data point indicate significant differences according to Tukey's test ($p \leq 0.05$). Lowercase letters (row) comparing different data points for the same vintage and vineyard location indicate significant differences according to Tukey's test ($p \leq 0.05$).

For grape must yield, the results varied considerably between vintages and vineyard location. The 'Touriga Nacional' grapes collected in the Portuguese vineyard showed the significantly highest average yield values (74.99%) at harvest in the 2022 vintage, while in the 2023 vintage, grapes collected in the vineyards located in both Brazilian states showed the significantly highest values (75.63 and 78.53%, respectively). However, during this vintage, between the two Brazilian vineyard locations, no significant differences in the yield values were found. However, in the 2024 vintage, at harvest, grapes collected in the vineyard located in the state of Santa Catarina showed the significantly highest must yield values (79.78%) (Table 2).

Concerning the results for the estimated alcohol degree, an increase in the values was observed throughout all grape ripening for the three different vineyard locations studied (Table 2). Grapes collected in the vineyard located in the state of Rio Grande do Sul showed the significantly highest values in the 2023 and 2024 vintages at harvest (varied from 12.50 to 12.33% *v/v* in 2023 and 2024 vintages, respectively). For the 2022 vintage and

at harvest, grapes collected in the Portuguese vineyard and in the state of Santa Catarina showed similar values (varied from 12.29 to 12.85% *v/v*, respectively), but significantly higher compared to grapes collected in the Rio Grande do Sul vineyard (11.59% *v/v*). A similar trend was obviously detected for the results of total soluble solids (°Brix), which are presented as Supplementary Material (Table S1).

Regarding the tartaric acid content (Table 3), no consistent pattern was detected throughout ripening. In the 2022 vintage, at harvest, grapes collected in the Portuguese vineyard showed the significantly highest average values (0.91 mg·g⁻¹ of berry), while in the 2023 vintage, there were no significant differences between the grapes. However, in the 2024 vintage, at harvest, grapes collected in the Rio Grande do Sul state vineyard showed the significantly highest values of tartaric acid (0.48 mg·g⁻¹ of berry). Finally, concerning the malic acid content, at harvest, grapes collected in the Santa Catarina state vineyard showed the significantly highest concentrations in 2022 and 2023 vintages (2.11 and 3.44 mg·g⁻¹ of berry, respectively). On the other hand, in the 2024 vintage, grapes collected in the Rio Grande do Sul state vineyard showed the significantly highest concentration of malic acid (1.56 mg·g⁻¹ of berry) (Table 3).

Table 3. Acidity and organic acids content of ‘Touriga Nacional’ grape berries during ripening (2022 to 2024) from vineyards located in Portugal and Brazil.

Parameters	Vintages	Vineyard Location	Days After Veraison				
			14	28	42	49	56 *
pH	2022	Portugal	3.84 ± 0.01 Ac	3.86 ± 0.01 Ac	4.00 ± 0.00 Ab	4.07 ± 0.01 Aa	4.10 ± 0.01 Aa
		R. Grande Sul	3.88 ± 0.45 Aa	3.63 ± 0.01 Ba	3.62 ± 0.03 Ba	3.65 ± 0.01 Ca	3.54 ± 0.01 Ca
		Santa Catarina	3.36 ± 0.01 Bd	3.56 ± 0.04 Cc	3.68 ± 0.04 Ba	3.72 ± 0.02 Ba	3.63 ± 0.01 Bb
	2023	Portugal	3.21 ± 0.00 Bc	3.39 ± 0.01 Bb	3.63 ± 0.00 Aa	3.39 ± 0.01 Cb	3.21 ± 0.00 Bc
		R. Grande Sul	3.49 ± 0.01 Abc	3.46 ± 0.02 Ac	3.52 ± 0.03 Bab	3.54 ± 0.01 Aa	3.45 ± 0.01 Ac
		Santa Catarina	3.15 ± 0.01 Bc	3.32 ± 0.03 Cb	3.33 ± 0.01 Cb	3.42 ± 0.01 Ba	3.43 ± 0.05 Aa
	2024	Portugal	2.82 ± 0.00 Cd	2.98 ± 0.00 Cc	3.29 ± 0.00 Ca	3.27 ± 0.02 Ca	3.15 ± 0.01 Cb
		R. Grande Sul	3.61 ± 0.01 Ad	3.60 ± 0.02 Ad	3.72 ± 0.01 Ac	3.75 ± 0.01 Bb	3.78 ± 0.01 Ba
		Santa Catarina	3.31 ± 0.01 Bd	3.57 ± 0.01 Bc	3.67 ± 0.01 Bb	3.81 ± 0.01 Aa	3.82 ± 0.01 Aa
Titratable acidity (mg tartaric acid g ⁻¹ of berry) **	2022	Portugal	2.30 ± 0.02 Ba	2.16 ± 0.08 Bb	1.58 ± 0.03 Cc	1.58 ± 0.03 Cc	1.49 ± 0.03 Bc
		R. Grande Sul	2.38 ± 0.07 Bb	2.16 ± 0.08 Bb	2.32 ± 0.14 Bb	2.44 ± 0.04 Bb	3.33 ± 0.16 Aa
		Santa Catarina	3.96 ± 0.01 Aa	2.95 ± 0.09 Ac	2.92 ± 0.08 Ac	2.77 ± 0.20 Ac	3.33 ± 0.09 Ab
	2023	Portugal	10.12 ± 0.46 Aa	8.68 ± 1.46 Aa	4.24 ± 0.24 Ab	2.55 ± 0.02 Abc	2.37 ± 0.11 Ac
		R. Grande Sul	1.53 ± 0.05 Ca	1.44 ± 0.01 Bab	1.39 ± 0.04 Cb	1.33 ± 0.06 Cb	1.35 ± 0.04 Cb
		Santa Catarina	2.54 ± 0.09 Ba	2.24 ± 0.04 Bb	2.19 ± 0.06 Bb	1.89 ± 0.02 Bc	1.98 ± 0.06 Bc
	2024	Portugal	6.41 ± 0.15 Ba	4.96 ± 0.15 Ab	2.47 ± 0.02 Cc	1.94 ± 0.07 Cd	1.51 ± 0.00 Ce
		R. Grande Sul	4.44 ± 0.30 Ca	4.12 ± 0.09 Bab	3.31 ± 0.39 Bbc	2.89 ± 0.45 Bc	2.80 ± 0.17 Bc
		Santa Catarina	8.21 ± 0.25 Aa	5.00 ± 0.14 Ab	4.56 ± 0.05 Ac	3.81 ± 0.07 Ad	4.67 ± 0.10 Abc
Tartaric acid (mg·g ⁻¹ of berry) **	2022	Portugal	0.94 ± 0.01 Ba	0.52 ± 0.00 Ae	0.82 ± 0.01 Ac	0.80 ± 0.00 Bd	0.91 ± 0.00 Ab
		R. Grande Sul	1.13 ± 0.01 Aa	0.43 ± 0.01 Bd	0.83 ± 0.01 Ac	1.02 ± 0.03 Ab	0.87 ± 0.02 Bc
		Santa Catarina	0.69 ± 0.01 Ca	0.25 ± 0.01 Cd	0.49 ± 0.01 Bb	0.32 ± 0.02 Cc	0.34 ± 0.01 Cc
	2023	Portugal	1.51 ± 0.75 Aa	0.40 ± 0.49 Aa	0.88 ± 0.54 Aa	0.64 ± 0.21 Aa	1.17 ± 0.47 Aa
		R. Grande Sul	0.56 ± 0.04 Aa	0.39 ± 0.12 Aa	0.76 ± 0.05 Aa	0.58 ± 0.08 Aa	0.53 ± 0.06 Aa
		Santa Catarina	1.02 ± 0.27 Aa	0.74 ± 0.02 Aa	0.58 ± 0.02 Aa	0.75 ± 0.02 Aa	0.78 ± 0.39 Aa
	2024	Portugal	0.63 ± 0.13 Aa	0.76 ± 0.55 Aa	0.23 ± 0.22 Aba	0.11 ± 0.09 Aa	0.10 ± 0.11 Ba
		R. Grande Sul	0.52 ± 0.00 Aa	0.53 ± 0.02 Aa	0.46 ± 0.00 Aa	0.37 ± 0.24 Aa	0.48 ± 0.02 Aa
		Santa Catarina	0.29 ± 0.08 Ba	0.02 ± 0.01 Ac	0.13 ± 0.00 Bbc	0.07 ± 0.05 Ac	0.21 ± 0.00 Bab
Malic acid (mg·g ⁻¹ of berry) **	2022	Portugal	2.88 ± 0.05 Aa	2.48 ± 0.05 Ab	1.75 ± 0.09 Bc	1.77 ± 0.05 Ac	1.55 ± 0.19 Bd
		R. Grande Sul	1.57 ± 0.05 Ca	1.16 ± 0.02 Cbc	1.28 ± 0.03 Cb	1.05 ± 0.09 Bc	1.50 ± 0.12 Ba
		Santa Catarina	2.46 ± 0.00 Ba	2.32 ± 0.05 Bb	2.06 ± 0.01 Abc	1.91 ± 0.19 Ac	2.11 ± 0.06 Ab

Table 3. *Cont.*

Parameters	Vintages	Vineyard Location	Days After Veraison				
			14	28	42	49	56 *
Malic acid (mg·g ⁻¹ of berry) **	2023	Portugal	2.24 ± 0.85 Ba	2.18 ± 0.27 Aa	1.01 ± 0.09 Cb	0.82 ± 0.07 Bb	0.54 ± 0.24 Cb
		R. Grande Sul	2.33 ± 0.17 Ba	2.05 ± 0.18 Aa	2.34 ± 0.11 Ba	2.21 ± 0.12 Aa	2.17 ± 0.11 Ba
		Santa Catarina	3.91 ± 0.32 Aa	3.12 ± 0.92 Aa	4.06 ± 0.12 Aa	2.85 ± 0.49 Aa	3.44 ± 0.44 Aa
	2024	Portugal	0.55 ± 0.11 Aa	1.01 ± 0.41 Ba	0.96 ± 0.18 Ba	1.09 ± 0.08 Aa	0.91 ± 0.08 Ba
		R. Grande Sul	1.07 ± 0.21 Ad	2.74 ± 0.02 Aa	1.93 ± 0.03 Ab	1.31 ± 0.29 Acd	1.56 ± 0.01 Abc
		Santa Catarina	1.24 ± 0.53 Aa	1.10 ± 0.36 Ba	1.20 ± 0.27 Ba	1.04 ± 0.19 Aa	1.04 ± 0.27 Ba

* Harvested between 53 and 56 days after veraison. ** Values expressed in fresh weight (f.w.). All data expresses the average of three replicates ± standard deviation. Average values with different uppercase letters (column) comparing different vineyard locations for the same vintage and for each data point indicate significant differences according to Tukey's test ($p \leq 0.05$). Lowercase letters (row) comparing different data points for the same vintage and vineyard location indicate significant differences according to Tukey's test ($p \leq 0.05$).

3.2. Phenolic Parameters

The global phenolic composition of ‘Touriga Nacional’ grapes during ripening is shown in Table 4. Generally, significant variations were observed in the content of total phenols, flavonoid phenols, and non-flavonoid phenols throughout the grape ripening in all the vineyard locations studied. This behavior was observed during the three vintages considered.

For total phenols, at harvest, the significantly highest values were found for the grapes collected in the vineyard located in the state of Santa Catarina for the 2023 and 2024 vintages. For these two vintages, grapes collected in this Brazilian vineyard showed values in the range of 0.85 (2023 vintage) to 1.13 mg gallic acid equiv. g⁻¹ of berry (2024 vintage). However, in the 2022 vintage, grapes collected in the Portuguese vineyard showed the significantly highest value of total phenols (1.36 mg gallic acid equiv. g⁻¹ of berry). Regarding the non-flavonoid phenols, at harvest, grapes collected in the vineyard located in the state of Rio Grande do Sul showed the significantly highest concentrations in the 2022 and 2023 vintages (0.20 and 0.18 mg gallic acid equiv. g⁻¹ of berry, respectively). For the grapes collected in the vineyards located in the state of Santa Catarina and Portugal, in general, the values did not differ statistically among them over the three vintages studied (ranged between 0.11 and 0.23 mg gallic acid equiv. g⁻¹ of berry) (Table 4). For flavonoid phenols, particularly at harvest, an oscillation was observed in the values for the different vineyard locations over the three vintages. Thus, grapes collected in the vineyard located in the state of Santa Catarina showed significantly higher values in 2023 and 2024 vintages (varied from 0.70 to 0.94 mg of gallic acid equiv. g⁻¹ of berry, respectively), while in the 2022 vintage, grapes collected in the Portuguese vineyard showed the significantly highest value (1.21 mg of gallic acid equiv. g⁻¹ of berry). However, in the 2024 vintage, grapes collected in the Portuguese vineyard showed the lowest average value (0.02 mg of gallic acid equiv. g⁻¹ of berry) (Table 4).

Table 4. General phenolic composition of ‘Touriga Nacional’ grape berries during ripening (2022 to 2024) from vineyards located in Portugal and Brazil.

Parameters	Vintages	Vineyard Location	Days After Veraison				
			14	28	42	49	56 *
Total phenols (mg gallic acid equiv. g ⁻¹ of berry) **	2022	Portugal	1.01 ± 0.00 Ac	1.31 ± 0.00 Ab	1.33 ± 0.00 Ab	1.52 ± 0.00 Aa	1.36 ± 0.01 Ab
		R. Grande Sul	0.51 ± 0.00 Bc	0.50 ± 0.01 Cc	0.65 ± 0.01 Bb	0.64 ± 0.01 Cb	0.73 ± 0.03 Ca
		Santa Catarina	0.40 ± 0.01 Cc	0.59 ± 0.00 Bb	0.32 ± 0.01 Cc	1.00 ± 0.06 Ba	0.92 ± 0.03 Ba

Table 4. Cont.

Parameters	Vintages	Vineyard Location	Days After Veraison				
			14	28	42	49	56 *
Total phenols (mg gallic acid equiv. g ⁻¹ of berry) **	2023	Portugal	0.28 ± 0.00 Cb	0.30 ± 0.00 Cab	0.34 ± 0.00 Ba	0.20 ± 0.00 Cc	0.31 ± 0.00 Cab
		R. Grande Sul	0.70 ± 0.06 Aab	0.74 ± 0.06 Aab	0.66 ± 0.03 Ab	0.80 ± 0.01 Aa	0.47 ± 0.06 Bc
		Santa Catarina	0.53 ± 0.04 Bd	0.61 ± 0.03 Bc	0.70 ± 0.02 Ab	0.73 ± 0.03 Bb	0.85 ± 0.01 Aa
	2024	Portugal	0.17 ± 0.00 Cd	0.17 ± 0.00 Cd	0.27 ± 0.00 Cb	0.30 ± 0.00 Ba	0.25 ± 0.00 Cc
		R. Grande Sul	0.72 ± 0.07 Ab	0.72 ± 0.03 Ab	0.87 ± 0.05 Aa	0.83 ± 0.04 Aab	0.88 ± 0.03 Ba
		Santa Catarina	0.47 ± 0.03 Be	0.62 ± 0.03 Bd	0.78 ± 0.00 Bc	0.88 ± 0.02 Ab	1.13 ± 0.06 Aa
Non-flavonoid phenols (mg gallic acid equiv. g ⁻¹ of berry) **	2022	Portugal	0.16 ± 0.00 Ab	0.17 ± 0.00 Aa	0.18 ± 0.00 Aa	0.18 ± 0.00 Ba	0.15 ± 0.00 Bc
		R. Grande Sul	0.11 ± 0.00 Bd	0.15 ± 0.01 Bc	0.14 ± 0.00 Ac	0.18 ± 0.01 Bb	0.20 ± 0.01 Aa
		Santa Catarina	0.12 ± 0.00 Bb	0.16 ± 0.00 Bab	0.17 ± 0.00 Aab	0.21 ± 0.01 Aa	0.18 ± 0.01 Aab
	2023	Portugal	0.05 ± 0.01 Bb	0.05 ± 0.01 Bb	0.10 ± 0.01 Ba	0.10 ± 0.01 Ba	0.11 ± 0.01 Ba
		R. Grande Sul	0.15 ± 0.02 Aa	0.16 ± 0.01 Aa	0.14 ± 0.03 ABA	0.18 ± 0.01 Aa	0.18 ± 0.02 Aa
		Santa Catarina	0.13 ± 0.01 Ab	0.16 ± 0.02 Aab	0.17 ± 0.02 Aab	0.19 ± 0.02 Aa	0.13 ± 0.01 Bb
	2024	Portugal	0.03 ± 0.00 Ce	0.07 ± 0.00 Cd	0.14 ± 0.00 Bc	0.18 ± 0.00 Bb	0.23 ± 0.01 Aa
		R. Grande Sul	0.14 ± 0.01 Aab	0.14 ± 0.01 Bab	0.13 ± 0.01 Bab	0.13 ± 0.01 Cb	0.16 ± 0.01 Ba
		Santa Catarina	0.11 ± 0.01 Bc	0.17 ± 0.01 Ab	0.20 ± 0.01 Aa	0.20 ± 0.01 Aa	0.22 ± 0.02 Aa
Flavonoid phenols (mg gallic acid equiv. g ⁻¹ of berry) **	2022	Portugal	0.84 ± 0.00 Ae	1.14 ± 0.00 Ad	1.15 ± 0.00 Ac	1.34 ± 0.00 Aa	1.21 ± 0.00 Ab
		R. Grande Sul	0.40 ± 0.00 Bc	0.35 ± 0.00 Cd	0.51 ± 0.01 Bab	0.48 ± 0.02 Cb	0.53 ± 0.01 Ca
		Santa Catarina	0.28 ± 0.01 Cc	0.43 ± 0.00 Bb	0.15 ± 0.01 Cd	0.79 ± 0.05 Ba	0.74 ± 0.03 Ba
	2023	Portugal	0.22 ± 0.01 Bab	0.25 ± 0.01 Ca	0.24 ± 0.01 Ba	0.10 ± 0.01 Cc	0.20 ± 0.01 Bb
		R. Grande Sul	0.55 ± 0.07 Aa	0.59 ± 0.05 Aa	0.51 ± 0.05 Aa	0.63 ± 0.01 Aa	0.29 ± 0.08 Bb
		Santa Catarina	0.39 ± 0.05 Bc	0.45 ± 0.03 Bbc	0.53 ± 0.04 Ab	0.53 ± 0.03 Bb	0.70 ± 0.05 Aa
	2024	Portugal	0.14 ± 0.00 Ca	0.11 ± 0.00 Cd	0.13 ± 0.00 Cb	0.12 ± 0.00 Bc	0.02 ± 0.00 Ce
		R. Grande Sul	0.58 ± 0.08 Aa	0.58 ± 0.04 Aa	0.72 ± 0.06 Aa	0.68 ± 0.07 Aa	0.73 ± 0.03 Ba
		Santa Catarina	0.37 ± 0.02 Bd	0.44 ± 0.05 Bd	0.57 ± 0.02 Bc	0.67 ± 0.01 Ab	0.94 ± 0.03 Aa

* Harvested between 53 and 56 days after veraison. ** Values expressed in fresh weight (f.w.). All data expresses the average of three replicates ± standard deviation. Average values with different uppercase letters (column) comparing different vineyard locations for the same vintage and for each data point indicate significant differences according to Tukey's test ($p \leq 0.05$). Lowercase letters (row) comparing different data points for the same vintage and vineyard location indicate significant differences according to Tukey's test ($p \leq 0.05$).

The total anthocyanin evolution is shown in Table 5. During ripening, the total anthocyanin content generally increased progressively until 49 days after veraison, followed by a slight decrease when maturation ended. However, for the grapes collected in the vineyards located in the states of Rio Grande do Sul (2022 vintage) and Santa Catarina (2023 and 2024 vintages), the total anthocyanin content increased progressively until harvest. However, at harvest, in the 2022 vintage, grapes collected in the Portuguese vineyard showed the significantly highest concentration of total anthocyanins (0.97 mg of malvidin-3-monoglucoside equiv. g⁻¹ of berry). This occurred despite a clear reduction in technological maturity. On the other hand, in the 2023 vintage, grapes collected in the vineyard located in the state of Santa Catarina showed the significantly highest total anthocyanin content (0.60 mg of malvidin-3-monoglucoside equiv. g⁻¹ of berry). In 2024, regardless of the vineyard location, all grapes showed similar total anthocyanin values (varied between 0.63 and 0.67 mg of malvidin-3-monoglucoside equiv. g⁻¹ of berry).

Finally, regarding the total tannins, in general, the highest values were found in grapes collected in the Portuguese vineyard. This tendency occurred during the three vintages considered (Table 5). In this case, at harvest, total tannins found for the grapes collected in the Portuguese vineyard varied from 0.59 (2023 vintage) to 1.53 mg catechin equiv. g⁻¹ of berry (2024 vintage). Grapes collected in the vineyard located in the state of Rio Grande do Sul showed, at harvest, the lowest total tannin values for the three

vintages analyzed (varied from 0.29 to 0.41 mg catechin equiv. g⁻¹ of berry, respectively for the 2024 and 2022 vintages).

Table 5. Total anthocyanins and total tannins of ‘Touriga Nacional’ grape berries during ripening (2022 to 2024) from vineyards located in Portugal and Brazil.

Parameters	Vintages	Vineyard Location	Days After Veraison				
			14	28	42	49	56 *
Total anthocyanins (mg of malvidin-3-monoglucoside equiv. g ⁻¹ of berry) **	2022	Portugal	0.68 ± 0.00 Ae	0.98 ± 0.00 Ab	0.81 ± 0.00 Ad	1.13 ± 0.00 Aa	0.97 ± 0.00 Ac
		R. Grande Sul	0.22 ± 0.00 Bc	0.20 ± 0.00 Cd	0.28 ± 0.01 Ca	0.25 ± 0.01 Cb	0.27 ± 0.01 Cab
		Santa Catarina	0.17 ± 0.00 Cd	0.27 ± 0.00 Bc	0.31 ± 0.00 Bb	0.37 ± 0.02 Ba	0.33 ± 0.01 Bb
	2023	Portugal	0.19 ± 0.01 Cd	0.30 ± 0.00 Bc	0.42 ± 0.00 Ba	0.34 ± 0.00 Bb	0.30 ± 0.00 Bc
		R. Grande Sul	0.53 ± 0.01 Aab	0.48 ± 0.04 Ab	0.50 ± 0.02 Aab	0.56 ± 0.01 Aa	0.34 ± 0.03 Bc
		Santa Catarina	0.32 ± 0.01 Bc	0.46 ± 0.02 Ab	0.54 ± 0.04 Aa	0.59 ± 0.01 Aa	0.60 ± 0.02 Aa
	2024	Portugal	0.03 ± 0.00 Cd	0.16 ± 0.00 Cc	0.70 ± 0.02 Ab	0.92 ± 0.04 Aa	0.65 ± 0.01 Ab
		R. Grande Sul	0.58 ± 0.10 Aa	0.60 ± 0.03 Aa	0.67 ± 0.04 Aa	0.55 ± 0.03 Ba	0.63 ± 0.04 Aa
		Santa Catarina	0.30 ± 0.02 Bd	0.45 ± 0.05 Bc	0.52 ± 0.03 Bbc	0.55 ± 0.02 Bb	0.67 ± 0.02 Aa
Total tannins (mg catechin equiv. g ⁻¹ of berry) **	2022	Portugal	0.77 ± 0.04 Ad	1.21 ± 0.01 Aa	1.17 ± 0.01 Aa	0.93 ± 0.02 Ac	1.00 ± 0.02 Ab
		R. Grande Sul	0.28 ± 0.01 Bc	0.44 ± 0.01 Bab	0.48 ± 0.01 Ba	0.21 ± 0.00 Cd	0.41 ± 0.03 Cb
		Santa Catarina	0.30 ± 0.00 Bc	0.09 ± 0.01 Ce	0.21 ± 0.02 Cd	0.58 ± 0.03 Ba	0.52 ± 0.02 Bb
	2023	Portugal	1.43 ± 0.29 Aa	1.18 ± 0.30 Aa	1.17 ± 0.18 Aa	0.46 ± 0.07 Ab	0.59 ± 0.13 Ab
		R. Grande Sul	0.42 ± 0.02 Bbc	0.46 ± 0.02 Bab	0.41 ± 0.02 Bbc	0.53 ± 0.08 Aa	0.32 ± 0.01 Bc
		Santa Catarina	0.50 ± 0.07 Ba	0.47 ± 0.05 Ba	0.62 ± 0.31 Ba	0.52 ± 0.07 Aa	0.56 ± 0.06 Aa
	2024	Portugal	1.79 ± 0.01 Ab	1.23 ± 0.01 Ae	1.36 ± 0.00 Ad	2.41 ± 0.00 Aa	1.53 ± 0.00 Ac
		R. Grande Sul	0.31 ± 0.08 Ba	0.27 ± 0.02 Ba	0.06 ± 0.04 Cb	0.35 ± 0.03 Ba	0.29 ± 0.05 Ba
		Santa Catarina	0.28 ± 0.02 Ba	0.21 ± 0.03 Ca	0.23 ± 0.09 Ba	0.25 ± 0.03 Ca	0.30 ± 0.05 Ba

* Harvested between 53 and 56 days after veraison. ** Values expressed in fresh weight (f.w.). All data express the average of three replicates ± standard deviation. Average values with different uppercase letters (column) comparing different vineyard locations for the same vintage and for each data point indicate significant differences according to Tukey’s test ($p \leq 0.05$). Lowercase letters (row) comparing different data points for the same vintage and vineyard location indicate significant differences according to Tukey’s test ($p \leq 0.05$).

3.3. Chromatic Characteristics

The chromatic characteristics of grapes during ripening from the vineyards located in Portugal and Brazil are shown in Table 6.

Regarding color intensity, an increase in the values was detected, followed by a slight decrease at the end of maturation in all three vineyards and vintages. This tendency was particularly evident for the grapes collected in the vineyard located in the state of Rio Grande do Sul, in the 2022 and 2023 vintages. It is important to note that the results obtained for color intensity, during the three vintages, followed the same trend already detected for total anthocyanins. This tendency was irrespective of the vineyard locations. At harvest, grapes collected in the Portuguese vineyard in the 2022 vintage, and grapes from the vineyard located in the state of Santa Catarina, in the 2023 and 2024 vintages, showed the significantly highest color intensity values (varied between 18.23 and 23.51 abs. units). Grapes collected in the vineyard located in the state of Rio Grande do Sul in the 2022 vintage showed the significantly lowest color intensity values (11.39 abs. units).

Table 6. Chromatic characteristics of ‘Touriga Nacional’ grape berries during ripening (2022 to 2024) from vineyards located in Portugal and Brazil.

Parameters	Vintages	Vineyard Location	Days After Veraison				
			14	28	42	49	56 *
Color intensity (abs. units $\times 10$)	2022	Portugal	12.59 $\pm$ 0.09 Ae	15.69 $\pm$ 0.05 Ac	14.63 $\pm$ 0.13 Ad	16.95 $\pm$ 0.05 Ab	18.23 $\pm$ 0.03 Aa
		R. Grande Sul	11.08 $\pm$ 0.18 Bc	12.66 $\pm$ 0.07 Bb	13.44 $\pm$ 0.19 Ca	12.32 $\pm$ 0.15 Cb	11.40 $\pm$ 0.03 Cc
		Santa Catarina	11.08 $\pm$ 0.05 Bd	12.81 $\pm$ 0.05 Bc	14.19 $\pm$ 0.04 Bb	15.29 $\pm$ 0.52 Ba	13.97 $\pm$ 0.03 Bb
	2023	Portugal	6.39 $\pm$ 0.79 Bc	10.24 $\pm$ 1.07 Bab	12.64 $\pm$ 0.92 Ba	10.13 $\pm$ 0.91 Bb	12.64 $\pm$ 0.92 Ba
		R. Grande Sul	13.67 $\pm$ 0.86 Aa	14.72 $\pm$ 0.97 Aa	15.38 $\pm$ 0.94 Aa	15.60 $\pm$ 1.55 Aa	12.65 $\pm$ 2.73 Ba
		Santa Catarina	12.68 $\pm$ 0.89 Ad	14.53 $\pm$ 0.04 Ac	16.45 $\pm$ 0.56 Ab	17.71 $\pm$ 0.28 Ab	19.68 $\pm$ 0.24 Aa
	2024	Portugal	2.04 $\pm$ 0.16 Ce	6.61 $\pm$ 0.19 Cd	17.33 $\pm$ 0.22 Bb	14.20 $\pm$ 0.16 Cc	18.62 $\pm$ 0.53 Ba
		R. Grande Sul	14.02 $\pm$ 0.12 Ad	12.92 $\pm$ 0.20 Be	14.95 $\pm$ 0.14 Cc	15.50 $\pm$ 0.24 Bb	18.62 $\pm$ 0.09 Ba
		Santa Catarina	12.42 $\pm$ 0.09 Bd	19.45 $\pm$ 0.26 Ac	21.52 $\pm$ 0.60 Ab	22.37 $\pm$ 0.36 Ab	23.51 $\pm$ 0.48 Aa
Color hue (abs. units)	2022	Portugal	0.80 $\pm$ 0.00 Aa	0.55 $\pm$ 0.00 Ad	0.59 $\pm$ 0.01 Ac	0.65 $\pm$ 0.00 Ab	0.65 $\pm$ 0.00 Ab
		R. Grande Sul	0.64 $\pm$ 0.02 Ba	0.50 $\pm$ 0.01 Bb	0.47 $\pm$ 0.02 Bbc	0.51 $\pm$ 0.02 Bb	0.46 $\pm$ 0.01 Cc
		Santa Catarina	0.50 $\pm$ 0.01 Ca	0.50 $\pm$ 0.00 Ba	0.48 $\pm$ 0.00 Ba	0.52 $\pm$ 0.06 Ba	0.50 $\pm$ 0.00 Ba
	2023	Portugal	0.42 $\pm$ 0.00 Bb	0.42 $\pm$ 0.00 Cb	0.51 $\pm$ 0.01 Aa	0.40 $\pm$ 0.03 Bb	0.51 $\pm$ 0.01 Ba
		R. Grande Sul	0.53 $\pm$ 0.02 Aa	0.47 $\pm$ 0.02 Ba	0.49 $\pm$ 0.01 Ba	0.51 $\pm$ 0.07 Aa	0.50 $\pm$ 0.03 Ba
		Santa Catarina	0.50 $\pm$ 0.07 Abc	0.50 $\pm$ 0.01 Ac	0.54 $\pm$ 0.02 Abc	0.59 $\pm$ 0.02 Aab	0.67 $\pm$ 0.01 Aa
	2024	Portugal	0.84 $\pm$ 0.02 Aa	0.53 $\pm$ 0.01 Ac	0.50 $\pm$ 0.00 Bd	0.56 $\pm$ 0.00 Bb	0.53 $\pm$ 0.00 Bc
		R. Grande Sul	0.51 $\pm$ 0.01 Bd	0.51 $\pm$ 0.01 Bd	0.59 $\pm$ 0.00 Ab	0.61 $\pm$ 0.00 Aa	0.57 $\pm$ 0.00 Ac
		Santa Catarina	0.38 $\pm$ 0.00 Cd	0.40 $\pm$ 0.00 Ccd	0.43 $\pm$ 0.01 Cbc	0.46 $\pm$ 0.01 Cab	0.48 $\pm$ 0.02 Ca
CIELAB coordinate <i>a</i> * (abs. units)	2022	Portugal	41.21 $\pm$ 0.20 Ba	36.70 $\pm$ 0.44 Aab	36.17 $\pm$ 0.94 Bab	17.58 $\pm$ 4.53 Bc	32.12 $\pm$ 0.10 Bb
		R. Grande Sul	42.54 $\pm$ 0.44 Ab	35.16 $\pm$ 0.70 Bd	38.04 $\pm$ 0.01 Ac	34.27 $\pm$ 0.19 Ad	44.43 $\pm$ 0.04 Aa
		Santa Catarina	39.64 $\pm$ 0.24 Ca	36.96 $\pm$ 0.06 Ab	26.12 $\pm$ 0.02 Cd	15.27 $\pm$ 0.03 Be	30.87 $\pm$ 0.09 Cc
	2023	Portugal	28.23 $\pm$ 1.37 Bb	37.02 $\pm$ 1.63 Aa	40.46 $\pm$ 1.67 Aa	27.36 $\pm$ 0.94 Cb	30.55 $\pm$ 1.58 Bb
		R. Grande Sul	33.49 $\pm$ 0.82 Ab	25.60 $\pm$ 1.18 Bc	30.61 $\pm$ 0.29 Bb	39.50 $\pm$ 1.65 Ba	23.73 $\pm$ 1.21 Cc
		Santa Catarina	36.28 $\pm$ 2.41 Ab	35.92 $\pm$ 1.68 Ab	42.49 $\pm$ 2.06 Aa	46.21 $\pm$ 1.40 Aa	42.64 $\pm$ 0.30 Aa
	2024	Portugal	7.98 $\pm$ 0.32 Cd	29.25 $\pm$ 0.29 Cc	50.03 $\pm$ 0.08 Aa	48.91 $\pm$ 0.52 Ab	49.01 $\pm$ 0.58 Aab
		R. Grande Sul	28.80 $\pm$ 1.09 Bc	32.57 $\pm$ 1.81 Bbc	35.04 $\pm$ 1.12 Bb	32.50 $\pm$ 1.33 Cbc	39.18 $\pm$ 1.85 Ba
		Santa Catarina	39.60 $\pm$ 1.27 Ac	48.70 $\pm$ 1.35 Aab	49.51 $\pm$ 1.45 Aa	45.79 $\pm$ 1.35 Bb	49.47 $\pm$ 1.18 Aa

* Harvested between 53 and 56 days after veraison. All data express the average of three replicates $\pm$ standard deviation. Average values with different uppercase letters (column) comparing different vineyard locations for the same vintage and for each data point indicate significant differences according to Tukey's test ($p \leq 0.05$). Lowercase letters (row) comparing different data points for the same vintage and vineyard location indicate significant differences according to Tukey's test ($p \leq 0.05$).

Concerning the results for color hue, in general, a tendency toward a slight increase, including a few oscillations of the values throughout the grape ripening, was detected. However, at the technological maturity for the three vintages studied, there was no clear pattern among the results for the color hue of the grapes harvested in the three vineyard locations. In the 2022 vintage, grapes collected in the Portuguese vineyard showed the significantly highest color hue value (0.65 abs. units), while in 2023, the highest color hue values were obtained for the grapes from the Brazilian vineyard located in the state of Santa Catarina (0.67 abs. units). In the 2024 vintage, grapes collected in the vineyard located in the state of Rio Grande do Sul showed the significantly highest color hue value (0.57 abs. units).

Finally, with respect to the chromatic coordinate a^* values (redness), in general, an increase was detected in the values throughout maturation, interspersed with some variations, particularly in the last weeks of ripening (Table 6). At harvest, grapes collected in the vineyard located in the state of Santa Catarina in the 2023 and 2024 vintages showed the significantly highest a^* values (varied between 42.64 and 49.47 abs. units, respectively). In the 2022 vintage, grapes collected in the Portuguese vineyard showed the significantly highest a^* values (44.43 abs. units). In general, all these results followed the same trend already observed for color intensity.

3.4. Antioxidant Capacity

The antioxidant capacity evolution during ‘Touriga Nacional’ grapes ripening is shown in Figure 3. According to the results obtained, antioxidant capacity was characterized by an oscillation of the values over the ripening. However, these oscillations tended to be greater when the ABTS^{•+} method was used. This trend was irrespective of the vineyard locations and vintages. In addition, the antioxidant capacity values obtained using the ABTS method were higher compared to those obtained using the DPPH method.

Considering the ABTS^{•+} method, in general, grapes collected in the Portuguese vineyard showed significantly higher values of antioxidant capacity during ripening (values ranging from 0.92 to 3.16 mg of trolox g⁻¹ of berry), compared to those obtained for the grapes collected in both Brazilian vineyards. However, regarding the DPPH method, this trend was observed only in 2022. In this vintage, grapes collected in the Portuguese vineyard showed significantly higher values of antioxidant capacity than the grapes collected in the two Brazilian vineyard locations throughout the whole ripening period (values ranging from 1.44 to 2.38 mg trolox g⁻¹ berry). In the 2023 and 2024 vintages, grapes from the two Brazilian vineyard locations showed the highest antioxidant capacity values using the DPPH method, particularly in the last two weeks of ripening.

At harvest, grapes collected in the Portuguese vineyard, in all the vintages, showed the highest values of antioxidant capacity using the ABTS^{•+} method (2.79, 1.57 and 2.13 mg trolox g⁻¹ berry, for the 2022, 2023 and 2024 vintages, respectively). The significantly lowest values were found in grapes collected in the vineyard located in the state of Santa Catarina (1.51, 0.73 and 0.39 mg trolox g⁻¹ berry, for the 2022, 2023 and 2024 vintages, respectively). Moreover, using the DPPH method only in the 2022 vintage, grapes collected in the Portuguese vineyard showed the significantly highest values (1.88 mg trolox g⁻¹ berry). In the 2023 and 2024 vintages, using the DPPH method, grapes collected in the vineyard located in the state of Santa Catarina showed the significantly highest values (1.12 and 2.18 mg trolox g⁻¹ berry, respectively).

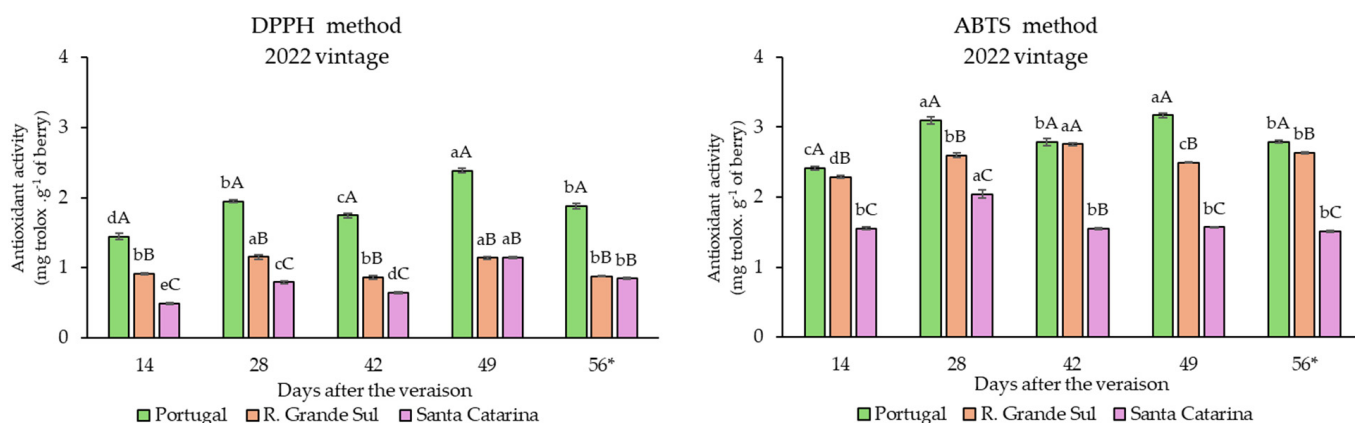


Figure 3. Cont.

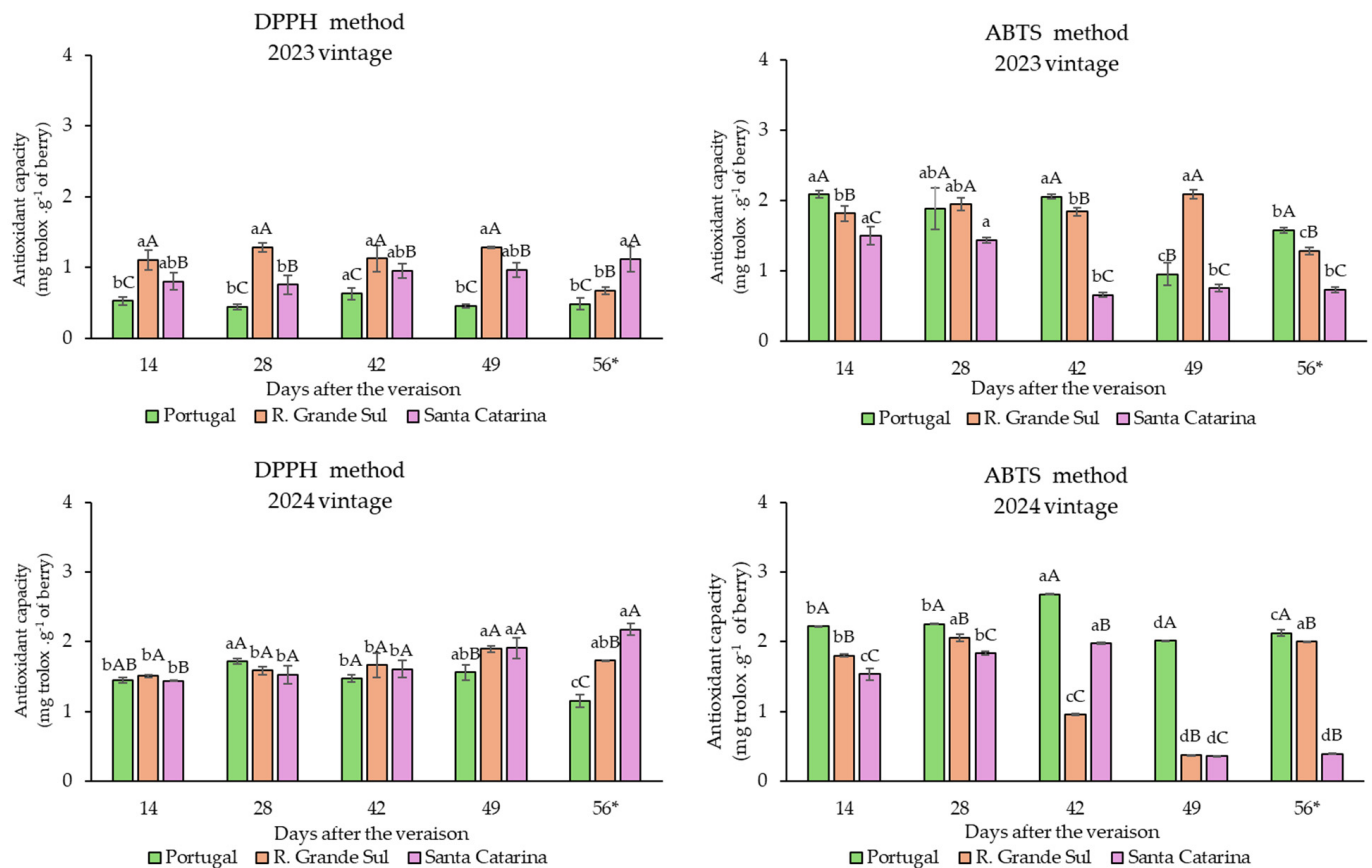


Figure 3. Antioxidant capacity (DPPH and ABTS^{•+} methods) of ‘Touriga Nacional’ grape berries during ripening (2022 to 2024) from vineyards located in Portugal and Brazil. * Harvested between 53 and 56 days after veraison. Values expressed in fresh weight (f.w.). All data express the average of three replicates, and error bars represent the standard deviation. Average values with different uppercase letters comparing different vineyard locations for the same vintage and for each data point indicate significant differences according to Tukey’s test ($p \leq 0.05$). Lowercase letters comparing different data points for the same vintage and vineyard location indicate significant differences according to Tukey’s test ($p \leq 0.05$).

3.5. Principal Components and UPGMA Cluster Analyses

Principal component analysis (PCA) was performed to understand the adaptability of ‘Touriga Nacional’ cultivated in vineyards located at three different locations (two in Brazil and one in Portugal) during three consecutive vintages (Figure 4). This statistical treatment was performed using the data obtained at harvest. The PC1 and PC2 explained 84.81% of the data variability (Figure 4A). The first principal component (PC1, 58.95% of the variance) was highly correlated with the variables: must yield, pH, estimated alcohol degree, total phenols, flavonoid phenols, total anthocyanins, antioxidant capacity by the DPPH methodology, and color intensity. The second principal component (PC2, 25.86% of the variance) was correlated with the color parameter a^* (redness) (Figure 4A).

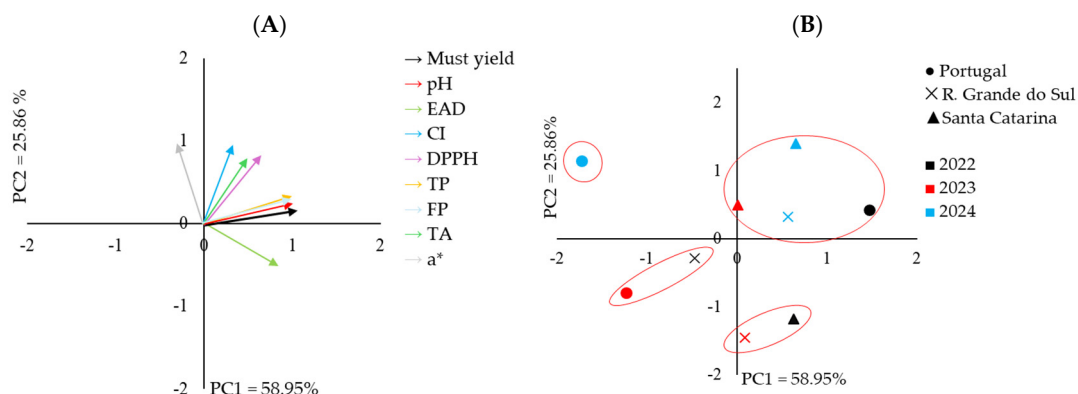


Figure 4. Principal component analysis of parameters analyzed for ‘Touriga Nacional’ grape berries at harvest over three vintages (2022 to 2024) from vineyards located in Portugal and Brazil. (A) Score graphs of the variables; (B) loading graphs of the locations. pH = hydrogen potential; EAD = estimated alcohol degree; CI = color intensity; DPPH = antioxidant capacity method; TP = total phenols; FP = flavonoid phenols; TA = total anthocyanins; a* = coordinate a* (redness).

The spatial distribution of the three vineyards in combination with the three consecutive vintages is shown in Figure 4B. Grapes collected in the vineyards located in Portugal in the 2022 vintage, in Rio Grande do Sul in the 2024 vintage, and in Santa Catarina in the 2023 and 2024 vintages were positioned on the positive side of the PC1 axis. These grapes showed higher values of total phenols, flavonoid phenols, total anthocyanins, antioxidant activity (DPPH), pH, must yield and color intensity. On the other hand, grapes collected in the vineyards located in the states of Santa Catarina in the 2022 vintage and Rio Grande do Sul in the 2023 vintage, positioned themselves on the negative side of the PC1 axis, showing a high estimated alcohol degree. Meanwhile, grapes collected in the Portuguese vineyard in the 2023 vintage were positioned on the positive side of the PC2 axis, showing good correlation with high color intensity values. Finally, grapes collected in Portugal in the 2024 vintage and in Rio Grande do Sul in the 2022 vintage were positioned on the negative side of the PC2 axis. These grapes were characterized by low values of total phenols, flavonoid phenols, total anthocyanins, antioxidant activity (DPPH), pH, must yield and color intensity.

Based on the Euclidean distance values and the resulting grouping, four main clusters were formed, indicating possible similarities between the ‘Touriga Nacional’ grapes collected in the three different vineyard locations (Figure 5).

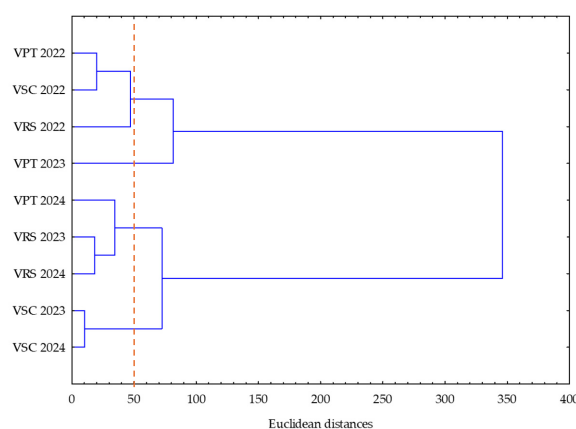


Figure 5. UPGMA cluster analysis for ‘Touriga Nacional’ grape berries at harvest over three vintages (2022 to 2024) from the vineyards located in Portugal and Brazil, as a function of all variables analyzed, using Euclidean distances. VPT = Vineyard in Viseu, Portugal; VRS = vineyard in the state of Rio Grande do Sul, Brazil; VSC = vineyard in the state of Santa Catarina, Brazil.

The first cluster was formed by grape samples collected in the vineyard located in the state of Santa Catarina (VSC 2023 and VSC 2024) in the 2023 and 2024 vintages, showing a high similarity between these two harvests from the same vineyard. This group stood out as it contained grapes with different characteristics at harvest, when compared to the other groups. This fact can be observed by the smaller Euclidean distance, thus showing consistency of the grape composition collected in the vineyard located in the state of Santa Catarina (VSC) during two consecutive vintages (Figure 5).

The second cluster of grouped samples was formed by grape samples collected in the Portuguese vineyard in the 2024 vintage (VPT 2022) and in the state of Rio Grande do Sul in the 2023 and 2024 vintages (VRS 2023 and VRS 2024), which demonstrated that despite being vineyards with different locations, at harvest, grapes showed very similar physicochemical and phenolic characteristics. Meanwhile, the third cluster consisted exclusively of grape samples collected in the Portuguese vineyard, in the 2023 vintage (VPT 2023), which demonstrated that the grapes in this vintage showed different characteristics compared to the remaining grapes from the other vineyard locations and vintages. Finally, the fourth cluster was formed by the grape samples collected in the vineyards located in Portugal and in both Brazilian states, all in the 2022 vintage. In this case, during the 2022 harvest, all the grapes analyzed, regardless of the vineyard location, showed similar characteristics for the parameters analyzed.

4. Discussion

In general, the results of this research confirm that vineyard location and vintage significantly influenced the physicochemical composition of the ‘Touriga Nacional’ grapes during ripening. In fact, it is well known that through grape ripening, several physical and biochemical changes occur in grapes [55–57]. All these changes are strongly modulated by different factors, such as temperature, rainfall, altitude, soil composition, and viticultural practices, among others [23,58–60].

4.1. General Physicochemical Composition of Grapes

Plant water status plays a crucial role in modulating berry weight, must volume, and must yield. Therefore, the amount and distribution of rainfall throughout grape ripening could determine these grape physicochemical parameters [61–63]. According to several studies, water uptake is largely responsible for the increase in berry weight [64] and must volume [65].

In our study, high rainfall during ripening was associated with increased berry growth collected in the vineyards located in the two Brazilian states. The higher rainfall values in these locations likely contributed to differences observed in the various grape physical parameters compared to what was observed for the grapes collected in the vineyard located in Portugal (Table 2). In the latter case, the meteorological data obtained over the three years clarify that grape ripening in the Portuguese vineyard occurred under much lower rainfall values (Figure 2). The data obtained in our study corroborate the findings reported by De La Hera-Orts et al. [66]. These authors demonstrated that for ‘Monastrell’ grapes cultivated in Spanish vineyards under a regime of greater water availability, they presented a greater berry weight compared to ‘Monastrell’ grapes grown in vineyards with less water availability. The combination of less accumulated rainfall values and higher average temperatures in 2023 and 2024 in the vineyard located in Portugal may have influenced the lower must yield per grape observed, compared to the values obtained in grapes collected in the vineyards located in Brazilian states (with higher rainfall and lower average temperature values). This may have led to greater dehydration of the grapes collected in the Portuguese vineyard. It should also be noted that, with low rainfall and high temperatures,

the transpiration rate increases, and the vine may even draw water from the grapes to survive, leading to grape desiccation.

Regarding the estimated alcohol degree, in 2023 and 2024, grapes collected in the vineyard from the state of Rio Grande do Sul showed the significantly highest estimated alcohol degree values. Furthermore, it was also clear that in all three vintages studied, grapes from both Brazilian vineyards were always those that showed the significantly highest values (Table 2) at harvest. However, irrespective of the vintage analyzed and vineyard location, in general, the estimated alcohol degree values obtained were slightly lower compared to the alcoholic potential values reported by other authors in several Portuguese wine regions [30,67]. Instead, Rodrigues et al. [68] reported high variability of estimated alcohol degree values over the years for ‘Touriga Nacional’ grapes cultivated also in the Viseu region (Portugal). Nevertheless, Barreto de Oliveira et al. [36] reported similar alcoholic potential values at harvest time also for ‘Touriga Nacional’ grapes cultivated under a tropical semi-arid climate in the Brazilian state of Pernambuco, compared to the values obtained in our investigation. In fact, it is also important to highlight that for the three vintages under study and regardless of the location of the vineyard studied, in the month in which the harvest took place, there were always notable amounts of rainfall (Figure 2). This fact was particularly evident in the vineyards located in both Brazilian states, which could also have contributed to the low estimated alcohol content. Another factor that may help to explain these results could be related to the altitude of the vineyards studied, particularly those that are in the two Brazilian states (located between 843 and 1200 m above sea level). In this case, some authors reported that the increase in altitude (between 873 and 1150 m above sea level) can contribute to a reduction in estimated alcohol degree values in grapes [69,70]. This fact can be explained mainly by two mechanisms. At high altitudes, low temperatures, particularly at night, delay the ripening process and consequently the sugar accumulation. On the other hand, increased UV-B radiation at high altitudes can reduce photosynthetic activity, inhibiting sugar production in the leaves and reducing translocation to the grapes. However, Mercenaro et al. [71] reported higher concentrations of sugars for ‘Cannonau’ grapes in vineyards situated at high altitudes (700 m above sea level) in the state of Minas Gerais (Brazil).

Regarding the pH values, in general, at harvest, it was also clear that the ‘Touriga Nacional’ grapes showed a clear differentiation between the samples collected in both Brazilian vineyards compared to the samples collected in the Portuguese vineyard, for the 3 years studied (Table 3). Thus, except for 2022, grapes from Brazilian vineyards presented significantly higher pH values. The occurrence of higher rainfall values during the ripening period in the Brazilian vineyards compared to the values observed for the vineyard located in Portugal may help to explain this difference in pH values of grapes. It is important to note that rainfall affects the pH values of grapes, but the exact relationship can be complex and vary by region and grape variety. While there is a general correlation between higher rainfall and higher pH values, this is not always consistent, and other factors such as temperature and soil potassium levels play a significant role in the final pH in a grape harvest [72]. Higher pH levels in grapes reduce the effectiveness of SO₂ as an antibacterial agent, requiring higher amounts to protect musts and wines from premature spoilage [73]. Therefore, low pH values in grape musts have a positive effect on the microbiological stability and sensory characteristics of musts and wines.

Furthermore, the high altitude of the two Brazilian vineyards may also have contributed to obtaining the higher titratable acidity values, particularly for the harvests in the 2022 and 2024 vintages. This occurs because vines grown at high altitudes, such as those found in the state of Santa Catarina, have cold winters and summers with mild temperatures, which results in slower ripening. This process favors the synthesis and accumulation

of organic acids, contributing to higher total acidity in the grapes [74–78]. De Oliveira et al. [79], who studied ‘Syrah’ grapes, reported that higher temperatures at the lower altitude regions (350 m above sea level) led to a high malic acid degradation compared to the vineyards located at high altitudes (1100 above sea level). Other authors reported that ‘Pinot Noir’ grapes from a high-site vineyard (1150 above sea level) characterized by lower temperatures, showed higher concentrations of malic acid compared to the same variety cultivated at 873 m above sea level [69]. Instead, the low titratable acidity found in ‘Touriga Nacional’ grapes collected in the Portuguese vineyard in the harvests of 2022 and 2024 (Table 3) can be explained by the high temperatures observed throughout the whole ripening period, which could have led to an increase in respiratory activity of the plants and consequently an intensification in the break of organic acids [77,80]. Arrizabalaga-Arriazu et al. [81] found that high temperatures throughout the ripening of ‘Tempranillo’ grapes promoted greater degradation of organic acids, resulting in lower total acidity. Similarly, Leeuwen et al. [82] also reported a total acidity decrease as a result of high temperatures.

4.2. Global Phenolic Composition and Chromatic Characteristics

The concentrations of total phenolic compounds and anthocyanin content varied over the three vintages studied and vineyard locations. These variations can be attributed mostly to the vintage effect, in which the year can be highly modulated by variations in temperature, rainfall, water balance and solar radiation, which greatly affect the grape maturation and consequently their phenolic composition [83,84].

In the vineyard located in the state of Santa Catarina, where high altitude (1200 m above sea level) and mild average monthly temperatures during grape ripening (between 13.5 and 18.4 °C) predominated, grapes showed the highest concentrations of total phenols, flavonoid phenols and total anthocyanins at harvest during two consecutive vintages (2023 and 2024). This tendency can be explained because at high altitudes, the atmospheric air mass is lower, and consequently, the levels of solar UV radiation are higher than in areas located at lower altitudes. According to several authors, higher UV radiation has the potential to positively regulate the synthesis of phenolics and consequently induce a positive effect on grape phenolic content [22,60,85]. Other authors also reported that mild temperatures can extend the grape ripening period, favoring the biosynthesis of the phenolic compounds [76,77]. Urvieta et al. [86] reported that the occurrence of high thermal amplitude between day and night is one of the factors that enhances the synthesis and accumulation of phenolics. According to these authors, lower night temperatures increase the expression of several key anthocyanin-regulating genes, such as the *VviMYBA1* gene. Billet et al. [87] also demonstrated that several polyphenol compounds found in grapes are highly influenced by temperature. Thus, lower temperatures have been shown to upregulate transcripts that accelerate the biosynthesis of several individual polyphenols, such as resveratrol.

González-Neves et al. [88] and Brighenti et al. [75] analyzed several grape varieties cultivated in vineyards located in the state of Santa Catarina, Brazil. According to these authors, the occurrence of mild daytime temperatures and cold nights contributes to higher accumulation of phenolic compounds, particularly anthocyanins. Therefore, grape ripening under conditions of average monthly temperatures not exceeding 18.1 °C, as observed in the vineyard located in the state of Santa Catarina (Figure 2), could also help to explain that, in general, ‘Touriga Nacional’ grapes from this vineyard location showed high levels of total anthocyanins. Previously, other authors reported a similar tendency in wine regions with similar temperature conditions [89,90]. The results obtained for total anthocyanins in grapes collected in the vineyard located in the state of Santa Catarina also induced higher values of red components (color intensity and coordinate *a**). However, it is important to highlight

that neither the high color of the must nor the high anthocyanin content necessarily results in intensely colored red wines, probably due to differences in the extraction of anthocyanins from the grape skins [91].

For the grapes collected in the Portuguese vineyard, in the 2022 vintage, high average monthly temperatures during grape ripening (23.1 °C in July, 21.7 °C in August and 17.1 °C in September), combined with low average monthly rainfall (0.7 mm in July, 0.1 mm in August and 4.5 mm in September), positively determined the synthesis of phenolic compounds. According to several authors, temperatures around 25 °C promote the biosynthetic pathway of phenolic compounds, especially flavonoids such as anthocyanins [92–94]. Additionally, these temperatures can induce high activity of the enzymes that are responsible for the sugar's biosynthesis [95,96], which are one of the great precursors of phenolic compounds [97,98]. This is because phenolic compounds originate from the same precursor, L-phenylalanine, which originates from glucose, via the shikimate pathway [92]. In addition to the temperature factor, water deficit can also be a major modulator of the concentration of phenolics in grapes. Under water stress conditions, secondary metabolism is activated, and consequently, the synthesis of phenolic compounds increases as part of the plant's defense mechanism against abiotic stress via the phenylpropanoid pathway [99,100]. According to Watrelot et al. [101], under these conditions, there is a higher activation of the phenylpropanoid pathways, which are responsible for the synthesis of flavonoids, which includes anthocyanins and condensed tannins. These environmental conditions (characterized by low average rainfall conditions) could also help to explain the significantly high content of total tannins in all vintages found in grapes collected in the Portuguese vineyard, compared to what was detected for the grapes collected in the remaining two vineyard locations. This result follows the same tendency reported by other authors who studied different grape varieties. For example, Chavarria et al. [20] found that the lower water availability increased the accumulation of total tannins in 'Cabernet Sauvignon' grapes, while Gouot et al. [92] reported high content of total tannins in 'Shiraz' grapes cultivated under high temperatures. Other authors demonstrated that for 'Cabernet Sauvignon', regulated deficit irrigation increases the contents of most monomeric anthocyanins and a high proportion of malvidin forms [98]. According to Castellarin et al. [99], water deficits promote higher values of anthocyanins in red grapes. This increase resulted from an increase of 3'4'5'-hydroxylated forms through the differential regulation of F3'H and F3'5'H. However, water deficits show a limited effect on flavonol synthesis.

'Touriga Nacional' grapes collected in the vineyard located in the state of Rio Grande do Sul showed the lowest total tannin concentrations at harvest, during the three vintages studied. However, these same grapes stood out for showing significant levels of non-flavonoid phenols in the 2022 and 2023 vintages. The combination of mild average monthly temperatures during grape ripening in this location (that did not exceed the average monthly temperature of 22.3 °C), high accumulated monthly rainfall (77.6 to 229.4 mm), and high altitude (843 m above sea level) may be largely responsible for this trend. This is because the hydration status of the vines has an important impact on the solutes in the berries [66]. Moreover, the high availability of water can cause the dilution of these solutes, primarily tannins and organic acids, among others [102]. On the other hand, mild temperatures, associated with high altitudes contributes to a slower ripening of the grapes, which allows for the greater accumulation and preservation of polyphenolic compounds such as flavonoids and non-flavonoids [76,77]. This shows that each *terroir* enhances the formation of different groups of compounds in grape berries due to the combination of elements that characterize the production locations. Corroborating these findings, Costa et al. [8] analyzed seven Portuguese red grape varieties cultivated in two Portuguese wine-growing regions and observed that for most of the varieties studied, the highest

concentrations of non-flavonoid phenols were achieved in grapes grown in the 'Douro' region compared to those grown in the 'Dão' region. These results highlight the action of *terroir* in the biosynthesis of some specific compounds in grapes.

4.3. Grapes Antioxidant Capacity

In general, 'Touriga Nacional' grapes with higher concentrations of phenolic compounds also showed a tendency for higher antioxidant capacity values. This trend was particularly evident for the grapes collected in the Portuguese vineyard, particularly when antioxidant capacity values were quantified using the ABTS^{•+} method. It is important to highlight that in the literature, there is generally a large variation in values of antioxidant capacities obtained for diverse grape varieties and grape berry fractions as a result of different analytical methods, different sample preparation and extraction processes [103–105]. Several studies indicate significant correlations between antioxidant capacity and concentrations of phenolic compounds in different grape cultivars. Vilanova et al. [106] reported high correlations between antioxidant capacity and total anthocyanin concentrations in different Spanish grape varieties, while Panceri et al. [107] also found high correlations between antioxidant capacity, total phenol concentrations and several individual phenolic compounds in 'Merlot' and 'Cabernet Sauvignon' varieties. Previously, Jordão and Correia [105] described a positive correlation between different proanthocyanidin fractions and the antioxidant capacity of 'Touriga Nacional' and 'Tinta Roriz' grapes. In contrast, during the grape ripening process, an inverse association was observed for individual anthocyanins. More recently, Jordão et al. [103] also reported significantly higher levels of antioxidant capacity in grapes from the 'Merlot' variety compared to 'Syrah' and 'Saborinho' cultivated under the specific conditions of the Azores Islands over three vintages.

In general, it was found that the antioxidant capacity values quantified by the ABTS^{•+} method were higher than those from the DPPH method in all three vintages studied. This tendency was also irrespective of the vineyard's location. On the other hand, it also became clear that the application of the two methods revealed a different trend depending on the location of the vineyard from which the samples were collected. This last point was particularly evident in the results obtained at harvest. Indeed, it is well known that total antioxidant capacity values can vary according to the analytical assay applied for their quantification. This variation stems from the fact that ABTS^{•+} and DPPH radicals have different stereochemical configurations, as well as different formation mechanisms, which generate different inactivation responses of these radicals when they react with different compounds [108,109]. According to Benbouguerra et al. [110], the lower detection of antioxidant activity using the DPPH method can be explained by the variation in the reaction time of phenolic compounds with the DPPH radical. Depending on the phenolic compound, it can react quickly or slowly with the radical under study, influencing the amount of detection of antioxidant capacity. Danilewicz [111] reported that caffeic acid reacted quickly with the DPPH radical, while (+)-catechin reacted very slowly, influencing the results obtained. Following the same trend observed in our work, Burin et al. [112] reported higher antioxidant capacity values using the ABTS^{•+} method compared to the values obtained using the DPPH method. However, other studies reported an opposite tendency, where the highest values were obtained by the use of the ABTS^{•+} method [104].

5. Conclusions

This study provides one of the first comparative assessments of the performance of 'Touriga Nacional' grapes under contracting vineyard conditions from Southern Brazil. Furthermore, comparing the results obtained in Brazil with grapes of the same variety culti-

vated in their region of origin in Portugal provides a comparative context that strengthens the relevance of this research.

Despite considerable interannual variation identified among all the studied harvests and for most of the evaluated parameters, the results seem to show that the two vineyards located in the two different Brazilian states considered may also present suitable conditions for the cultivation of the ‘Touriga Nacional’ variety. This tendency was particularly evident for the grapes collected in the vineyard located in the state of Santa Catarina. These grapes showed a trend towards higher total phenolic content, mainly flavonoid phenols and total anthocyanins, as well as higher concentrations of organic acids and total titratable acidity compared to the grapes collected from the other two vineyard locations (Portugal and Rio Grande do Sul). However, it is of great importance to highlight that the results found for ‘Touriga Nacional’ grapes did not exhibit homogeneous behavior across the three vintages studied. This demonstrates that, in addition to the genotype, interannual environmental variability of vintage also affects the physicochemical characteristics of the grapes.

Finally, despite interannual variability, ‘Touriga Nacional’ seems to demonstrate phenotypic plasticity under different growing conditions, which could be a differentiating factor in relation to the red wines that could be produced in those regions. However, the results obtained in our work need to be treated with caution, because vintage effects and harvest timing likely influenced the physicochemical characteristics of the grapes produced.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijpb17030022/s1>, Table S1: Total soluble solids of ‘Touriga Nacional’ grape berries during ripening over three vintages (2022 to 2024) from vineyards located in Portugal and Brazil (States of Rio Grande do Sul and Santa Catarina).

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