agriculture

MDPI

Article

The Influence of Sulfur Dioxide Concentration on Antioxidant Activity, Total Polyphenols, Flavonoid Content and Individual Polyphenolic Compounds in White Wines during Storage

Jiří Mlček [1,*], Tunde Jurikova [2,*], Růžena Bednaříková [1], Lukáš Snopek [1,3], Sezai Ercisli [4,5] and Ondřej Tureček [1]

[1] Department of Food Analysis and Chemistry, Faculty of Technology, Tomas Bata University in Zlin, Vavreckova 5669, 76001 Zlin, Czech Republic; rvaskova@utb.cz (R.B.); lsnopek@utb.cz (L.S.); o_turecek@utb.cz (O.T.)
[2] Institute for Teacher Training, Faculty of Central European Studies, Constantine the Philosopher University in Nitra, 94974 Nitra, Slovakia
[3] Department of Environmental Security, Faculty of Logistics and Crisis Management, Tomas Bata University in Zlin, nám. T. G. Masaryka 5555, 76001 Zlin, Czech Republic
[4] Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum TR-25240, Turkey; sercisli@gmail.com
[5] HGF Agro, Ata Teknokent, Erzurum TR-25240, Turkey
* Correspondence: mlcek@utb.cz (J.M.); tjurikova@ukf.sk (T.J.); Tel.: +420-9576033030 (J.M.); +421-875110 (T.J.)

Abstract: Wines represent a rich source of bioactive compounds, especially polyphenolic compounds, which mostly contribute to the antioxidant activity. Sulfur dioxide (SO_2) has mostly been used as a preservative in winemaking to prevent oxidation during storage. The aim of this paper is to evaluate the changes in SO_2 levels and the influence of sulfur dioxide addition at seven different concentrations on the antioxidant activity (detected by DPPH-2,2-diphenyl-1-picrylhydrazyl and the ABTS Trolox equivalent antioxidant capacity methods), total polyphenols, flavonoid content and individual polyphenolic compounds (determined by the HPLC high-performance liquid chromatography method) of white wines during 5 months of storage. The assayed sulfur dioxide concentrations show a decreasing tendency with time, with a final decrease of more than 50% in comparison with the start of the experiment. Between the first and second measurements, the average decrease in sulfur dioxide was 16%. In the following interval, it was found that the maximum decline in SO_2 was 26%. The changes in SO_2 levels cannot be considered statistically significant. At the same time, we observed a decreasing tendency in the TPC content during storage. The antioxidant activity determined by the DPPH method at the beginning of the experiment ranged from 116.75 up to 270.62 mg·L^{-1}, while the antioxidant activity increased with sulfur dioxide concentration. The AA detected by the ABTS method displayed a decreasing tendency during storage. In the case of the TFC content, we observed a significant influence of sulfur dioxide on the concentration. No addition or addition of high SO_2 concentrations negatively influenced the flavonoid content in the samples. During storage, we observed a highly variable content of phenolic compounds in relation to SO_2 addition. The most abundant compounds were chlorogenic acid, caffeic acid and epigallocatechin.

Keywords: white wines; storage; sulfur dioxide

check for updates

Citation: Mlček, J.; Jurikova, T.; Bednaříková, R.; Snopek, L.; Ercisli, S.; Tureček, O. The Influence of Sulfur Dioxide Concentration on Antioxidant Activity, Total Polyphenols, Flavonoid Content and Individual Polyphenolic Compounds in White Wines during Storage. *Agriculture* **2023**, *13*, 1439. https://doi.org/10.3390/agriculture13071439

Academic Editor: Jianle Chen

Received: 22 June 2023
Revised: 13 July 2023
Accepted: 14 July 2023
Published: 21 July 2023

1. Introduction

Wines can be characterized by their high content of bioactive substances, including flavonoids and non-flavonoid compounds, displaying a wide range of health-promoting activities [1]. Flavonoid compounds, such as flavonols, monomeric catechins, proanthocyanidins, anthocyanins and anthocyanidins, along with non-flavonoid phenolic compounds, such as resveratrol, have been increasingly studied with respect to their structure and metabolism/bioavailability [2]. Phenolic acids include benzoic acid, cinnamic acid and

their derivatives [3,4]. Moderate wine consumption contributes to a healthy lifestyle [5], improves lipoprotein metabolism, lowers cardiovascular mortality risk [6,7], protects the brain and nerve cells and reduces platelet aggregation [8]. Red wine polyphenolic extract consumption can be used in the prevention and treatment of chronic diseases, such as metabolic syndrome, degenerative pathologies and cancer [9]. The study of the chemical composition of grapes confirms the widespread use of grape and fruit wines in medical, nutritional and other fields [10].

One of the biggest problems for winemakers is wine preservation. Antioxidant activity and the effects of polyphenols can be inhibited by the addition of preservatives to wine. Sulphur dioxide thus represents the most widely used effective preservative in enology. Sulfur dioxide prevents oxidation and browning [11]. It also displays antioxidant and antimicrobial effects, preventing microbial growth and blocking the reproductive cycle of bacteria in the digestive tract. SO_2 prevents the conversion of sugars and alcohol derivates in the liver by blocking vitamin B [8,12]. Moreover, it helps to control undesirable fermentation, inhibits oxidase activity and protects against fungal infections [13]. From a sensory point of view, it helps to eliminate unpleasant odors resulting from oxidation [14] and neutralizes any aroma that gives wines a characteristic aroma defect [15].

On the other hand, sulfites also display side effects. Firstly, they can be considered a strong allergen that can cause headaches, stomach problems, swimmer's dermatitis, diarrhea, bronchoconstriction and anaphylaxis [16,17]. A high concentration of SO_2 can also delay the malolactic fermentation of wine, especially in wines with a low pH [18].

This is a reason for legislative regulation of its concentration with the allowed values for its addition. The limits of SO_2 content in wines (EC, 606/2009) according to the European Commission and OIV (International Organisation of Vine and Wine) are between concentrations of 150 mg·L^{-1} and 200 mg·L^{-1} in conventional red and white wines, and organic red and white wines cannot exceed values between 100 mg·L^{-1} and 150 mg·L^{-1} [15,19]. The main aim of producers is to produce wines with lower amounts of SO_2 and SO_2-derived compounds (sulfites) or sulfite-free wines. However, despite growing interest and the attempts of wine producers to substitute sulfites with phenolics or natural extracts, the results so far have been discouraging. In summary, there are currently no substances, treatments or green technologies that are able to effectively substitute the use of SO_2 entirely [20].

Therefore, the aim of this research paper is to evaluate the effect of the addition of different doses of SO_2 on the nutritive quality of wines—the total polyphenol content, the total flavonoid content and antioxidant activity determined by DPPH and ABTS methods. Moreover, we also examined the changes in chemical parameters during 5 months of storage. This paper offers an evaluation of changes in individual polyphenolic compounds presented in wines—gallic acid, protocatequie acid, naochlorogenic, 4-hydroxybenzoic acid, vanillic acid, chlorogenic acid, coffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid and ethylesters of protocatequie acid.

2. Materials and Methods

2.1. Sample Preparation

To achieve our goal, 40 L of white wine was prepared—Moravian Zemské. For processing, the grapes of cultivars 'Pálava' and 'Irsai Oliver' were used in a 1:1 ratio, as shown in Table 1.

The grapes for the preparation of the samples were collected on 14 September 2021, when 40 kg of grapes of the Pálava variety and 40 kg of the Irsai Oliver variety were collected. Consequently, the grapes were crushed and stemmed with a grape crusher and stalk remover and pressed using a vertical press with a wood basket and a hydraulic press. From 80 kg of grapevines, we obtained 55 L of grape must which corresponded to a 68.75% yield. The normalized must scale ("Normalizovaný moštoměr" °NM) is a scale used in the Czech Republic and Slovakia to measure the sweetness of wine must. A measurement of 1 °NM indicates 1 kg of sugar in 100 L of must. The extracted must was de-sludged

for 18 h. The loss after sludge coiling was 5 L with the remaining 50 L of must. The sugar content was increased by the addition of 1.1 kg saccharose at 20.2 °NM. Fermentation of the improved must was conducted in a 150 L plastic bowl with a floating lid and a fermentation plug at a temperature of 15–16 °C using spontaneous fermentation. After the fermentation was completed on 25 October 2021, the vine was centrifuged from fine sludge and filtrated with 8 to 3 microns desk retention. The filtered vine was adjusted in 8 glass bowls with a volume of 5 L. Consequently, ammonium hydrogensulfide was added in the following amounts (Table 2).

Table 1. Overview of grape cultivars used for preparation of samples.

Varieties	Registration No. of Vineyard	Cultivation Year	Conduction	Region	Subregion	Village	Localization
Pálava	628964/0198	2011	high	Morava	Mikulovská	Dolní Dunajovice	Zimní vrch
Irsai Oliver	628964/0198	2009	high	Morava	Mikulovská	Dolní Dunajovice	Zimní vrch

Table 2. Total content of sulfur dioxide at wines.

Sample No	Amount of Added 40% Solution of Ammonium Hydrogensulfide per 5 L of Wine (mL)
1	0.000
2	0.375
3	0.750
4	1.125
5	1.500
6	1.875
7	2.250
8	2.625

For the collection of wine samples, the standard SN 56 0216 method was used, using Tokay wine and malt wine. This Czech technical standard applies to the sampling and testing of natural, sparkling, dessert and spicy grape wines, Tokay wines and malt wines of domestic and foreign origin. The ČSN standard comprises standardized methods and describes the procedure for performing standardized tests. The standard sets out the procedures for carrying out certain tests (methods) to detect and/or verify quality characteristics relevant to nutritional hygiene.

2.2. Determination of SO_2 by OIV-MA-AS323-04B: R 2009

Chemicals: sulfuric acid (H_2SO_4), starch (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic), sodium hydroxide (NaOH), EDTA 3, acetaldehyde, iodine (I_2) (Ing. Petr Lukeš, Uherský Brod, Czech Republic). Ordinary laboratory glassware and equipment: stopwatch, 25 mL burette, digital lamp.

The free sulfur dioxide was determined by direct titration with iodide. After alkali hydrolysis, bonded sulfur dioxide was detected as well. The total amount of sulfur dioxide was calculated by addition of its free and bonded forms. In the samples, free and bonded SO_2 was detected. Standardization of the volume solution was provided by sodium thiosulfate standardized on potassium dichromate.

2.2.1. Determination of Free SO_2

An amount of 50 mL of the wine sample was pipetted into a 500 mL volumetric flask, and 3 mL 16% H_2SO_4 and 1 mL of EDTA 3 solution with a concentration of 1% was added. An amount of 5 mL of starch solution was titrated against a white background I_2 solution

with a concentration 0.02 mol·L^{-1} until a blue color was observed. The obtained power consumption was used in the final calculation (V1).

2.2.2. Determination of Total SO_2

After titration of free SO_2, an 8 mL NaOH solution at a concentration 4 mol·L^{-1} was added to the sample, and after 5 min, we added 10 mL of a 16% H_2SO_4 solution titrated with iodine. We used the final consumption to calculate (V2). Then, 20 mL of NaOH and 200 mL of distilled water were added, and after 5 min, 30 mL of 16% H_2SO_4 solution was added and titrated with iodine until a blue color was observed. We obtained a consumption of V3.

Correction for reductones.

A 50 mL wine sample and 1 mL of 1% formaldehyde were measured, and after 30 min, 3 mL of 16% H_2SO_4, 1 ml of 1% EDTA 3 solution and 5 mL of starch solution were added and titrated against a white background with an I_2 solution at 0.02 mol·L^{-1} until a blue color was observed. This step provides a consumption of V4.

Calculation concentration SO_2 (mg·L^{-1})

Concentration of free SO_2 c = (V1 − V2).f.12,8

Concentration of total SO_2 c = (V1 + V2 + V3 − V2).f.12,8

12.8—coefficient for conversion to SO_2 when used 0.025 M I_2.

2.3. Determination of Total Polyphenol Compounds (TPC)

Chemicals: distilled water, Folin–Ciocalteu reagent (FCR) (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic), sodium carbonate (Na_2CO_3) (Ing. Petr Lukeš, Uherský Brod, Czech Republic). To determine the total content of phenolic compounds (TPC), a spectrophotometric method was conducted using Folin–Ciocalteu reagent based on the reduction of the phosphomolybdate–tungsten complex by phenolic substances in an alkaline medium. The modified method of Singleton and Rossi (1965) according to Sumczynski et al. (2015) was used [21,22]. Determination was performed at a wavelength of 765 nm after a 30 min incubation. The total content of phenolic substances was expressed as gallic acid equivalents (GAE) in mg·L^{-1}. The repeatability of the assay was verified on 10 parallel determinations for cm = 0.5 g·L^{-1} of tannin. The calibration dependence A = f (cm) was constructed using six calibration solutions. For the preparation of calibration solutions, we dispensed approximately 20 mL of distilled water into four 50 mL volumetric flasks, pipetted 0.4; 0.6; 0.8; and 1.0 mL of the standard solution, added 1 mL Folin–Ciocalteu reagent and mixed the solution. After 3 min, 5 mL of 20% Na_2CO_3 solution was added, made up to the mark with distilled water and mixed. After 60 min, the intensity of the staining in a 10 mm cuvette at 765 nm was measured against a blank spectrophotometrically. In the same way, the absorbance of the samples was determined. According to the regression curve equation, the polyphenol content was calculated and expressed as mg gallic acid (GAE)·L^{-1}.

2.4. Determination of Total Flavonoids Content (TFC)

Determination of the total flavonoids content was performed spectrophotometrically according to a modified method by Li et al. (2009) and Saeed et al. (2012) [23,24].

A volume of 0.425 mL of wine sample and 4.25 mL of 20% ethanol were pipetted into a test tube. Then, 0.19 mL of 0.5 M $NaNO_2$ was added to the mixture. Into this mixture, 0.19 mL of 0.3 M $AlCl_3 \cdot 6H_2O$ was added after 5 min and the solution was incubated at 20 °C for 5 min. This process was followed by the addition of 1.25 mL of 1 M NaOH. The mixture was allowed to stand for 10 min. Subsequently, the solution was measured at a wavelength of 506 nm on a Lambda 25 spectrometer.

For evaluation, the calibration curve method to the routine standard was used. The results were expressed in mg of rutin equivalent (RE·L^{-1}) of the sample.

2.5. Determination of Total Antioxidant Activity by DPPH and ABTS Methods

Chemicals: methanol, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and (2,2′-azinobis(3-etyl-2,3-dihydrobenzotiazol-6-sulphonic acid)) (ABTS) (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic).

The total antioxidant activity was assessed by the modified method of Orsavova et al. (2019) [25]. First, a stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. A working solution was then prepared from the prepared stock solution by mixing 10 mL of the stock solution and 45 mL of methanol. Subsequently, the working solution thus prepared was spectrophotometrically measured at a wavelength of 515 nm against methanol as a blank. A sample of 450 µL of wine was pipetted into a test tube and then 8.55 mL of DPPH working solution was added. After 60 min of incubation in the dark, the sample was measured spectrophotometrically at said wavelength. The absorption loss was recalculated using the linear regression equation to equivalent Trolox (TE)·L^{-1}. The statistical analysis results are reported as mean values with standard deviation (SD). Differences between observed results were detected by a t-test (Statistica, 2018, StatSoft, Inc., Tulsa, OK, USA).

The final extract solutions prepared from the grapevine samples were employed in the antioxidant activity assays based on the quenching of the synthetic radical ABTS+. Prior to the analysis, an ABTS stock solution was prepared using 7 mol·L^{-1} ABTS and 60 mmol·L^{-1} $K_2S_2O_8$ in a volume ratio of 1:50 and then incubated at room temperature for 16 h. Then, an ABTS working solution was prepared by mixing 2.5 mL of ABTS stock solution and 97.5 mL of acetic buffer (pH 4.3). Two hundred microliters of the sample extract was mixed with 24.0 mL of ABTS working solution. Thereafter, the depletion of absorbance was measured spectrophotometrically (Lambda 25, Perkin Elmer, Waltham, MA, USA) at 734 nm after being incubated for 30 min.

2.6. Determination of Individual Phenolic Compounds

High-performance liquid chromatography with a diode array detector (HPLC–DAD) was used to separate and identify individual phenolic compounds in wines.

Prior to measurement, the wine samples were diluted with distilled water in a ratio of 1:10 (wine/DW) and then filtered through nylon microfilters (0.45 µm Nylon Syringe Filter). The determination was performed by reverse-phase high-performance liquid chromatography (RP-HPLC) on an UltiMate® 3000 instrument (Dionex, Sunnyvale, CA, USA) with a DAD using a Kinetex C-18 column (150 × 4.6 mm; 2.6 µm) (Phenomenex, Torrance, CA, USA). Gradient elution was performed using mobile phases comprising eluent A—distilled water/acetic acid (99: 1, *v*/*v*) and eluent B—distilled water/acetonitrile/acetic acid (67: 32: 1, *v*/*v*/*v*). The gradient program was 0–10 min: 90% A + 10% B; 10–16 min: 80% A + 20% B; 16–20 min: 60% A + 40% B; 20–25 min: 50% A + 50% B; 25–27 min: 60% A + 40% B; and 27–35 min: 90% A + 10% B. The flow rate was 1 mL/min, the injection volume was 10 µL and the analysis time was 35 min. Detector responses were recorded at 275 nm as described by de Quirós et al. (2009) [26]. Twenty-two individual phenolic compounds were separated and identified. However, only groups of selected substances are described in the text and in the results. Due to the large scope of analyses, the listing of individual phenolic compounds for each sample would mean a disproportionate extension of this scientific work.

- Phenolic acids:
  - Benzoic acid derivatives (gallic, vanilla, syringic, protocatechuic, ellagic, 4-hydrox ybenzoic acid and protocatechuic acid ethyl ester).
  - Cinnamic acid derivatives (trans-cinnamic, ferulic, caffeic, hydroxycinnamic, chlorogenic, neochlorogenic, sinapic and p-coumaric acids).
- Flavonoids:
  - Flavonols (quercetin, rutin and kaempferol).
  - Flavanols (epigallocatechin, epicatechin and catechin).

- Stilbenes:
  - (resveratrol).

Qualitative evaluation was performed using the standards analysis of individual polyphenolic compounds. Quantitative evaluation, where the final value was determined as the average of six measurements ($n = 6$), was performed by the method of a calibration curve and subsequent calculation of the substance's concentration in the sample. The individual polyphenol content was expressed as the equivalent concentration of mg standard in 1 L of the sample.

2.7. Statistical Evaluation

The obtained data were expressed as arithmetic means ± standard deviation. All analyses were performed twice in triplicate. The values of Pearson correlation coefficients (r), determined by the methods described by Snedecor and Cochran (1994) [27], were calculated to detect the linear dependences between different quantities determined by different methods. The statistical methods used include an analysis of variance (ANOVA, $\alpha = 0.05$), which examines whether there is a statistically significant difference between at least two mean values.

3. Results and Discussion

3.1. Detection of Sulfur Dioxide in White Wines

In samples 2–8, we detected changes in the concentration of sulfur dioxide regularly in the interval of one month from 29 October 2021 to 25 September 2022. Sample 1 contained no added SO_2.

Detected values of sulfur dioxide during storage are given in Table 3.

Table 3. Changes of free SO_2 during storage time.

Sample No	Date of Detection				
	29 October 2021	**25 November 2021**	**04 January 2022**	**26 January 2022**	**25 September 2022**
	The Content of Free SO_2 (mg·L^{-1}) ± Standard Deviation				
2	12.10 ± 1.07	10.59 ± 1.07	9.08 ± 1.85	5.3 ± 1.07	4.54 ± 1.07
3	29.5 ± 0.00	26.48 ± 1.07	16.64 ± 1.07	15.13 ± 1.07	9.08 ± 1.07
4	54.47 ± 0.00	41.61 ± 1.07	26.48 ± 1.07	26.48 ± 1.07	20.42 ± 1.07
5	80.19 ± 2.14	63.54 ± 0.00	46.14 ± 1.07	46.14 ± 1.07	34.04 ± 1.07
6	103.64 ± 1.07	87.75 ± 1.07	67.33 ± 1.07	64.3 ± 1.07	52.2 ± 0.00
7	133.89 ± 0.00	110.44 ± 1.07	84.72 ± 1.07	83.21 ± 1.07	68.08 ± 1.07
8	155.08 ± 1.07	137.68 ± 1.07	105.15 ± 1.07	104.39 ± 1.85	86.24 ± 1.07

According to the achieved results, the free sulfur dioxide content at the beginning of the storage period reached values ranging from 1210 to 155.08 mg·L^{-1}. Snopek et al. (2018) determined the content of free SO_2 in three samples of white wine in concentrations from 5.37 ± 0.32 to 11.14 ± 1.88 mL.L^{-1} [8].

Between the first measurement on 29 October 2021 and the 25 November 2021 measurement, an average decrease of 16% in sulfur dioxide was recorded. In the subsequent interval from 25 November 2021 to 4 January 2022, a maximum decrease of 26% in SO_2 was recorded. On the other hand, the lowest decrease was registered between 4 January 2022 and 26 January 2022, at 8% on average. During this period, the biggest drop was detected in sample 2 (42%), which at the same time represented the highest value of free sulfur dioxide decline during the full time of storage. On the other hand, the rest of samples displayed the lowest value of SO_2 by 0–9%. In the period between 26 January 2022 and 25 February 2022, we observed a 56% decline; after 4 months of storage, the content of free SO_2 reached up to 4.54 and 86.24 mg·L^{-1}.

Snopek (2019) observed the average decline in free sulfur dioxide concentration in wines after one month of storage (3.34%), which represents a value of 12.66% less than the

results of our measurement [28]. In the same way, the authors noticed an average decline in SO_2 concentration by 14.53%, which was lower value than the decline measured after 4 months of storage. According to Fišera et al. (2022), the average loss of SO_2 in wines after 150 days of storage was 37% [29]. On the other hand, the decline in free sulfur dioxide can be related to applied technologies, the conditions of storage and the content of carbonyl compounds bound to SO_2 [30].

A 2% decrease in SO_2 concentration was observed between the two measurements. This value corresponds with the experimental work of Snopek (2019), who found approximately identical values of total sulfur dioxide after 1 month and 6 months of storage in comparison with the beginning of storage [28]. A more significant decrease of 10.73% was observed after 12 months of storage.

The detected amounts of total sulfur dioxide concentration are given in Table 4.

Table 4. Content of total SO_2.

Sample No	Date of Detection	
	25 November 2021	25 February 2022
	Total SO_2 Content ($mg \cdot L^{-1}$) ± Standard Deviation	
2	32.53 ± 1.07	31.63 ± 1.85
3	62.79 ± 1.07	60.77 ± 1.07
4	92.29 ± 1.07	91.18 ± 1.07
5	111.20 ± 0.00	109.12 ± 1.07
6	135.41 ± 1.07	133.89 ± 1.07
7	161.88 ± 1.07	161.44 ± 0.00
8	189.12 ± 1.07	188.64 ± 1.85

3.2. Impact of Sulfur Dioxide Addition on Total Phenolic Content (TPC) during Storage

The content of total content polyphenols (TPC) in assayed samples during storage is presented below in Table 5.

Table 5. The changes in TPC during storage.

Sample No	Date of Measurement				
	26 October 2021	24 November 2021	3 January 2022	25 January2022	23 February 2022
	TPC Content (mg GAE·L^{-1}) ± Standard Deviation				
1	150.00 ± 1.70 ^{a,A}	131.75 ± 2.75 ^{b,A}	128.35 ± 0.75 ^{b,A}	120.73 ± 0.61 ^{c,A}	138.55 ± 1.45 ^{d,A}
2	191.65 ± 7.35 ^{a,B}	165.75 ± 3.35 ^{b,B}	149.10 ± 0.30 ^{bc,B}	142.15 ± 8.16 ^{c,B}	170.30 ± 5.00 ^{b,B}
3	197.05 ± 7.35 ^{a,B}	184.55 ± 0.05 ^{a,BC}	158.55 ± 4.15 ^{b,BC}	146.18 ± 5.60 ^{b,BC}	168.35 ± 1.65 ^{b,B}
4	212.35 ± 8.85 ^{a,B}	199.45 ± 3.35 ^{ab,CD}	159.60 ± 5.10 ^{c,BD}	155.58 ± 3.41 ^{c,CD}	183.65 ± 8.15 ^{b,B}
5	250.50 ± 1.50 ^{a,C}	221.95 ± 0.35 ^{b,DE}	171.15 ± 5.25 ^{c,CDE}	170.68 ± 0.83 ^{c,E}	197.50 ± 6.40 ^{d,BC}
6	246.75 ± 12.95 ^{a,C}	234.15 ± 16.55 ^{ab,E}	179.20 ± 1.40 ^{c,E}	164.03 ± 2.24 ^{c,DE}	201.85 ± 13.65 ^{bc,CD}
7	254.65 ± 15.25 ^{a,C}	259.15 ± 6.05 ^{a,F}	188.15 ± 5.55 ^{b,F}	165.68 ± 6.56 ^{b,DE}	227.40 ± 3.50 ^{c,D}
8	254.60 ± 5.50 ^{a,C}	259.85 ± 0.35 ^{a,F}	182.70 ± 5.40 ^{b,E}	167.50 ± 5.74 ^{b,DE}	217.15 ± 15.85 ^{c,CD}

Notes: values with different indexes with lowercase letters mean statistically significant differences among the dates of determination and uppercase letters represent significant differences among the samples (1–6) at the >0.05 level.

At the begging of experiment (before the storage (26 October 2021)), the TPC content in assayed samples ranged from 150.00 up to 254.60 $mg \cdot L^{-1}$. The TPC content in white wine was determined by Mitrevska et al. (2020) as 169.48 to 434.27 $mg \cdot L^{-1}$ in studied samples of white wine from north Macedonia [31]. Čeryová (2021) examined muscat cultivars of grapevines from Slovenia, determining a content of 226.80 to 568.30 $mg \cdot L^{-1}$ [32]. Snopek et al. (2018) determined values of 203.06 (Riesling) up to 445.45 mg GAE·L^{-1} in white wine originating from the territory of Moravia [8]. In contrast to the mentioned studies, a lower TPC value was achieved. Paixao et al. (2007) evaluated the content of

total polyphenols in Portuguese white wines and found an average content of 369 mg GAE·L^{-1} [33]. Another study by Hurtado et al. (1997) reported the average content in white wines as 292 mg GAE·L^{-1} [34].

These differences may be due to different geographical origins, cultivars, ripening stages, detection methods or different technologies of sampling such as maceration, duration molding, etc.

Mitić et al. (2010) compared the content of TPC in selected wines originating from Europe and South Africa [35]. According to published data, the wines from the territory of Czech Republic achieved on average a TPC content of 103–125 mg·L^{-1}. In our research work. we determined lower values of TPC.

Castellari et al. (1998) compared the TPC content in organic wines without SO_2 addition and conventional wines and found a similar content of TPC without statistically significant differences [36]. On the other hand, Nardini et al. (2018) found that the addition of sulfites (at 1–10 µg levels) to three samples of white wines resulted in a significant and positive interference in the Folin–Ciocalteu assay used for polyphenol detection [37]. Abramovič et al. (2015) found that the sulfur dioxide content influenced the content of TPC using Folin–Ciocalteu reagent; they noticed an increased content of TPC with an excess sulfur dioxide concentration [38]. In the same way, Ivanova et al. (2012) found a higher content of TPC, as determined by an FC reducing capacity, and an anthocyanin level in conventional wine that prevented phenolic oxidation at the highest SO_2 concentration [39]. In the majority of samples, SO2-treated wines showed higher concentrations of total polyphenols. The increase in sulfur dioxide correlated with the TPC content in the samples assayed.

Between the first and second measurement dates, we observed a 29% decline in TPC content. Subsequently, we registered a 22% increase in the TPC content between 25 January 2022 and 23 February 2022. In general, over the entire duration of the experiment, sample 1 showed the lowest TPC loss of 8%. The TPC loss in samples 2–8 reached 11–21%, with the most significant decrease in sample 5. The decrease in TPC between 26 October 2021 and 23 February 2022 can be considered as statistically significant in all assayed samples ($p < 0.05$) (Table 5).

Snopek (2019) reported a 6% decrease in TPC content in observed white wines during 6 months of storage [28]. In our research work, we similarly observed a 14% decline in TPC content during the first six months.

3.3. Impact of Sulfur Dioxide Addition on the Total Flavonoid Content (TFC) during Storage

The total flavonoid content in the samples during the storage time is given in Table 6.

Table 6. Total flavonoid content during storage.

Sample No	Date of Measurement				
	27 October 2021	**25 November 2021**	**3 January 2022**	**26 January 2022**	**23 September 2022**
	Total Flavonoids Content (mg·L^{-1}) ± Standard Deviation				
1	61.74 ± 0.49 ^{a,A}	54.38 ± 2.71 ^{b,A}	58.76 ± 0.68 ^{ab,A}	60.73 ± 1.89 ^{a,A}	61.19 ± 0.55 ^{a,A}
2	91.13 ± 1.81 ^{a,B}	84.59 ± 1.61 ^{b,B}	92.86 ± 0.03 ^{a,B}	93.05 ± 1.37 ^{a,B}	91.82 ± 2.11 ^{a,B}
3	86.70 ± 0.74 ^{a,BC}	81.20 ± 2.05 ^{a,BC}	87.38 ± 0.30 ^{a,C}	87.06 ± 3.48 ^{a,C}	87.27 ± 1.51 ^{a,BC}
4	83.91 ± 2.55 ^{a,CD}	80.02 ± 0.33 ^{a,BD}	85.74 ± 1.29 ^{a,C}	85.99 ± 1.37 ^{a,C}	86.51 ± 2.33 ^{a,C}
5	85.22 ± 0.08 ^{a,BD}	77.7 ± 0.25 ^{b,CD}	85.77 ± 1.70 ^{a,C}	76.38 ± 0.14 ^{b,D}	84.62 ± 2.13 ^{a,C}
6	88.8 ± 3.04 ^{a,B}	75.56 ± 0.47 ^{b,DE}	79.69 ± 0.44 ^{bc,D}	85.74 ± 2.65 ^{a,C}	83.63 ± 0.38 ^{ac,C}
7	81.77 ± 1.89 ^{a,D}	72.09 ± 0.33 ^{b,EF}	77.64 ± 1.56 ^{c,D}	83.52 ± 0.16 ^{a,CE}	83.47 ± 0.05 ^{a,C}
8	80.09 ± 1.11 ^{a,D}	68.50 ± 0.90 ^{b,F}	77.97 ± 0.14 ^{ac,D}	77.81 ± 0.19 ^{c,DE}	77.97 ± 0.30 ^{c,D}

Notes: values with different lowercase letters indicate statistically significant differences among the dates of determination, uppercase letters indicate significant differences among the samples (1–6) at the >0.05 level.

At the begging of the experiment, on 27 October 2021, the total flavonoid content in analyzed samples ranged from 61.74 to 91.13 mg·L^{-1}. According to the data published

by Mitić et al. (2010), the average concentration of flavonoids in wines originating from Serbia reached 55.08 mg·L^{-1}. Snopek (2019) observed 36.00 mg·L^{-1} TFC in Moravian wines [28,35]. Differences could be caused by different geographical conditions, different cultivations of grapevine or different technological procedures in wine production.

A decrease in flavonoid content by an average of 10% was observed between 27 October 2021 and 25 November 2021. In the following period, an increase or stagnation in flavonoid content was observed. Generally, during the total storage time (27 October 2021–23 February 2022), the content of flavonoids did not change significantly (p >0.05) (Table 6). At the end of experiment on 23 February 2022, the flavonoid content reached a concentration of 61.19–91.82 mg·L^{-1}.

Comparing the content of flavonoids and the content of sulfur dioxide in observed samples, it is evident that the lowest concentration of TFC was in sample 1 (without addition of sulfur dioxide) and the highest was in sample 2. In most samples, we observed that the increased content of sulfur dioxide was accompanied by a decline in TFC content. Thus, it can be concluded that the addition of sulfur dioxide influenced the flavonoid content in wines. It is very important to find the proper concentration of sulfur dioxide, as not adding it or adding a very high concentration could lead to a adverse decrease in TFC. From this point of view, sample 2 appears to be ideal; however, on the other hand, in sensory evaluations, it showed the traits of oxidation. This, in this way, samples 3 and 4 can be concluded as more ideal because they did not display the marks or traits of oxidation after sulfur dioxide addition.

With respect to TFC, the addition of sodium sulfite or sodium metabisulfite to the three examples of organic wines resulted in a negative interference. In all samples, the TFC of wines decreased with increasing additions of sodium sulfite [37]. Morena et al. (2018) detected a lower level of flavonoids and flavonols with an increasing SO_2 content [20]. Garaguso and Nardini (2015) compared organic red wine produced without SO_2 addition ($\leq$2 mg·L^{-1}) with wines from conventional practices and found comparable results for wines produced without SO_2 addition and those with 50 mg·L^{-1} of SO_2 added [15].

The results of Morena et al. (2018) demonstrated that conventional wine exhibited a significantly higher FC reducing capacity (p < 0.05 vs. LS0) and higher amount of anthocyanins (p < 0.001 vs. LS0 and LS50), while containing lower levels of flavonoids (p < 0.01 vs. LS0 and LS50) and flavonols (p < 0.05 vs. LS0 and LS50) compared to wines obtained without SO_2 addition or those with the lowest dose added [20].

3.4. Impact of Sulfur Dioxide Addition on Antioxidant Activity Determined by the DPPH Method

The importance of sulfur dioxide in increasing the total antioxidant capacity of wines has been demonstrated in an evaluation of the technological and potentially health-promoting properties of different products [40]. Morena et al. (2018) highlighted higher ORAC values in white wines with SO_2 addition both at low and high doses [20].

Testing of the antioxidant activity by the DPPH method was conducted in five intervals. The detected values of antioxidant activity in the samples during storage are shown in Table 7.

As can be seen from the table, the antioxidant activity at the beginning of the experiment (26 October 2021) ranged from 116.75 up to 270.62 mg·L^{-1}, and the antioxidant activity increased with the sulfur dioxide concentration.

A study by Abramovič et al. (2015) pointed to the fact that the content of sulfur dioxide in wine led to an increase in antioxidant activity, detected by the DPPH method [38]. After the storage period, a mean value of 214.06 mg·L^{-1} was determined in the samples. Lachman et al. (2007) detected a mean value 227 mg·L^{-1} in white wines originating from the territory of Czech Republic [41]. The results showed that sulfur dioxide plays a significant role in increasing the antioxidant activity of white wines, especially those with a low AA. Similar results were reported by Long et al., 2000, and Mitsuhashi et al., 2001 [42,43]. On the other hand, other studies have found that sulfur dioxide plays a minor role in the antioxidant capacity of wines [44,45].

Table 7. Antioxidant activity detected by the DPPH method.

Sample No	Date of Detection				
	26 October 2021	**24 November 2021**	**3 January 2022**	**25 January 2022**	**23 February 2022**
	Antioxidant Activity–Method DPPH (mg·L^{-1}) ± Standard Deviation				
1	116.75 ± 3.87 ^{a,A}	105.64 ± 4.79 ^{a,A}	88.63 ± 3.36 ^{b,A}	118.12 ± 7.89 ^{ac,A}	132.58 ± 0.22 ^{c,A}
2	183.77 ± 0.86 ^{a,B}	188.41 ± 8.98 ^{a,B}	137.8 ± 0.01 ^{b,B}	176,47 ± 8.11 ^{a,B}	175.07 ± 4.10 ^{a,B}
3	213.55 ± 5.32 ^{a,C}	198.03 ± 2.77 ^{a,B}	168.91 ± 11.39 ^{b,C}	210.14 ± 0.15 ^{a,C}	202.00 ± 0.48 ^{a,C}
4	230.32 ± 5.53 ^{a,D}	219.35 ± 1.36 ^{a,C}	187.48 ± 4.07 ^{b,CD}	227.14 ± 5.29 ^{a,CD}	221.5 ± 3.81 ^{a,D}
5	245.19 ± 4.07 ^{a,DE}	237.82 ± 6.52 ^{a,C}	206.43 ± 2.23 ^{b,DEF}	245.07 ± 6.95 ^{a,DE}	244.06 ± 0.63 ^{a,E}
6	259.16 ± 7.90 ^{a,EF}	258.56 ± 10.61 ^{a,D}	218.25 ± 3.24 ^{b,EF}	249.74 ± 3.59 ^{a,E}	261.87 ± 3.62 ^{a,F}
7	268.33 ± 1.23 ^{a,F}	265.96 ± 1.32 ^{a,D}	222.91 ± 12.32 ^{b,FG}	269.78 ± 1.90 ^{a,F}	280.78 ± 6.02 ^{a,G}
8	270.62 ± 2.80 ^{a,F}	267.02 ± 3.98 ^{a,D}	241.85 ± 0.03 ^{b,G}	269.7 ± 3.13 ^{ab,F}	277.53 ± 1.86 ^{c,G}

Notes: Values with different lowercase indexes indicate statistically significant differences among the dates of determination, uppercase letters indicate significant differences among the samples (1–6) at the >0.05 level.

The addition of sulfites to organic white wines (at 25–200 mg·L^{-1} wine) clearly resulted in a significant overestimation of the antioxidant activity and polyphenol content [37].

According to Mattia et al. (2015), the contribution of added SO_2 to the overall antioxidant activity of wines was higher than that of naturally occurring antioxidants [40].

In the period between 26 October 2021 and 3 January 2022, we noticed 19% decline in the mean value of antioxidant activity. The most significant loss was registered within the samples with lower sulfur dioxide values. The largest was observed in sample 1 with 26%, followed by sample 2 with 24%. On the other hand, the lowest loss of antioxidant activity was measured in sample 8, with a 10% decrease. Consequently, we noticed an increase in antioxidant activity of 24% between 3 January 2022 and 23 February 2022. The most significant increase was observed in sample 1, at 45%. We registered that with the increase in sulfur dioxide concentration in samples, the antioxidant activity values declined in sequence, except for sample 7.

Considering the period between 26 October 2021 and 23 February 2022, the antioxidant activity in samples 2–5 decreased by 0.5–5%. In contrast to this trend, in samples 6–8, antioxidant activities rose by 1–5%. The mentioned changes in AA between 26 October 2021 and 23 February 2022 (except for sample 8) cannot be evaluated as statistically significant ($p > 0.05$), as given in Table 7. The statistically significant change ($p < 0.05$) we registered between 26 October 2021 and 23 February 2022 was in sample 1, with an increase of 14%.

3.5. Impact of Sulfur Dioxide Addition on Antioxidant Activity Determined by the ABTS Method

The analysis of AA during storage was conducted at five intervals, as shown in Table 8.

Table 8. Antioxidant activity detected by the ABTS method.

Sample No	Date of Detection				
	26 October 2021	**24 November 2021**	**4 January 2022**	**26 January 2022**	**23 February 2022**
	Antioxidant Activity–Method ABTS (mg·L^{-1}) ± Standard Deviation				
1	117.74 ± 0.09 ^{a,A}	104.18 ± 2.31 ^{a,A}	93.51 ± 2.59 ^{b,A}	99.81 ± 2.31 ^{b,A}	115.18 ± 9.23 ^{ab,A}
2	203.78 ± 0.09 ^{a,B}	147.57 ± 10.46 ^{b,B}	145.64 ± 1.50 ^{bc,B}	130.94 ± 1.32 ^{c,B}	184.50 ± 3.30 ^{d,B}
3	268.85 ± 2.26 ^{a,C}	181.72 ± 10.86 ^{b,C}	193.56 ± 1.68 ^{c,C}	174.58 ± 0.47 ^{b,C}	195.75 ± 1.60 ^{c,B}
4	330.91 ± 0.16 ^{a,D}	206.34 ± 15.60 ^{b,C}	241.89 ± 2.59 ^{c,D}	206.37 ± 0.41 ^{b,D}	258.13 ± 5.51 ^{d,C}
5	380.58 ± 12.07 ^{aE}	263.6 ± 2.43 ^{b,D}	294.20 ± 1.08 ^{c,E}	258.73 ± 1.62 ^{b,E}	309.7 ± 2.98 ^{c,D}
6	422.25 ± 0.56 ^{a,F}	268.02 ± 2.22 ^{b,D}	334.42 ± 9.56 ^{c,F}	281.61 ± 0.69 ^{b,F}	307.01 ± 1.25 ^{d,D}
7	466.78 ± 1.07 ^{a,G}	294.11 ± 2.89 ^{b,DE}	376.87 ± 7.09 ^{c,G}	334.71 ± 5.44 ^{d,G}	349,57 ± 0.99 ^{e,E}
8	482.3 ± 0.66 ^{a,H}	309.57 ± 10.78 ^{b,E}	393.79 ± 1.83 ^{c,H}	325.88 ± 6.52 ^{b,G}	391.91 ± 4.81 ^{c,F}

Notes: values with different lowercase letters indicate statistically significant differences among the dates of determination, uppercase letters indicate significant differences among the samples (1–6) at the >0.05 level.

The antioxidant activity measured by the ABTS method was highly variable and showed repeated decreases and increases.

On the 26 October 2021, we registered an AA of sample 1 of 111,774 mg·L$^{-1,}$ with the increased values of sulfur dioxide increasing this value to 482.30 mg·L^{-1} in sample 8. Between 26 October 2021 and 24 November 2021, we noticed a mean decline in the AA in all samples of 31%. Subsequently, between 24 November 2021 and 4 January 2021, we detected an increase in the AA of samples 2–8 of 19%. On the other hand, a decline in the AA was determined in sample 1 by 10% and in sample 2 by 1%. Between 4 January 2022 and 26 January 2022, we measured a loss in AA in samples 2–8 by on average 11%, except for sample 1, which exhibited a 7% increase. Between 26 January 2022 and 23 February 2022, we detected an increase in the AA by on average 18% in all assayed samples.

In general, with the exception of mentioned variations, the AA of all observed samples declined on average by 19% during storage.

The detected reduction in AA between 26 October 2021 and 23 February 2022 (except for sample 1) can be considered as statistically significant (Table 8). The lowest decreases were observed in sample 1 (2%) and sample 2 (9%), which correlated with content of free sulfur dioxide. In the case of sample 2, there was a decrease at the end of storage and no addition of sulfur dioxide was made.

On the detection date of 23 February 2022, the AA ranged from 115.18 up to 391.91 mg·L^{-1}. The average AA of samples during the storage time was 261.16 mg·L^{-1}.

According to Floegel et al. (2011), the ABTS method displayed higher correlations with the TPC in comparison with the DPPH method [46]. This trend was also confirmed in our research work. A decreased level of TPC was correlated with the AA detected by the ABTS method.

In comparison with the results of a study by Fernández-Pachón et al. (2004), the mean value of Spanish white wine was 115.87 mg·L^{-1}, which is in agreement with the value found in sample 1 [47].

Snopek (2019) noticed an average decline in the AA determined by the ABTS method of 10.15% during 6 months of storage, which shows the same trend of AA decline as found in our research work [28].

In comparison with the AA detected by the DPPH method, a different trend was observed. Samples 2–7 showed approximately the same values after 4 months of storage compared to the values at the beginning of the experiment; in addition, a slight increase was observed in samples 1 and 8.

A comparison of the AA determined by the ABTS and DPPH methods was conducted by Floegel et al. (2011) [46]. Their results pointed to the fact that the ABTS method was more suitable for the determination of the AA of beverages, including wine, in comparison with the DPPH method.

3.6. The Detection of Phenolic Compounds in Relation to Sulfur Dioxide Addition

During white wine storage, we noticed a total of 18 phenolic compounds.

From the group of phenolic acids, we detected the following as the most abundant: gallic acid, protocatequie acid, naochlorogenic, 4-hydroxybenzoic acid, vanilic acid, chlorogenic acid, coffeic acid, syringic acid, p-coumaric acid, ferulic, sinapic and ethylesters of protocatequie acid.

From the group of flavonoids, we detected epigallocatechin, catechin, epicatechin, quercetin, and kaempferol and from stilbenes, we detected resveratrol.

During storage, we observed a highly variable content of phenolic compounds. The most abundant compounds were chlorogenic acid, caffeic acid and epigalocatechin.

The contents of individual polyphenols in assayed samples during storage are given in Table 9.

Table 9. Content of individual phenolic compounds.

	Gallic Acid					Protocatechic Acid					Neochlorogenic Acid				
Date of Measurement	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**
Sample No	**The Content of Individual Phenolic Compounds (μg·mL^{-1}) ± Standard Deviation**														
1	0.03 ± 0.01	0.12 ± 0.02	N.D.	N.D.	N.D.	0.34 ± 0.08	0.10 ± 0.03	0.10 ± 0.02	0.34 ± 0.02	0.70 ± 0.09	0.90 ± 0.29	0.41 ± 0.03	0.02 ± 0.00	0.04 ± 0.01	0.22 ± 0.06
2	0.01 ± 0.00	0.04 ± 0.03	N.D.	1.24 ± 0.01	N.D.	0.14 ± 0.05	0.04 ± 0.03	0.09 ± 0.03	0.26 ± 0.04	0.64 ± 0.09	1.24 ± 0.08	0.26 ± 0.22	0.03 ± 0.02	0.04 ± 0.00	0.24 ± 0.05
3	N.D.	0.02 ± 0.01	0.01 ± 0.00	1.37 ± 0.02	N.D.	0.17 ± 0.03	0.02 ± 0.00	0.11 ± 0.01	0.21 ± 0.02	0.55 ± 0.11	0.97 ± 0.22	0.28 ± 0.13	0.03 ± 0.01	0.04 ± 0.01	0.22 ± 0.06
4	0.01 ± 0.01	0.05 ± 0.03	0.01 ± 0.00	1.27 ± 0.03	N.D.	0.13 ± 0.04	0.01 ± 0.01	0.08 ± 0.02	0.26 ± 0.12	0.63 ± 0.13	0.97 ± 0.19	0.22 ± 0.15	0.03 ± 0.01	0.04 ± 0.01	0.18 ± 0.04
5	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	1.27 ± 0.02	N.D.	0.09 ± 0.08	0.01 ± 0.00	0.03 ± 0.02	0.21 ± 0.03	0.89 ± 0.21	0.79 ± 0.35	0.14 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.27 ± 0.03
6	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	1.22 ± 0.05	N.D.	0.13 ± 0.05	0.03 ± 0.02	0.04 ± 0.03	0.20 ± 0.03	0.98 ± 0.10	1.03 ± 0.38	0.13 ± 0.07	0.05 ± 0.02	0.01 ± 0.00	0.25 ± 0.01
7	0.05 ± 0.05	0.03 ± 0.01	0.02 ± 0.00	1.13 ± 0.02	N.D.	0.15 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.21 ± 0.05	0.97 ± 0.06	1.10 ± 0.31	0.16 ± 0.05	0.04 ± 0.01	0.03 ± 0.02	0.30 ± 0.04
8	0.01 ± 0.00	0.07 ± 0.02	0.01 ± 0.00	1.03 ± 0.04	N.D.	0.10 ± 0.04	0.10 ± 0.03	0.05 ± 0.01	0.16 ± 0.03	0.99 ± 0.17	0.85 ± 0.28	0.23 ± 0.09	0.03 ± 0.01	0.06 ± 0.02	0.24 ± 0.04
Phenolic compounds	4-hydroxybenzoic acid					epigallocatechin					catechin				
1	0.52 ± 0.11	0.08 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	N.D.	0.84 ± 0.33	0.98 ± 0.22	0.08 ± 0.02	1.29 ± 0.04	0.12 ± 0.04	N.D.	N.D.	0.15 ± 0.04	N.D.	0.09 ± 0.01
2	1.00 ± 0.03	0.04 ± 0.03	0.03 ± 0.02	0.05 ± 0.01	N.D.	1.42 ± 0.14	0.50 ± 0.35	0.18 ± 0.12	1.29 ± 0.03	0.10 ± 0.06	N.D.	N.D.	0.17 ± 0.07	N.D.	0.08 ± 0.01
3	0.86 ± 0.13	0.02 ± 0.01	0.05 ± 0.00	0.04 ± 0.01	N.D.	0.19 ± 0.09	0.45 ± 0.17	0.44 ± 0.03	1.14 ± 0.03	0.47 ± 0.18	N.D.	N.D.	0.19 ± 0.02	N.D.	0.07 ± 0.01
4	0.52 ± 0.09	0.02 ± 0.01	0.04 ± 0.01	0.05 ± 0.00	N.D.	0.42 ± 0.25	0.31 ± 0.15	0.37 ± 0.05	1.20 ± 0.06	0.44 ± 0.02	N.D.	N.D.	0.21 ± 0.05	N.D.	0.06 ± 0.01
5	0.23 ± 0.09	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	N.D.	0.76 ± 0.33	0.12 ± 0.06	0.21 ± 0.11	1.24 ± 0.06	0.24 ± 0.07	N.D.	N.D.	0.13 ± 0.04	N.D.	0.06 ± 0.03
6	0.06 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	N.D.	1.33 ± 2.02	0.01 ± 0.01	0.27 ± 0.13	1.27 ± 0.06	0.11 ± 0.05	N.D.	N.D.	0.17 ± 0.11	N.D.	0.05 ± 0.01
7	0.10 ± 0.03	0.02 ± 0.01	0.04 ± 0.01	N.D.	N.D.	0.01 ± 0.01	0.46 ± 0.25	0.39 ± 0.03	1.55 ± 0.21	0.05 ± 0.01	N.D.	N.D.	0.26 ± 0.02	N.D.	0.03 ± 0.01
8	0.10 ± 0.05	0.03 ± 0.01	0.03 ± 0.01	N.D.	N.D.	3.82 ± 4.31	1.06 ± 0.27	0.31 ± 0.11	1.74 ± 0.08	0.05 ± 0.01	N.D.	N.D.	0.25 ± 0.10	N.D.	0.01 ± 0.01
Phenolic compound	Vanilic acid					Chlorogenic acid					Caffeic acid				
Date of mesurement	31 October 2021	26 November 2021	5 January 2022	26 January 2022	1 March 2022	31 October 2021	26 November 2021	5 January 2022	26 January 2022	1 March 2022	31 October 2021	26 November 2021	5 January 2022	26 January 2022	1 March 2022
Sample No	The content of individual phenolic compounds (μg·mL^{-1}) ± standard deviation														
1	0.95 ± 0.31	0.31 ± 0.06	0.04 ± 0.01	0.03 ± 0.01	0.08 ± 0.02	2.58 ± 1.06	0.93 ± 0.03	0.39 ± 0.08	0.29 ± 0.04	0.46 ± 0.06	2.21 ± 0.75	8.41 ± 4.26	0.15 ± 0.03	0.11 ± 0.01	N.D.
2	1.19 ± 0.12	0.23 ± 0.18	0.05 ± 0.01	0.19 ± 0.02	0.11 ± 0.03	3.64 ± 0.31	0.64 ± 0.51	0.38 ± 0.03	0.21 ± 0.01	0.43 ± 0.05	2.52 ± 0.31	0.87 ± 0.73	0.12 ± 0.02	0.08 ± 0.01	N.D.
3	1.02 ± 0.25	0.24 ± 0.11	0.07 ± 0.01	0.15 ± 0.01	0.08 ± 0.01	2.70 ± 0.85	0.53 ± 0.26	0.45 ± 0.04	0.24 ± 0.02	0.45 ± 0.03	2.36 ± 0.61	0.67 ± 0.29	0.12 ± 0.00	0.04 ± 0.02	N.D.

Table 9. *Cont.*

	Gallic Acid					Protocatechic Acid					Neochlorogenic Acid				
Date of Measurement	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**
Sample No	**The Content of Individual Phenolic Compounds (μg·mL^{-1}) ± Standard Deviation**														
4	1.00 ± 0.23	0.21 ± 0.13	0.05 ± 0.01	0.16 ± 0.04	0.11 ± 0.02	2.87 ± 0.62	0.44 ± 0.31	0.34 ± 0.06	0.19 ± 0.04	0.52 ± 0.05	2.03 ± 0.47	0.52 ± 0.35	0.10 ± 0.02	N.D.	N.D.
5	0.83 ± 0.37	0.14 ± 0.06	0.04 ± 0.01	0.12 ± 0.03	0.10 ± 0.01	2.99 ± 1.38	0.33 ± 0.12	0.20 ± 0.06	0.21 ± 0.03	0.39 ± 0.04	1.42 ± 0.67	0.35 ± 0.15	0.07 ± 0.01	N.D.	N.D.
6	0.94 ± 0.34	0.15 ± 0.07	0.03 ± 0.01	0.14 ± 0.02	0.07 ± 0.01	2.87 ± 1.21	0.32 ± 0.15	0.11 ± 0.02	0.25 ± 0.03	0.43 ± 0.03	1.67 ± 0.71	0.37 ± 0.15	0.06 ± 0.02	N.D.	N.D.
7	1.10 ± 0.30	0.19 ± 0.07	0.04 ± 0.01	0.18 ± 0.05	0.08 ± 0.02	3.14 ± 0.70	0.40 ± 0.09	0.15 ± 0.02	0.40 ± 0.07	0.50 ± 0.03	1.17 ± 0.40	0.49 ± 0.11	0.03 ± 0.00	N.D.	N.D.
8	0,.2 ± 0.26	0.26 ± 0.10	0.03 ± 0.01	0.13 ± 0.02	0.07 ± 0.01	2.98 ± 1.01	0.65 ± 0.20	0.13 ± 0.02	0.69 ± 0.17	0.42 ± 0.09	0.57 ± 0.24	0.56 ± 0.28	0.03 ± 0.01	N.D.	N.D.
Phenolic compounds	Syringic acid					Epicatechin					trans-p-coumaric acid				
1	0.11 ± 0.06	0.13 ± 0.03	N.D.	0.08 ± 0.02	0.15 ± 0.01	2.46 ± 0.87	0.69 ± 0.02	0.12 ± 0.02	0.15 ± 0.02	0.04 ± 0.01	0.25 ± 0.10	0.14 ± 0.01	0.01 ± 0.00	0.21 ± 0.01	0.06 ± 0.02
2	0.18 ± 0.01	0.07 ± 0.06	0.08 ± 0.02	0.12 ± 0.01	0.19 ± 0.02	2.63 ± 0.36	0.48 ± 0.39	0.11 ± 0.05	0.12 ± 0.01	0.04 ± 0.01	0.39 ± 0.03	0.10 ± 0.08	0.02 ± 0.00	0.17 ± 0.01	0.04 ± 0.01
3	0.15 ± 0.05	0.07 ± 0.03	0.13 ± 0.01	0.12 ± 0.01	0.21 ± 0.01	1.79 ± 0.45	0.49 ± 0.24	0.10 ± 0.01	0.12 ± 0.01	0.04 ± 0.00	0.39 ± 0.10	0.10 ± 0.04	0.02 ± 0.00	0.15 ± 0.01	0.15 ± 0.02
4	0.19 ± 0.06	0.07 ± 0.05	0.11 ± 0.02	0.13 ± 0.03	0.16 ± 0.02	1.97 ± 0.98	0.40 ± 0.26	0.08 ± 0.02	0.12 ± 0.01	0.03 ± 0.00	0.41 ± 0.10	0.10 ± 0.06	0.02 ± 0.00	0.15 ± 0.01	0.13 ± 0.03
5	N.D.	0.05 ± 0.02	0.06 ± 0.02	0.19 ± 0.04	0.21 ± 0.02	1.13 ± 0.66	0.26 ± 0.11	0.06 ± 0.02	0.17 ± 0.09	0.03 ± 0.01	0.34 ± 0.15	0.08 ± 0.03	0.01 ± 0.00	0.16 ± 0.01	0.25 ± 0.04
6	N.D.	0.05 ± 0.02	0.07 ± 0.05	0.16 ± 0.03	0.19 ± 0.02	0.95 ± 0.76	0.18 ± 0.07	0.05 ± 0.03	0.17 ± 0.06	0.04 ± 0.01	0.48 ± 0.22	0.07 ± 0.03	0.01 ± 0.01	0.19 ± 0.02	0.25 ± 0.01
7	N.D.	0.06 ± 0.02	0.14 ± 0.05	0.14 ± 0.02	0.16 ± 0.04	0.28 ± 0.13	0.30 ± 0.08	0.08 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.48 ± 0.11	0.10 ± 0.03	0.02 ± 0.00	0.19 ± 0.02	0.28 ± 0.04
8	0.16 ± 0.06	0.08 ± 0.04	0.13 ± 0.05	0.17 ± 0.04	0.12 ± 0.04	0.33 ± 0.11	0.18 ± 0.19	0.08 ± 0.02	0.13 ± 0.01	0.06 ± 0.01	0.55 ± 0.20	0.13 ± 0.04	0.01 ± 0.00	0.19 ± 0.01	0.29 ± 0.02
Phenolic compounds	Ferrulic acid					Sinapic acid					Elagic acid				
Datas of measurement	31 October 2021	26 November 2021	5 January 2022	26 January 2022	1 March 2022	31 October 2021	26 November 2021	5 January 2022	26 January 2022	1 March 2022	31 October 2021	26 November 2021	5 January 2022	26 January 2022	1 March 2022
Sample No	The content of individual phenolic compounds (μg·mL^{-1}) ± standard deviation														
1	0.08 ± 0.03	0.02 ± 0.00	N.D.	N.D.	0.15 ± 0.01	0.13 ± 0.06	0.05 ± 0.02	0.02 ± 0.01	N.D.	0.04 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.
2	0.12 ± 0.01	0.02 ± 0.02	N.D.	N.D.	0.15 ± 0.02	0.15 ± 0.05	0.01 ± 0.01	N.D.	N.D.	0.02 ± 0.01	N.D.	N.D.	N.D.	N.D.	N.D.
3	0.10 ± 0.03	0.02 ± 0.01	N.D.	N.D.	0.10 ± 0.02	0.05 ± 0.01	00,2 ± 0.01	N.D.	N.D.	0.14 ± 0.01	N.D.	N.D.	N.D.	N.D.	N.D.
4	0.10 ± 0.02	0.02 ± 0.01	N.D.	N.D.	0.04 ± 0.01	0.07 ± 0.02	0.01 ± 0.01	N.D.	N.D.	0.21 ± 0.09	N.D.	N.D.	N.D.	N.D.	N.D.
5	0.07 ± 0.04	0.01 ± 0.00	N.D.	N.D.	0.02 ± 0.00	0.06 ± 0.03	0.01 ± 0.01	N.D.	N.D.	0.31 ± 0.03	N.D.	N.D.	N.D.	N.D.	N.D.
6	0.09 ± 0.03	0.01 ± 0.01	N.D.	N.D.	0.01 ± 0.02	0.08 ± 0.03	0.01 ± 0.00	N.D.	N.D.	0.18 ± 0.11	N.D.	N.D.	N.D.	N.D.	N.D.

Table 9. *Cont.*

	Gallic Acid					Protocatechic Acid					Neochlorogenic Acid				
Date of Measurement	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**
Sample No	**The Content of Individual Phenolic Compounds (μg·mL^{-1}) ± Standard Deviation**														
7	0.09 ± 0.01	0.02 ± 0.00	N.D.	N.D.	N.D.	0.09 ± 0.02	0.02 ± 0.00	N.D.	N.D.	0.17 ± 0.03	N.D.	N.D.	N.D.	N.D.	N.D.
8	0.09 ± 0.03	0.03 ± 0.01	N.D.	N.D.	N.D.	0.08 ± 0.03	0.02 ± 0.01	N.D.	N.D.	0.15 ± 0.03	N.D.	N.D.	N.D.	N.D.	N.D.
Phenolic compound	Rutin					Hydroxycinnamic acid					Ethylester of protocatequie acid				
1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.20 ± 0.06	0.04 ± 0.00	N.D.	N.D.	N.D.
2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.60 ± 0.04	0.06 ± 0.06	N.D.	N.D.	N.D.
3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.48 ± 0.14	0.03 ± 0.02	N.D.	N.D.	N.D.
4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.46 ± 0.11	0.01 ± 0.01	N.D.	N.D.	N.D.
5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.37 ± 0.13	0.01 ± 0.00	N.D.	N.D.	N.D.
6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.44 ± 0.15	0.01 ± 0.00	N.D.	N.D.	N.D.
7	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.41 ± 0.10	N.D.	N.D.	N.D.	N.D.
8	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.34 ± 0.11	0.01 ± 0.00	N.D.	N.D.	N.D.
Phenolic compounds	Resveratrol					trans-cinnamic acid					Kaempferol				
Data of measurement	31 October 2021	26 November 2021	5 January 2022	26 January 2022	01 March 2022	31 October 2021	26 November 2021	5 January 2022	26 January 2022	1 March 2022	31 October 2021	26 November 2021	5. January 2022	26 January 2022	1 March 2022
Sample No	The content of individual phenolic compounds (μg·mL^{-1}) ± standard deviation														
1	0.03 ± 0.01	0.03 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.18 ± 0.07	0.08 ± 0.01	N.D.	N.D.	N.D.
2	0.13 ± 0.02	0.03 ± 0.03	0.04 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.22 ± 0.02	0.05 ± 0.04	N.D.	N.D.	N.D.
3	0.13 ± 0.02	0.03 ± 0.01	0.06 ± 0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.23 ± 0.05	0.05 ± 0.02	N.D.	N.D.	N.D.
4	0.14 ± 0.01	0.03 ± 0.02	0.05 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.23 ± 0.06	0.05 ± 0.03	N.D.	N.D.	N.D.
5	0.12 ± 0.03	0.02 ± 0.01	0.03 ± 0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.21 ± 0.08	0.04 ± 0.02	N.D.	N.D.	N.D.
6	0.11 ± 0.06	0.02 ± 0.01	0.05 ± 0.04	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.23 ± 0.08	0.04 ± 0.01	N.D.	N.D.	N.D.
7	0.17 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.25 ± 0.07	0.05 ± 001	N.D.	N.D.	N.D.
8	0.12 ± 0.05	0.03 ± 0.01	0.04 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.22 ± 0.07	0.07 ± 0.02	N.D.	N.D.	N.D.

Table 9. *Cont.*

	Gallic Acid					Protocatechic Acid					Neochlorogenic Acid				
Date of Measurement	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**	**31 October 2021**	**26 November 2021**	**5 January 2022**	**26 January 2022**	**1 March 2022**
Sample No	**The Content of Individual Phenolic Compounds (μg·mL^{-1}) ± Standard Deviation**														
Phenolic compounds	Quercetin														
1	0.59 ± 0.17	0.07 ± 0.01	N.D.	N.D.	N.D.										
2	0.76 ± 0.07	0.04 ± 0.04	N.D.	N.D.	N.D.										
3	0.61 ± 0.16	0.02 ± 0.01	N.D.	N.D.	N.D.										
4	0.34 ± 0.18	0.02 ± 0.02	N.D.	N.D.	N.D.										
5	0.03 ± 0.02	0.02 ± 0.01	N.D.	N.D.	N.D.										
6	0.04 ± 0.02	0.01 ± 0.01	N.D.	N.D.	N.D.										
7	0.03 ± 0.01	0.01 ± 0.01	N.D.	N.D.	N.D.										
8	0.03 ± 0.01	N.D.	N.D.	N.D.	N.D.										

N.D.: Not detected.

Garaguso et al. (2015) suggested that the phenolic profile of conventional wines with sulfur dioxide addition and organic wines without SO_2 addition did not differ quantitively, except for caffeic acid, rutin, resveratrol and quercetin, which exhibited a higher content in organic wines [15]. In our samples, rutin was not detected, but the same trend can be seen in the caffeic acid and quercetin contents, except for samples 2 and 3 detected on 31 October 2021. In the case of quercetin, we found a decrease in the quercetin content in samples 2 and 3, then a rapid decline is evident, which is in accordance with the observations of Morena et al. (2018) [20]. Moreno et al. (2018) noticed an excess in gallic acid (from 1.42 up to 1.98 $\mu g \cdot g^{-1}$) and in resveratrol (0.13 to 0.25 $\mu g \cdot g^{-1}$) concentrations with higher amounts of sulfur dioxide, but in our work, this trend was not evident; concentrations are capped [20]. Moreover, we noticed a decline in caffeic acid concentration with the gradual addition of SO_2, but the mentioned authors observed the opposite trend, with the concentration increasing from 0.84 up to 1.86 $\mu g \cdot g^{-1}$.

Nardini et al. (2018) reported that the gallic acid concentration increased with increasing levels of sulfite addition; at the sulfite level of 10 μg, they observed an increase in GA of 31% compared to the control without sulfite addition [37]. On the other hand, catechins were negatively affected by sulfite addition at the maximum level of 20 μg; they noticed a decrease of 84% compared to the control sample level.

According to Castellari et al. (1998) [36], sulfur dioxide reduced the resveratrol concentration in red wines, but this is not consistent with our study.

An analogous trend was observed for trans-resveratrol, an important antioxidant compound in red wine, and for values reported in other papers [15,20,48]. The increasing concentration of bioactive compounds determined in this work at higher levels of sulfur dioxide is statistically significant and is in agreement with previous works [36].

4. Conclusions

Our research work was aimed at the determination of antioxidant activity, the total phenolic and flavonoid content and individual phenolic compounds in relation to the addition of sulfur dioxide at seven different concentrations.

Moreover, approximately every month, we conducted analyses of assayed parameters to determine changes during storage time. The assayed concentrations of sulfur dioxide show a decreasing tendency with time, with a final decrease of more than 50% in comparison with the starting data. This observation confirms the fact that the samples were exposed to oxidative processes. The changes in SO_2 levels cannot be considered statistically significant. We observed a decreasing trend in the TPC content during storage. Simultaneously, we also observed an increase in TPC with an increasing concentration of sulfur dioxide, mostly contributed to AA. The AA detected by the ABTS method displayed a decreasing tendency during storage, while in the case of the DPPH method, we did not observe significant changes during 4 months of storage for most of the samples assayed. In the case of the TFC content, we noticed a significant influence of sulfur dioxide on the concentration. No addition or addition of high concentrations of SO_2 negatively influenced the flavonoid content of the samples.

Sulfur dioxide represents an essential preservative in the process of wine making, despite the attempts to integrate new technologies or natural products as alternative possibilities. The greatest challenge is to determine the appropriate amount of sulfur dioxide that can preserve and improve the quality of the wine. An adequate concentration of sulfur dioxide is necessary for wine stability and the preservation of wine's aroma. On the other hand, the negative impact of sulfides on human health must also be noted. Thus, adding the right amount of sulfur dioxide can improve the quality of beverages.

Author Contributions: Conceptualization, O.T.; formal analysis, O.T. and R.B.; investigation, T.J.; methodology, J.M.; supervision, J.M. and S.E.; writing—original draft, T.J. and O.T.; writing—review and editing, J.M. and L.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the internal grant of Tomas Bata University in Zlin (No. IGA/FT/2023/003).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author (T.J.).

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

References

1. Fernandes, R.; Pérez-Gregorio, S.; Soares, N.; De Freitas, V. Wine flavonoids in health and disease prevention. *Molecules* **2017**, *22*, 292. [CrossRef] [PubMed]
2. Ferraz da Costa, D.C.; Rangel, L.P.; Quarti, J.; Santos, R.A.; Silva, J.L.; Fialho, E. Bioactive Compounds and Metabolites from Grapes and Red Wine in Breast Cancer Chemoprevention and Therapy. *Molecules* **2020**, *25*, 3531. [CrossRef] [PubMed]
3. Escarpa, A.; Gonzalez, M.C. An Overview of Analytical Chemistry of Phenolic Compounds in Foods. *Crit. Rev. Anal. Chem.* **2001**, *31*, 57–139. [CrossRef]
4. Rosa, L.A.; Alvarez-Parrilla, E.; González-Aguilar, G. *Fruit and Vegetable Phytochemicals: Chemistry, Nutritional Value and Stability*; Wiley-Blackwell: Ames, IA, USA, 2010.
5. Radeka, S.; Rossi, S.; Bestulić, E.; Budić-Leto, I.; Kovačević Ganić, K.; Horvat, I.; Lukić, I.; Orbanić, F.; Zaninović Jurjević, T.; Dvornik, Š. Bioactive Compounds and Antioxidant Activity of Red and White Wines Produced from Autochthonous Croatian Varieties: Effect of Moderate Consumption on Human Health. *Foods* **2022**, *11*, 1804. [CrossRef]
6. German, J.B.; Walzen, R.L. The health benefits of wine. *Annu. Rev. Nutr.* **2022**, *20*, 561–593. [CrossRef]
7. Castaldo, L.; Narváez, A.; Izzo, L.; Graziani, G.; Gaspari, A.; Minno, G.D.; Ritieni, A. Red Wine Consumption and Cardiovascular Health. *Molecules* **2019**, *24*, 3626. [CrossRef]
8. Snopek, L.; Mlcek, J.; Sochorova, L.; Baron, M.; Hlavacova, I.; Jurikova, T.; Kizek, R.; Sedlackova, E.; Sochor, J. Contribution of Red Wine Consumption to Human Health Protection. *Molecules* **2018**, *23*, 1684. [CrossRef]
9. Amor, S.; Châlons, P.; Aires, V.; Delmas, D. Polyphenol Extracts from Red Wine and Grapevine: Potential Effects on Cancers. *Diseases* **2018**, *6*, 106. [CrossRef]
10. Cehula, M.; Baron, M.; Jurikova, T.; Alumbro, A.; Perrocha, M.; Ondrasek, I.; Mlcek, J.; Adamkova, A.; Sochor, J. The Study of Selected Components of Grape and Fruit Wines. *Potravin. Slovak J. Food Sci.* **2020**, *14*, 759–766. [CrossRef]
11. Grogan, K.A. The Value of Added Sulfur Dioxide in French Organic Wine. *Agric. Food Econ.* **2015**, *3*, 19. [CrossRef]
12. Monro, T.M.; Moore, R.L.; Nguyen, M.C.; Ebendorff-Heidepriem, H.; Skouroumounis, G.K.; Elsey, G.M.; Taylor, D.K. Sensing Free Sulfur Dioxide in Wine. *Sensors* **2012**, *12*, 10759–10773. [CrossRef] [PubMed]
13. Raposo, R.; Ruiz-Moreno, M.J.; Garde-Cerdán, T.; Puertas, B.; Moreno-Rojas, J.M.; Gonzalo Diago, A.; Guerrero, R.F.; Ortiz, V.; Cantos-Villar, E. Effect of hydroxytyrosol on quality of sulfur dioxide-free red wine. *Food Chem.* **2016**, *192*, 25–33. [CrossRef] [PubMed]
14. Ivanova, V.; Dörnye, A.; Mark, L.; Vojnosk, B. Polyphenolic content of Vranec wines produced by different vinification conditions. *Food Chem.* **2011**, *124*, 316–325. [CrossRef]
15. Garaguso, I.; Nardini, M. Polyphenols content, phenolics profile and antioxidant activity of organic red wines produced without sulfur dioxide/sulfites addition in comparison to conventional red wines. *Food Chem.* **2015**, *179*, 336–342. [CrossRef] [PubMed]
16. Raposo, R.; Ruiz-Moreno, M.J.; Garde-Cerdán, T.; Puertas, B.; Moreno-Rojas, J.M.; Gonzalo Diago, A.; Guerrero, R.F.; Ortiz, V.; Cantos-Villar, E. Grapevine-shoot stilbene extract as a preservative in red wine. *Food Chem.* **2016**, *197 Pt B*, 1102–1111. [CrossRef]
17. Vally, H.; Misso, N.L.; Madan, V. Clinical effects of sulphite additives. *Clin. Exp. Allergy* **2009**, *39*, 1643–1651. [CrossRef] [PubMed]
18. Peynaud, E. *Knowing and Making Wine*; John Wiley & Sons: New York, NY, USA, 1984; pp. 135–136, 267–269.
19. Ruiz-Moreno, M.J.; Raposo, R.; Moreno-Rojas, J.M.; Zafrilla, P.; Cayuela, J.M.; Mulero, J.; Puertas, B.; Guerrero, R.F.; Piñeiro, Z.; Giron, F.; et al. Efficacy of olive oil mill extract in replacing sulfur dioxide in wine model. *LWT-Food Sci. Technol.* **2015**, *61*, 117–123. [CrossRef]
20. Morena, G.; Gerardi, C.; Lucejko, J.J.; Longo, V.; Pucci, L.; Domenici, V. Effects of low sulfur dioxide concentrations on bioactive compounds and antioxidant properties of Aglianico red wine. *Food Chem.* **2018**, *245*, 1105–1112.
21. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [CrossRef]
22. Sumczynski, D.; Bubelová, Z.; Sneyd, J.; Erb-Weber, S.; Mlcek, J. Total phenolics, flavonoids, antioxidant activity, crude fibre and digestibility in non-traditional wheat flakes and muesli. *Food Chem.* **2015**, *174*, 319–325. [CrossRef]
23. Li, H.; Wang, X.; Li, Y.; Li, P.; Wang, H. Polyphenolic compounds and antioxidant properties of selected China wines. *Food Chem.* **2009**, *112*, 454–460. [CrossRef]
24. Saeed, N.; Khan, M.R.; Shabir, M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement. Altern. Med.* **2012**, *12*, 221. [CrossRef]

25. Orsavová, J.; Hlaváčová, I.; Mlček, J.; Snopek, L.; Mišurcová, L. Contribution of phenolic compounds, ascorbic acid and vitamin E to antioxidant activity of currant (*Ribes* L.) and gooseberry (*Ribes uva-crispa* L.) fruits. *Food Chem.* **2019**, *284*, 323–333. [CrossRef] [PubMed]
26. De Quirós, A.; Lage-Yusty, R.B.; López-Hernández, M.A. HPLC analysis of polyphenolic compounds in Spanish white wines and determination of their antioxidant activity by radical scavenging assay. *Food Res. Int.* **2009**, *42*, 1018–1022. [CrossRef]
27. Snedecor, G.W.; Cochran, W.G. *Statistical Methods*, 8th ed.; Iowa State University Press: Ames, IA, USA, 1994.
28. Snopek, L. Changes in Bioactive and Sensory Important Substances of Grape Wines during Storage. Ph.D. Thesis, Tomas Bata University in Zlin, Zlin, CR, USA, 2019.
29. Fišera, M.; Šustová, K.; Tvrzník, P.; Velichová, H.; Fišerová, L.; Lukášová, E.; Kráčmar, S. Reduce the Sulphur Dioxide Content of Wine by Biological Process in Relation to the Content of Polyphenolic Substances. *J. Microbiol. Biotechnol. Food Sci.* **2022**, *11*, e59752. [CrossRef]
30. Michlovský, M. *Sulphur Dioxide in Oenology*; Vinselekt Michlovský: Rakvice, Czech Republic, 2012.
31. Mitrevska, K.; Grigorakis, S.; Loupassaki, S.; Calokerinos, C. Antioxidant Activity and Polyphenolic Content of North Macedonian Wines. *Appl. Sci.* **2020**, *10*, 2010. [CrossRef]
32. Čeryová, N.; Bajčan, D.; Lidiková, J.; Musilová, J.; Šnirc, M.; Jančo, I.; Franková, H.; Bláhová, M. Phenolic Content and Antioxidant Activity of Slovak Varietal Wines of Muscat Type. *J. Microbiol. Biotechnol. Food Sci.* **2021**, *10*, e4292. [CrossRef]
33. Paixao, N.; Perestrelo, R.; Marques, J.C.; Camara, J.S. Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines. *Food Chem.* **2007**, *105*, 204–214. [CrossRef]
34. Hurtado, I.; Caldu, P.; Gonzalo, A.; Ramon, J.M.; Minguez, S.; Fiol, C. Antioxidative capacity of wine on human LDL oxidation in vitro: Effect of skin contact in winemaking of white wine. *J. Agric. Food Chem.* **1997**, *45*, 1283–1289. [CrossRef]
35. Mitić, M.N.; Obradović, M.V.; Grahovac, Z.B.; Pavlović, A.N. Antioxidant Capacities and Phenolic Levels of Different Varieties of Serbian White Wines. *Molecules* **2010**, *15*, 2016–2027. [CrossRef]
36. Castellari, M.; Spinabelli, U.; Riponi, C.; Amati, A. Influence of some technological practices on the quantity of resveratrol in wine. *Z. Lebensm.-Unters. Und-Forsch. A* **1998**, *206*, 151–155. [CrossRef]
37. Nardini, M.; Garaguso, I. Effect of Sulfites on Antioxidant Activity, Total Polyphenols, and Flavonoid Measurements in White Wine. *Foods* **2018**, *7*, 35. [CrossRef] [PubMed]
38. Abramovič, H.; Košmerl, T.; Poklar Ulrich, N.; Cigić, B. Contribution of SO_2 to antioxidant potential of white wine. *Food Chem.* **2015**, *174*, 147–153. [CrossRef] [PubMed]
39. Ivanova, V.; Vojnoski, B.; Stefova, M. Effect of winemaking treatment and wine aging on phenolic content in Vranec wines. *J. Food Sci. Technol.* **2012**, *49*, 161–172. [CrossRef]
40. Di Mattia, C.D.; Piva, A.; Matruscelli, M.; Mastrocola, D.; Sacchetti, G. Effect of Sulfities on the in vitro Antioxidant Activity of Wines. *Ital. J. Food Sci.* **2015**, *27*, 505–512.
41. Lachman, K.; Šulc, M.; Schilla, M. Comparison of the total antioxidant status of Bohemian wines during the wine-making process. *Food Chem.* **2007**, *103*, 802–807. [CrossRef]
42. Long, L.H.; Kwee, D.C.; Halliwell, B. The antioxidant activities of seasonings used in Asian cooking. Powerful antioxidant activity of dark soy sauce revealed using the ABTS assay. *Free Rad. Res.* **2000**, *32*, 18. [CrossRef]
43. Mitsuhashi, H.; Ikeuchi, H.; Nojima, Y. Is sulfite an antiatherogenic compound in wine? *Clin. Chem.* **2001**, *47*, 1872. [CrossRef]
44. Manzocco, L.; Mastrocola, D.; Nicoli, M.C. Chainbreaking and oxygen scavenging properties of wine as affected by some technological procedures. *Food Res. Int.* **1998**, *31*, 673. [CrossRef]
45. Cimino, F.; Sulfaro, V.; Trombetta, D.; Saija, A.; Tomaino, A. Radical-scavenging capacity of several Italian red wines. *Food Chem.* **2007**, *103*, 75. [CrossRef]
46. Floegel, A.; Kim, D.; Chung, S.; Koo, S.I.; Chun, O.K. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J. Food Compos. Anal.* **2011**, *24*, 1043–1048. [CrossRef]
47. Fernández-Pachón, M.S.; Villaño, D.; García-Parrila, M.C.; Troncoso, A.M. Antioxidant activity of wines and relation with their polyphenolic composition. *Anal. Chim. Acta* **2004**, *513*, 113–118. [CrossRef]
48. García-Ruiz, A.; Moreno-Arribas, M.V.; Martín-Álvarez, P.J.; Bartolomé, B. Comparative study of the inhibitory effects of wine polyphenols on the growth of enological lactic acid bacteria. *Int. J. Food Microbiol.* **2011**, *145*, 426–431. [CrossRef]