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Effect of Heat Pasteurization and Enzymatic Maceration on Yield, Color, Sugars, Organic Acids, and Phenolic Content in the 'Merlot Kanthus' Grape Juice

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Abstract: This study researched the combined effects of heat treatment and varying concentrations of the pectolytic enzyme on the improvement of yield, color, and extraction of sugars, acids, and bioactive compounds of the 'Merlot Kanthus' grape juice. Application of low (0.05 IU/mL) and high (0.09 IU/mL) enzyme treatment substantially increased the yield of unpasteurized and pasteurized juice. Color intensity significantly improved in the pasteurized juice with a high enzyme concentration (CIRG 4.4) and the pasteurized juice without enzymes (4.5). No considerable differences in the total sugar concentration between the treatments were observed; however, the concentration of organic acids was improved by 27 and 13% in unpasteurized and pasteurized juice with a high enzyme concentration, respectively. A total of 78 individual phenolic compounds were identified, and the treatment with a high enzyme concentration had the most notable effect on the total anthocyanins, improving their concentration by 33.6% and increasing the concentration of 18 individual compounds. The heat treatment increased flavonol concentration by 41%. Overall, heat and enzyme treatment, mainly the high enzyme concentration, had a very favorable effect on the parameters of the analyzed 'Merlot Kanthus' grape juice, with a significant increase in the yield of bioactive components.

Keywords: heat treatment; enzyme concentration; juice processing; flavonoids; hydroxycinnamic acids

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1. Introduction

Grape juice is mainly produced in the United States of America, Brazil, and Spain and is consumed worldwide with a global production of approximately 11 to 12 million hectoliters [1]. The increase in beverage consumption, coupled with a considerable intensification of industrial production, is a result of the importance of grape juice in the diet as a healthy and functional food [2]. Grape and its products contain a wide range of polyphenolic constituents, mainly flavonols, procyanidins, anthocyanins, and phenolic acids, which are presumed to provide beneficial health effects [2]. These phenolic compounds, ubiquitous in plants, are an essential component of the human diet due to their health-promoting properties, which were shown to mitigate the symptoms of many chronic diseases, including diabetes and cardiovascular and neurodegenerative diseases [3–5]. These health benefits are mainly attributed to its antioxidant properties that complement and extend the functions of antioxidant vitamins and enzymes as a defense mechanism [6,7]. Additionally, based on studies indicating potential adverse effects of synthetic antioxidant consumption, such as skin allergies, gastrointestinal tract problems, DNA damage, and induced premature senescence [8], much interest has been expressed in the use of natural antioxidants in foods.

Challenges faced by the food industry include expanding the development of new products and convincing consumers to buy them [9]. Several studies have confirmed that consumers' eating and purchasing habits and preferences have changed as a result of the

COVID-19 pandemic, with an increase in consumer health awareness and adoption of healthy eating habits [10,11]. To respond to consumers' demands for natural and healthy products, it is, therefore, important to consider these demands when developing new and innovative products. Phenolic compounds are considered to be one of the most prominent quality parameters of grapes and their products as they contribute to their color and organoleptic properties, such as flavor, bitterness, and astringency; their concentration and stability in grape juices are thus very important [12,13].

Phenolic composition and antioxidant activity of grape berries and juice are affected by the cultivar, grape ripeness, weather, viticultural practices, as well as the environment [1,14]. Compared with the phenolic composition in grape berries [1,15], final phenolic composition of grape juice is significantly influenced by various methods and treatments used in its production, such as the type of extraction, contact time, and heat and enzyme treatments. This is a challenge for the food industry, especially for grape juice production, since most antioxidants in grapes are found in the pulp, skin, and seeds; however, most of them remain in the by-product after juice processing, and merely a small fraction is produced into juice [15]. To increase yield and storage stability and to prevent unfavorable properties of fruit juices, such as turbidity and cloudiness, pectolytic enzymes have shown to have great potential for use in the fruit juice industry [16]. The working mechanism of pectinase consists of hydrolyzation of pectin, a galacturonic acid-rich polymer responsible for maintaining the integrity and safety of plant tissues, which ruptures the plant cell wall and leads to a release of cell components [17]. For this reason, pectinases are among the most commonly used enzymes in juice production enhancing quality, increasing yield, and clarifying the extracted juice [17].

This study aimed to examine how heat treatment, along with varying concentrations of the pectolytic enzyme, impacted yield, color characteristics, concentrations of total sugars, organic acids, and phenolic compounds in grape juice.

2. Materials and Methods

2.1. Fruit Samples

The grapes of the cultivar 'Merlot Kanthus' (*V. vinifera* L.) from the Pouzelce vineyard (Institute for Agriculture and Forestry Nova Gorica, coordinates 45°50′02.6″ N 13°56′21.6″ E) were used. The vineyard has a low slope (on a plain), a sub-Mediterranean climate, and a space between rows, which is permanently green covered. The vines of VCR Rauscedo (Vitis Rauscedo Società Cooperativa Agricola, Italy) were planted in 2012 with a cultivar grafted on 5BB rootstock on a flysch soil type. The vines are grown according to the system of integrated pest management and as a single guyot training system.

2.2. Enzyme Treatment of the Mash and Juice Preparation

The formulation of the mash enzyme solution was prepared by using ROHAPECT® MC pectinase (AB Enzymes GmbH, Darmstadt, Germany). This pectinase was chosen since it is suitable for mash and juice treatments of various fruits, including berries, grapes, citrus, and pome fruits; what is more, it helps in attaining reduced viscosity, high juice yields, and fast clarification [18]. To prepare the enzyme solution, 1 mL of the enzyme with a declared activity of 650 PE/g was diluted in 100 mL of distilled water, mixed, and stored at +4 °C until use. The procedure for obtaining grape juice, which is presented in Figure A1, starts with the whole grapes of the 'Merlot Kanthus' cultivar, which were kept frozen at −18 °C until processing. Prior to juice processing, three equal amounts (6 kg) of each replicate of grapes were defrosted at room temperature, then crushed twice using a stainless-steel mill (Paul Arauner GmbH & Co. KG, Kitzingen, Germany), resulting in cold mash and pomace. The mash obtained from 6 kg of fruit was preheated in the oven until the internal temperature reached 50 °C, then divided among three tanks, each containing approximately 2 kg of hot mash for the three treatments applied. For a control treatment, no enzyme solution was added to the first batch of the grape mash. For a low enzyme concentration treatment, 13.5 mL of the prepared enzyme solution was added to

the second batch to achieve the required enzyme activity of 0.05 IU/mL. For a high enzyme concentration treatment, 26.9 mL of the solution was added to the third batch to achieve an enzyme activity of 0.09 IU/mL. The mash was further macerated in the oven at 50 °C for one hour, transferred into the Hydro PARA-Press stainless-steel hydraulic press (part no. 0423, Paul Arauner GmbH & Co. KG, Germany), and the juice was extracted under the pressure of 3.5 bar for 15 min. Pasteurization was performed in a PAS1-PS2-81-V2 pasteurizer (Mabo-Steuerungselemente GmbH, Eppingen, Germany) at 85 °C for 1 min. Upon pasteurization, the juice was filled into bottles and hermetically sealed with parafilm and caps.

2.3. Pressing Yield

The efficiency of juice pressing was calculated using the following equation [19]:

$$Wj(\%) = \frac{Mj}{Mi} \times 100$$

where *Wj* (%) represents the efficiency of pressing, *Mj* (kg) is the mass of the juice after pressing, and *Mi* (kg) is the mass of the input material, i.e., fruit mass.

2.4. Color Measurements

Color assessment was carried out with a colorimeter (CR-10 Chroma, Minolta, Osaka, Japan) and is expressed in L^*, C, and h° values. CIRG (Color Index of Red Grapes) by Carreño et al. (1996) [20] was registered for each of the samples using the following equation:

$$\text{CIRG} = \left(180 - h^\circ\right) / \left(L^* + C^*\right)$$

where L^* represents lightness from black to white on a scale of zero to 100, C^* represents chroma, and h° is the hue angle. The value of chroma is the distance from the lightness axis (L^*) and starts at 0 in the center. The hue angle starts on the $+a^*$ axis and is expressed in degrees (e.g., 0° is red, and 90° is yellow).

2.5. Sugar and Organic Acid Extraction and Determination

Extraction and determination of sugars and organic acids were performed according to the method previously described [21]. The juice samples were centrifugated in Eppendorf Centrifuge 5810 R (Eppendorf, Hamburg, Germany) for 5 min at 4 °C and 3226× *g* RCF (Relative centrifugal force), then filtered using 0.20 µm cellulose filters (Macherey-Nagel, Düren, Germany) into glass vials. The samples were then analyzed using a high-performance liquid chromatograph (HPLC Vanquish™ Flex UHPLC, Thermo Fisher Scientific, San Jose, CA, USA). For sugar determination, the Rezex RCM-monosaccharide Ca^+ (2%) column (150 mm × 7.8 mm) (Phenomenex, Torrance, CA, USA) was used, and the mobile phase was bidistilled water. For organic acids, a Rezex ROA—organic acid H^+ (8%) column (150 mm × 7.8 mm) (Phenomenex, Torrance, CA, USA) was used, and 4 mM sulfuric acid was used for the mobile phase. For sugar analysis, the following was applied: a flow rate of 0.8 mL/min, a column temperature of 80 °C, and a total run of 20 min; whereas for organic acids, the following was applied: the run of one sample was 25 min, the flow rate was 0.6 mL/min, and the working temperature of the column was 65 °C. A refractive index detector was used to identify and measure sugars, and a UV detector at 210 nm was used for the organic acid analysis. The concentration of an individual metabolite was calculated according to a calibration curve of corresponding standards and expressed in g/L of juice.

2.6. Phenolic Compounds Analysis on PDA–HPLC MSn System

The analysis of individual phenolic compounds in the grape juice samples was performed using an HPLC system (HPLC Finnigan Surveyor, Thermo Fischer Scientific, San Jose, CA, USA) with a photodiode array detector (PDA) at three wavelengths (280, 350, and 530 nm) using a mass spectrometer (MS), according to the method described in Mikulic-

Petkovsek (2020) [22]. Mobile phases consisted of bidistilled water/acetonitrile/formic acid (96.9/3/0.1, *v*/*v*/*v*) for mobile phase A and acetonitrile/bidistilled water/formic acid (96.9/3/0.1, *v*/*v*/*v*) for mobile phase B. The samples were eluted according to a linear gradient from 5 to 20% B in the first 15 min, followed by a linear gradient from 20 to 30% B for 5 min, then an isocratic mixture for 5 min followed by a linear gradient from 30 to 90% B for 5 min, and then an isocratic mixture for 15 min before returning to the initial conditions. A Gemini C18 column (Phenomenex, Torrance, CA, USA) set at 25 °C was used for the measurements. Phenolic compounds were determined in positive (for anthocyanins) and negative ionization (for all other phenolic compounds) modes, and the analyses were carried out using full-scan data-dependent MSn scanning from m/z 115 to 1900. The following source parameters were used: the capillary temperature was set at 250 °C; the sheath gas and auxiliary gas were 60 and 15 units, respectively; the source voltage was 3 kV; and the normalized collision energy was between 20 and 35%. Spectral data were elaborated using Thermo Scientific™ Xcalibur™ TM 4.3 software (Thermo Scientific, Waltham, MA, USA). Phenolic compounds were identified based on their retention times and PDA spectra compared with phenolic standards, and fragmentation patterns in different MSn modes compared with literature data. The content of individual phenolic compounds was calculated using standard curves of different phenolics. To obtain standard curves, five different concentrations for each phenolic compound were injected three times. The total concentration of phenolic compounds was expressed in mg/kg of mash and mg/L of juice.

2.7. Statistical Analysis

Samples were collected from the cold mash before enzyme addition, as well as from the hot mash, unpasteurized and pasteurized juice with no enzyme, a low enzyme concentration, and a high enzyme concentration (Figure A1). Each treatment was examined using three biological replicates, and each sample was measured in three technical replicates. A *t*-test was performed for the factor heat treatment to determine the differences between the cold and the hot mash without enzyme addition. One-way analysis of variance was performed for the factor enzyme treatment in the hot grape mash with added enzymes. For grape juice, a two-way analysis of variance (ANOVA) was performed for the factor heat and factor enzyme treatment using the statistical program R-commander version 4.3.0 (R Formation for Statistical Computing, Auckland, New Zealand). Significant differences between means ($p \leq 0.05$) were assessed using Tukey's test for significance. The means and standard errors are presented as mean ± SE with different letters indicating statistically significant differences between the treatments.

3. Results and Discussion

3.1. Yield

The lowest juice yield was obtained without the enzyme addition (59.3%). However, the addition of both low and high enzyme concentrations resulted in a yield of 71.9% and 74.8% respectively, significantly improving the average yield compared with the juice without enzymes. Since pectinase acts on the pectin structure and breaks the glycosidic bonds present between the galacturonic acid monomers, it decreases the water-holding capacity of pectin, consequently improving the yield [23]. Overall, these results imply that the enzyme addition process, especially the addition of a higher enzyme concentration, had a positive effect and substantially increased the yield by 12−15%. Similar yield results were reported when pectolytic enzymes were used in grape juice [24] and blueberry juice [25].

3.2. Color of Grape Juice

A color index for an objective determination of color in red grapes (CIRG) [20] was used to determine the color of grape juice treated with different heat and enzyme treatments. The results for the CIRG index are shown in Table 1. According to the criterion established by Carreño et al. (1996) [20], grapes can be classified into five groups based on their color: green-yellow (CIRG < 2), pink (2 < CIRG < 4), red (4 < CIRG < 5), dark red (5 < CIRG < 6),

and blue-black (CIRG > 6). In this case, although all the samples were categorized as red with the CIRG value between 4 and 5, considerable differences were observed in the CIRG index between the treatments. These results are in contrast to what was previously published [24], where no significant differences in color intensity were found between different maceration conditions, using three enzyme dosages and two temperatures (50 °C and 60 °C).

Table 1. Colorimetric values of differently treated grape juice cv. 'Merlot Kanthus'.

Heat Treatment	Enzyme Treatment	*L**	*C**	*h*°	CIRG Index	Color
Unpasteurized juice	No enzyme	23.3 ± 0.03	3.4 ± 0.07	63.2 ± 1.86	4.4 ± 0.06 ^{ab}	Red
	Low enzyme	23.1 ± 0.06	3.3 ± 0.03	64.9 ± 1.13	4.4 ± 0.04 ^{ab}	Red
	High enzyme	23.1 ± 0.07	3.3 ± 0.03	69.3 ± 2.49	4.2 ± 0.08 ^{a}	Red
Pasteurized juice	No enzyme	23.2 ± 0.12	3.3 ± 0.10	62.0 ± 1.17	4.5 ± 0.04 ^{b}	Red
	Low enzyme	23.2 ± 0.03	3.4 ± 0.18	64.5 ± 0.57	4.3 ± 0.04 ^{ab}	Red
	High enzyme	23.2 ± 0.1	3.3 ± 0.09	63.3 ± 2.49	4.4 ± 0.09 ^{b}	Red
	p heat treatment	0.726	0.582	0.111	0.030	
	p enzyme treatment	0.801	0.692	0.637	0.044	
	p INT	0.968	0.871	0.863	0.039	

The values are the means of triplicate samples. Means separation by Tukey's test. Mean values within a column followed by a different letter are significantly different ($p \leq 0.05$).

In this case, the lowest CIRG value measured was for the unpasteurized juice with a high enzyme concentration (4.2), while the pasteurized juice with a high enzyme concentration (4.4) and the pasteurized juice without enzymes had the highest CIRG value i.e., higher color intensity (4.5). From these results, it can be concluded that higher color intensity of red juice can be achieved by adding a higher enzyme concentration if accompanied by an appropriate heat treatment.

Color is a characteristic of grape juice that is directly dependent on the phenolic composition of the juice and the anthocyanins present in the grape skin. Anthocyanins are involved in many reactions that promote changes in the color of grape products, mainly through copigmentation and the formation of polymer pigments [12]. These results could, therefore, be due to the increased concentration of anthocyanins caused by cell disruption, as these compounds are responsible for a blue and purple color [25], and possibly made the color of the juice appear slightly darker after the heat and enzyme treatments.

3.3. Sugar and Organic Acid Content in Grape Juice

Average values of total sugars for the juice are presented in Figure 1. The average sugar content ranged from 186.2 g/L for the unpasteurized juice with a high enzyme concentration to 207.4 g/L for the pasteurized juice without the enzyme addition. These values are slightly lower compared with literature data, where the sugar content in the juice from grapes of the 'Merlot' variety was 222.0 g/L [26].

As for the effect of the treatments and their interaction, no considerable differences were observed between the total sugar content in the juice ($p \leq 0.05$). In a study published by Praskova et al. (2022) [27], in which the effect of enzymes on the clarification of juice from wild 'Amur' grapes was investigated, similar results were reported. The mass fraction of reducing sugars in the juice of the wild 'Amur' grape before and after the enzymatic treatment did not exhibit any significant changes. Other authors [28] reported similar results; they observed that the enzyme treatment had no effect on fructose, glucose, and total sugars in blueberry juice.

As shown in Figure 2, the heat treatment/pasteurization by itself did not exert a substantial effect on the total organic acid content ($p = 0.848$); however, there were statistically significant differences caused by different enzyme treatments ($p = 0.037$).

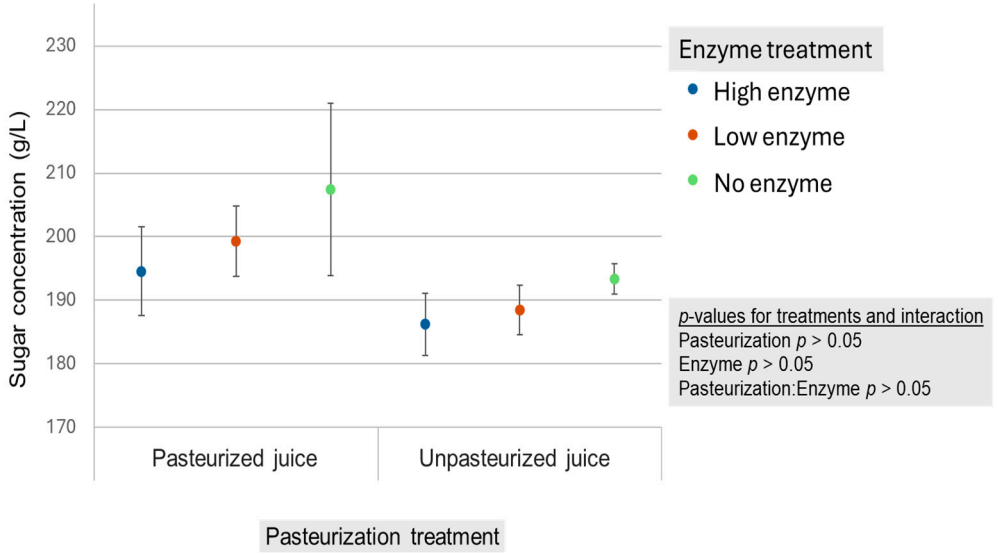

Figure 1. Average sugar concentration in the grape juice of the cultivar 'Merlot Kanthus' (g/L). The values are the means of triplicate samples. Means separation by Tukey's test ($p \leq 0.05$).

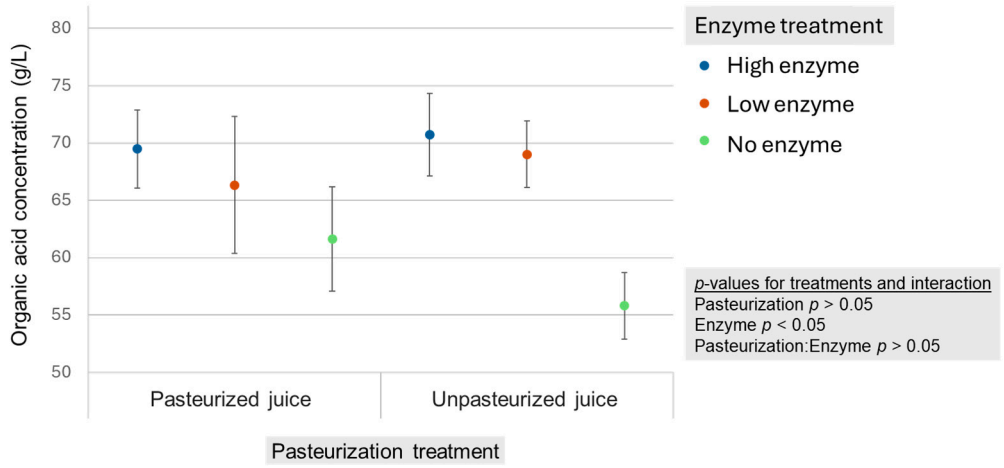

Figure 2. Average organic acid concentration in the grape juice of the cultivar 'Merlot Kanthus' (g/L). The values are the means of triplicate samples. Means separation by Tukey's test ($p \leq 0.05$).

In both the unpasteurized and pasteurized juice, the highest total organic acid content was achieved by adding a high enzyme concentration (70.7 and 69.5 g/L), while the addition of a lower enzyme concentration did not result in a significant difference between the samples. Thus, the total concentration of organic acids increased by 27% in the unpasteurized juice and by 13% in the pasteurized juice. These results are in agreement with the research findings of Lima et al. (2015) [24], in which the effect of two different temperatures (50 °C and 60 °C) and three enzyme dosages (0.0, 1.5, and 3.0 mL/100 kg) on the organic acid content of grape juice was tested. The results of this study showed that the combination of a temperature of 60 °C with the use of enzymatic poll led to an increase in almost all organic acids. Kim et al. (2017) [15] also reported a positive effect of pectin enzymes on organic acids in aronia juice, with a significant increase in the total acid content in the aronia juice obtained with three commercial pectinases.

3.4. Effect of Heat Treatment on Phenolic Compound Content in Grape Mash

To investigate potential losses caused by heat treatment during juice extraction, we initially examined the differences in the total phenolic compound content between the cold mash and the hot mash. In both the cold and the hot mash, all identified phenolic

compounds were assigned to the following phenolic groups: anthocyanins, flavonols, stilbenes, hydroxycinnamic acids, flavanols, and hydroxybenzoic acids. Anthocyanins were the most prominent phenolic group (Table A1, Appendix A), with the concentration ranging from 987.1 mg/kg in the hot mash to 1078.2 mg/kg in the cold mash, corresponding to 44.8 and 50.5% of the total phenolic content, respectively.

At a concentration of 1.2 mg/kg, stilbenes accounted for approximately 0.05% of the total phenolics analyzed, with no significant differences observed between the treatments. Guerrero et al. (2010) [29] reported higher levels of stilbenes, whereas in a comparative study between berries of three different grape varieties, 'Syrah', 'Tempranillo', and 'Merlot', the stilbene content in 'Merlot' was up to 3.6 mg/kg. According to Aguilar et al. (2016) [30], stilbenes are relatively unreactive, colorless, and almost insoluble in water, with poor diffusion properties, which could explain the results in our study.

The total phenolic content ranged from 2135.3 to 2202.4 mg/kg, but there were no differences between the treatments, meaning that the heat treatment did not significantly affect the total phenolic content at this stage of juice processing. The concentration of phenolic compounds in grape juice, especially anthocyanins, depends on factors related to the raw material, but the processing technology also exerts a significant influence on the anthocyanin concentration of grape juice, with the use of heat treatments being fundamental for better extraction of anthocyanins from the grape skins [1].

3.5. Effect of Enzyme Treatment on Phenolic Compound Content in Hot Grape Mash

Since no considerable differences were observed between the cold and the hot mash, the concentration of phenolic compounds in the mash with added enzymes was further investigated to observe the effect of the enzyme addition (Table A2, Appendix A). When enzymes were added to the hot mash, total flavanols were the most abundant group, accounting for more than 50% of the total phenolics. Their concentration in the hot grape mash with a low enzyme addition significantly improved by 36% in comparison with the hot mash without enzymes.

Overall, the addition of both low and high enzyme concentrations to the hot grape mash substantially increased the total phenolic content (no enzyme 1810.9 mg/kg, low enzyme 2352.8 mg/kg, and high enzyme 2287.9 mg/kg). The only two phenolic groups that did not undergo significant changes between the treatments were flavonols and hydroxycinnamic acids (Table A2, Appendix A). Although Guler (2023) [31] reported a similar content of total phenolics in grape mash compared with our results (1807.0 mg/kg), the authors did not observe significant differences following the enzymatic treatment in that study. According to Romero-Cascales et al. (2012) [32], the extraction of phenolic compounds can be further enhanced by extending the maceration time since the main effect of the pectolytic enzyme is related to the pectin fraction of the cell wall where it seems to provoke the greatest degradation.

3.6. Effect of Heat and Enzyme Treatments on the Content of Total and Individual Phenolic Compounds in Grape Juice

The results of the effect of the heat and the enzyme treatments and their interaction in the grape juice samples are presented in Table S1 in Supplementary Materials. Regarding the effect of the treatments, the differences in the total and individual concentrations of phenolic compounds were attributed to individual factors (i.e., the heat and enzyme treatments) rather than their interaction. A total of 78 different phenolic compounds were identified in the grape juice and classified into the following groups: anthocyanins, flavonols, stilbenes, hydroxycinnamic acids, flavanols, and hydroxybenzoic acids. Among the six phenolic groups identified, total anthocyanins (1065.4 to 2076.7 mg/L) and total flavanols (991.8 to 2327.6 mg/L) were the most abundant, representing 94% of the total phenolic content.

A total of 30 different individual compounds were identified; among them were anthocyanins, which primarily consisted of anthocyanin diglucosides and glucosides:

malvidin-3,5-diglucoside > delphinidin-3-glucoside > malvidin-3-glucoside and petunidin-3-glucoside. Furthermore, malvidin derivatives accounted for 60.7% of the total anthocyanin content, delphinidin derivatives 19.4%, petunidin derivatives 12.4%, and peonidin and cyanidin derivatives for less than 10%. Similarly, Kontic et al. (2016) [33] reported that the most abundant anthocyanin in the 'Léon Millot', 'Cabernet Cortis', and 'Monarch' grapes is malvidin 3,5-*O*-diglucoside. These results are notable because anthocyanins in grapes are usually in the form of 3-monoglucoside, with the dominant anthocyanin being malvidin-3-glucoside [34]. A very characteristic and similar anthocyanidin pattern was also observed in the 'Merlot' grape by Dimitrovska et al. (2011) [35], where 'Merlot' was abundant in delphinidin glycosides (13%) and petunidin glycosides (12%), which is consistent with this study's results. The heat treatment (p = 0.248) and the interaction between the heat and the enzyme treatments (p = 0.353) had no significant effect on the total anthocyanin concentration (Table S1 in Supplementary Materials). However, the enzyme treatment with a high concentration improved the total concentration of anthocyanins by 33.6% (p = 0.009) compared with the no-enzyme treatment. In addition, the high enzyme treatment substantially increased the concentration of 18 individual anthocyanin compounds out of 30 that were determined, implying that this enzyme treatment positively affects the efficiency of anthocyanin extraction from grape skins, where they are the main components [36].

A total of 19 individual flavanols were identified (Table S1 in Supplementary Materials). Regarding the flavanol profile, catechin and epicatechin derivatives accounted for 43% of the flavanol content, followed by procyanidin dimer derivatives with an average of 39.5%. A similar flavanol profile was previously reported [37], with catechin, epicatechin, and procyanidin dimers being the dominant components in the extracts from the 'Muscat of Alexandria' grape seeds. This is due to the high content of proanthocyanidins in grape seeds, which are oligomers of flavan-3-ol units, particularly catechin and epicatechin [38].

The interaction between the heat and enzyme treatments did not affect the concentration of the total analyzed flavanols. However, the heat treatment significantly increased only the concentration of some procyanidins (procyanidin trimer 3, procyanidin dimer gallate, and procyanidin tetramer 3), while the concentrations of catechin, epicatechin, and the majority of procyanidin derivatives did not substantially differ between the treatments. These results indicate that the heat treatment slightly affected the extraction of individual flavanols from the grape seeds present in the grape mash. Considering that derivatives of catechin and epicatechin are among the phenolic compounds responsible for the astringency of wine and grape juice [38], this can imply that the astringency of the juice was not affected either.

The enzyme treatment with a higher concentration had a considerable effect on total flavonols, improving their concentration by 79%, while the heat treatment significantly improved the concentration of ten individual flavonols, mainly quercetin derivatives in the pasteurized juice, suggesting the effectiveness of the extraction from the mash as flavonols are present in the berry skin [39]. Flavonols accounted for 2.4% of the total phenolic content, with a range of 0.3 to 22.7 mg/L, which is in the range reported by Talcott and Lee (2002) [40] for the flavonol content in juices from the Muscadine grapes. Regarding the phenolic profile, the derivatives of quercetin and myricetin were present in the highest concentration, with 43.2 and 37.6%, respectively. Among them, myricetin-3-glucoside (22.6 mg/L), quercetin-3-glucoside (17.8 mg/L), and quercetin-3-glucuronide (13.4 mg/L) were the most prominent ones.

A total of ten phenolic acids were identified, including seven hydroxycinnamic acids and three hydroxybenzoic acids. Among the individual hydroxycinnamic acids, caffeic acid was the most abundant, with an average concentration of 29.1 mg/L. It was mainly present in the hexose form as caffeic acid hexoside 1 and caffeic acid hexoside 2, with none of the treatments significantly affecting its concentration in the analyzed juice samples. Among hydroxybenzoic acids, gallic acid was the most abundant with an average concentration of 14.1 mg/L, followed by protocatechuic acid (6.2 mg/L) and *p*-hydroxybenzoic acid (0.5 mg/L). The heat treatment considerably affected only the concentration of gallic acid,

increasing it by 24%, while the enzyme treatment had no significant effect. Moreover, this profile is consistent with the findings in the literature, as gallic acid is considered the main phenolic acid in grape seeds and is also the precursor of all hydrolyzable tannins, while protocatechuic acid and *p*-hydroxybenzoic acid are also present in lower amounts [41].

The total content of stilbenes, with an average concentration of 0.15–1.6 mg/L, was the lowest among the phenolic compounds analyzed in grapes, which is within the range published by Leblanc et al. (2008) [42] for the red grape juice. Among them, only two resveratrol hexosides were identified (Table S1 in Supplementary Materials). For the total stilbenes, no significant differences between the treatments were observed. However, the heat treatment affected only individual stilbenes, significantly increasing the concentration of resveratrol hexoside 1 in the pasteurized juice. Although grape juice is considered a good source of resveratrol, its concentration in grape berries and consequently in grape juices depends on the grape variety, climatic and growing conditions, as well as the juice processing method used [1]. Since stilbenes are phytoalexins, their presence in grapes is also directly related to environmental stress [43]. Among them, resveratrol is a polyphenol mostly present in red grapes and wines as cis-and trans-isomers in free or glycosidic bound form [44], which is consistent with our results, as resveratrol was present in a hexosidic form in the analyzed juice samples.

4. Conclusions

The enzymatic treatment, especially at a high concentration, significantly increased the yield by 12–15% and improved the juice color intensity, particularly in the pasteurized samples. The sugar concentration did not considerably change during the individual treatments. In contrast, the enzyme treatment substantially improved the concentration of organic acids, especially at a high enzyme concentration, while the heat treatment had no significant effect.

The enzyme treatment notably increased the total anthocyanin concentration by 33.6%, while heat treatment primarily boosted the flavonol concentration by 41%. Overall, both the heat and high enzyme concentration treatments had positive effects on the yield, color, and the concentration of bioactive compounds in the 'Merlot Kanthus' grape juice. However, based on the results obtained for sugars and acids, future studies should investigate the sensory properties of juices produced using the described procedure.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages10030066/s1, Table S1: Content of individual phenolic compounds in analyzed grape juice samples subjected to heat and enzyme treatment.

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Appendix A

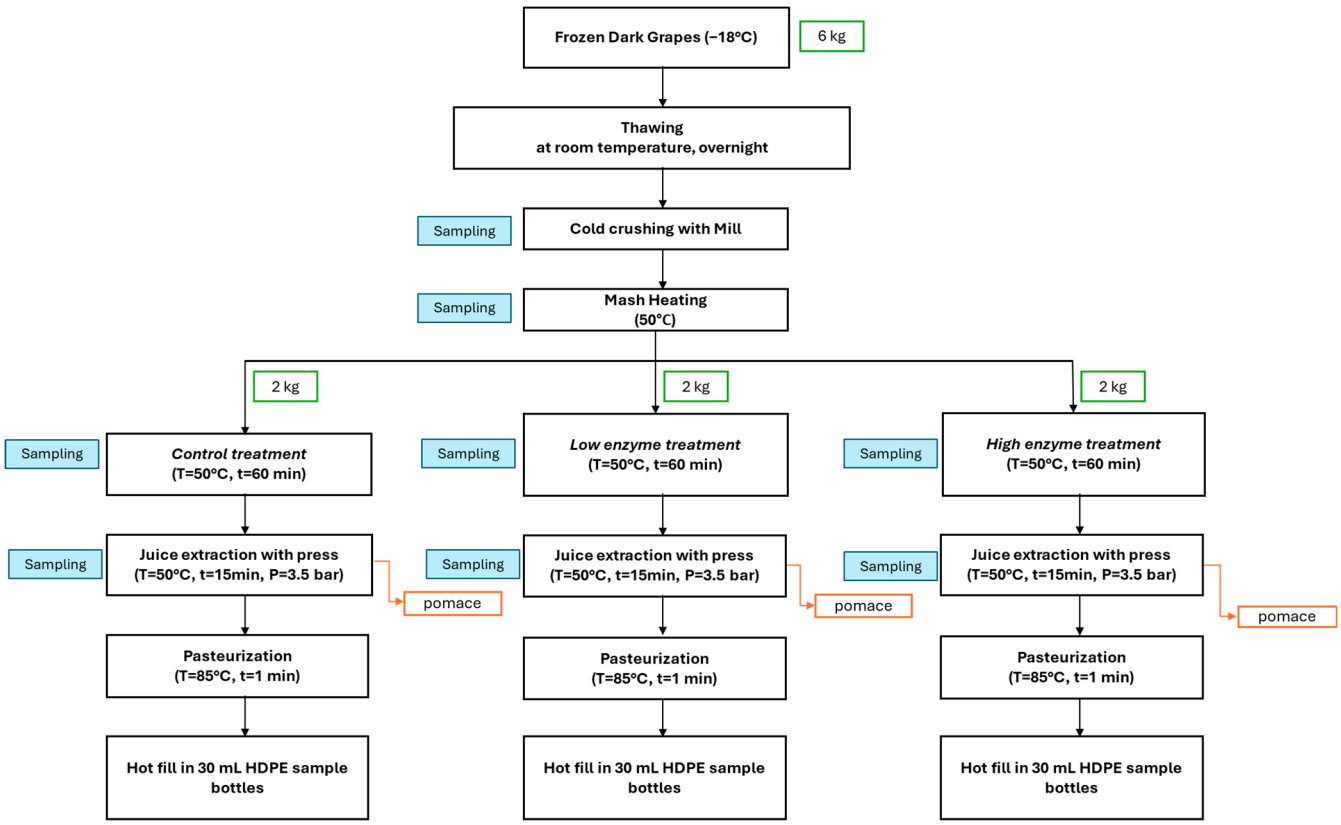

Figure A1. Schematic representation of the grape juice extraction procedure.

Table A1. Effect of heat treatment on the phenolic content of grape mash (mean ± SE in mg/kg).

	Total Anthocyanins			Total Flavonols			Total Stilbenes			Total Hydroxycinnamic Acid			Total Flavanols			Total Hydroxybenzoic Acid			Total Phenolics		
Heat Treatment	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**
Mash cold	1078.2	46.3	a	49.9	3.7	a	1.2	0.1	a	58.3	2.4	a	938.8	45.7	a	8.9	0.5	a	2135.3	93.7	a
Mash hot	987.1	59.6	a	49.4	4.5	a	1.2	0.2	a	63.9	3.9	a	1092.9	85.3	a	7.8	0.5	a	2202.4	152.5	a

Values are the mean of triplicate samples. Means separation by *t*-test ($p \leq 0.05$). Mean values within a column followed by letter "a" indicate significant difference ($p \leq 0.05$).

Table A2. Effect of enzyme treatment on the phenolic content (mean±SE in mg/kg FW) of hot grape mash.

	Total Anthocyanins			Total Flavonols			Total Stilbenes			Total Hydroxycinnamic Acid			Total Flavanols			Total Hydroxybenzoic Acid			Total Phenolics		
Enzyme Treatment	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**	**Mean**	**±SE**	**Sign.**
No enzyme	839.7	28.2	a	43.3	2.9	a	1.1	0.04	a	53.3	2.8	a	866.6	62.4	a	6.9	0.1	a	1810.9	90.5	a
Low enzyme	1046.6	40.6	b	57.6	5.7	a	1.2	0.3	b	67.8	1.2	a	1174.9	40.1	b	8.3	0.1	b	2352.8	42.3	b
High enzyme	1038.6	43.9	b	57.6	6.7	a	1.32	0.5	b	65.5	3.3	a	1116.3	25.1	ab	8.6	0.1	b	2287.9	79.7	b

The values are the mean of triplicate samples. Means separation by Tukey's test. Mean values within a column followed by a different letter are significantly different ($p \leq 0.05$).

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