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Diversity of Seed Flavan-3-Ols in Croatian Native Grapevine Cultivars (*Vitis vinifera* L.) Grown in Coastal Region

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Abstract: Seed extracts are becoming more important due to their beneficial biological activities. The main constituents of seed extracts are flavan-3-ols, compounds important in winemaking. The coastal region in Croatia is rich in native grapevine varieties, which are used in wine production. The aim of the research was to analyze the flavan-3-ol profiles of 20 native varieties, and to evaluate the potential use of grape seeds as a source of flavan-3-ols. The flavan-3-ols from seeds were analyzed by HPLC. The predicted yield of flavan-3-ols was calculated using the analyzed profiles. In total, eight compounds were identified, with the most abundant compounds being catechin, epicatechin, and procyanidin B2. In general, the red grape varieties had higher content of flavan-3-ols than the white varieties, which was confirmed by PCA. The coastal region could potentially yield up to 73.97 kg/ha of flavan-3-ols, depending on the variety. The results show the diversity of flavan-3-ol profiles among Croatian varieties and their potential usage as a source of valuable nutraceuticals.

Keywords: grape seeds; flavan-3-ols; grapevine varieties; Croatia

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1. Introduction

Croatia is a home to more than 120 native grapevine varieties grown in four different climate zones. The coastal region of Croatia is very important for its wealth of indigenous varieties, and about a hundred different varieties are grown in this area [1]. The Dalmatian subregion is characterized by a Mediterranean climate and karst relief, which enable the production of premium quality wines from indigenous *Vitis vinifera* varieties, such as Plavac mali, Grk, Posip, Babic, and Malvasija dubrovacka.

However, the quality of wines depends not only on the basic chemical parameters, such as total soluble solids, organic acids, and pH, but also on the secondary metabolites, polyphenolic, and volatile compounds. The polyphenolic compounds are important secondary metabolites, influencing the grape and wine quality, color, and sensory properties, such as astringency and bitterness [2]. Furthermore, these compounds are becoming more important as compounds with a beneficial influence on human health, due to their antioxidant, anti-cancer, and antimicrobial properties [3,4]. The polyphenols in grapes are distributed between skin (28–35%), seeds (60–70%), and pulp (<10%) [5], thus, the seeds represent a valuable source of polyphenolic compounds. The flavan-3-ols are present in grapes as monomers and polymers. The main monomers are catechin, epicatechin, epigallocatechin, and epicatechin-gallate [6]. The polymers are represented by procyanidins or condensed tannins, which are built from monomeric forms of flavan-3-ols. The procyanidins B1–B4 differ in the arrangement of catechin and epicatechin starter and extension units, and are under strict enzymatic control [7]. In grape seeds, the most abundant are procyanidins, followed by catechin, epicatechin, and epicatechin-gallate [8]. The flavan-3-ols, like other classes of polyphenolic compounds, also showed beneficial biological activities, such as antioxidant, antidiabetic, cardioprotection, which were extensively reviewed by several groups of authors [9–11]. Moreover, the phenolics in the grape seeds are important in improving the wine structure and enhancing the aging potential, as well as protecting the wine against oxidation and stabilizing wine color [12]. Only a few pieces of research on seed polyphenols were conducted that included the Croatian varieties. Curko et al. [13] investigated seed flavan-3-ols from Plavac mali and Babic, where the dominant compounds were catechin and epicatechin. Similar results were presented for Vranac seeds, where catechin was also the dominant compound [14].

During vine cultivation and winemaking, high amounts of waste are produced, including pomace, seeds, stems, prunings, yeast and bacteria lees, organic acids, CO_2, and wastewater [15]. The seeds are a relevant part of the waste, and represent 20–25% of the biomass generated by the wine industry, which in many countries is considered as a disposable material [16]. Furthermore, the conventional treatments of the wastes are expensive, as they require considerable amounts of economic and energy resources for their safe discharge into the environment [17]. Besides the polyphenols, the seeds contain beneficial polyunsaturated fatty acids and oils [16,18,19], thus representing a promising source of nutraceuticals. These physical–chemical properties of the waste material determine its subsequent use in order to minimize the ecological footprint [15]. Thus, these compounds used as grape seed extracts can demonstrate antioxidant and antimicrobial activities [20–23], which enables their usage in the food industry as natural additives, for increasing product quality, safety, and shelf life [24].

Since the research on the seed polyphenols is scarce, the aim of this research was to analyze polyphenolic compounds from the seeds of important native grapevine cultivars grown in the Dalmatia subregion. Another aim of this study was to evaluate the potential use of seeds as a valuable source of flavan-3-ols in commercial production. Thus, we calculated the potential yield of individual compounds based on the analyzed profiles of flavan-3-ols.

2. Materials and Methods

2.1. Grape Samples

The grape samples were collected at the germplasm collection of the Institute for Adriatic Crops and Karst Reclamation in Split (Dalmatia, Croatia) and belong to the wine-growing region of Central and South Dalmatia. In total, nineteen grape varieties (10 red varieties and 9 white varieties) were collected during two consecutive years, 2011 and 2012. All of the varieties were sampled in both years and subjected to the analysis of seed flavan-3-ols. In Table 1, the list of the collected *Vitis vinifera* cultivars is shown. The cultivars are important native varieties grown in Dalmatia and are included in the revitalization program. All of the grapevine cultivars are of the same age and pruning system (bilateral cordon training), grafted onto 1013 Paulsen with planting spacing 2.2 × 1.1 m. The samples of each cultivar consisted of five bunches randomly picked from five different vines.

Table 1. The list of sampled grapevine cultivars.

Red Cultivars	White Cultivars
Babica	Bogdanusa
Babic	Cetinka
Dobricic	Debit
Lasina	Gegic
Ljutun	Grk
Nincusa	Malvazija dubrovacka
Plavac mali	Posip
Plavina	Prc
Tribidrag	
Vranac	Zilavka

The picked bunches were used to record cluster weight, the number of berries per bunch, and the weight of 100 berries. Each cluster for every variety was weighed, and then the berries were removed from each cluster while counting them. The three samples of 100 berries were randomly counted and weighed. These batches of 100 berries were used for counting the seed number per berry, and the given numbers were then used to calculate the percentage of seeds in the berry and the bunch. In Table 2 are shown the mean values of the seed content in the analyzed varieties.

Table 2. Mean values of seed content in analyzed grape varieties.

Variety	Color	Mass Cluster (g)	Average Mass 1 Berry (g)	% Seeds in Berry	% Seeds in Bunch
Babica	Red	329.62 ± 91.66 abcd *	2.49 ± 0.65 abcde	2.00 ± 0.71 d	1.95 ± 0.63 d
Babic	Red	422.66 ± 92.83 abc	3.65 ± 1.15 a	2.00 ± 0.70 d	1.90 ± 0.56 d
Dobricic	Red	235.52 ± 86.38 bcd	1.77 ± 0.22 cdefg	2.90 ± 1.41 bcd	2.80 ± 1.41 bcd
Lasina	Red	302.86 ± 85.63 abcd	2.35 ± 0.35 abcde	8.00 ± 3.26 a	7.70 ± 3.48 a
Ljutun	Red	165.53 ± 27.52 d	1.34 ± 0.09 efg	2.50 ± 0.84 cd	2.40 ± 0.84 bcd
Nincusa	Red	343.06 ± 32.31 abcd	3.27 ± 0.61 ab	2.10 ± 0.56 d	2.05 ± 0.63 d
Plavac mali	Red	174.91 ± 49.83 cd	1.99 ± 0.52 abcdefg	2.10 ± 0.55 d	2.10 ± 0.55 d
Plavina	Red	177.33 ± 50.54 cd	1.59 ± 0.08 defg	5.60 ± 1.27 ab	5.30 ± 1.27 ab
Tribidrag	Red	166.86 ± 31.39 d	1.09 ± 0.33 fg	7.40 ± 2.26 a	7.15 ± 2.19 a
Vranac	Red	232.04 ± 55.04 bcd	1.68 ± 0.73 defg	5.35 ± 0.35 abc	5.10 ± 0.28 abc
Bogdanusa	White	294.33 ± 80.76 abcd	3.10 ± 1.12 abc	2.45 ± 0.91 cd	2.30 ± 0.84 cd
Cetinka	White	477.25 ± 190.77 a	2.93 ± 0.53 abcd	3.15 ± 0.77 bcd	3.10 ± 0.84 bcd
Debit	White	433.91 ± 162.02 ab	2.32 ± 0.33 abcdef	2.75 ± 0.49 bcd	2.65 ± 0.49 bcd
Gegic	White	276.06 ± 94.15 abcd	1.80 ± 0.41 cdefg	2.20 ± 0.28 d	2.10 ± 0.28 d
Grk	White	349.79 ± 32.31 abcd	2.28 ± 0.53 abcde	1.93 ± 0.56 d	0.58 ± 0.32 d
M. dubrovacka	White	143.07 ± 25.88 d	0.83 ± 0.26 g	7.00 ± 2.83 a	6.45 ± 2.47 a
Posip	White	244.40 ± 2.19 bcd	2.19 ± 0.37 abcdef	2.65 ± 1.20 bcd	2.55 ± 1.20 bcd
Prc	White	218.99 ± 37.75 cd	2.06 ± 0.65 abcdefg	2.20 ± 0.42 d	2.10 ± 0.42 d
Zilavka	White	223.38 ± 54.90 cd	1.98 ± 0.79 bcdefg	2.85 ± 0.49 bcd	2.75 ± 0.35 bcd
Pr > F		0.036	0.025	0.001	0.001

* Means with different letters in the same column differ significantly ($p \leq 0.05$).

2.2. Sample Preparation and Extraction

The grape seeds were manually removed from the pulp and skin, and air dried. To obtain the powder, the dried seeds were ground using a coffee-grinder (Tefal, France). The analysis for each sample was carried out in triplicate. The extraction procedure was carried out as described by Tomaz et al. [25]. In brief, the solid–liquid extraction was performed using 125 mg of grape seed powder and 10 mL of extraction solvent, comprising of acetonitrile: water: formic acid (20:79:1, $v/v/v$). The extraction was completed on a magnetic stirrer (RTC basic, IKA, Staufen, Germany) at 400 rpm and temperature of 50 °C. The extraction time was one hour. After the extraction was completed, the extracts were filtered with a Phenex-PTFE (polytetrafluorethylene) 0.20 µm, syringe filter (Phenomenex, Torrance, CA, USA), and analyzed by HPLC.

2.3. HPLC Analysis

The HPLC separation, identification and quantification of polyphenolic compounds were performed on an Agilent 1100 Series system (Agilent, Germany), according to the method described by Tomaz and Maslov [26]. The separation was performed with a reversed-phase column LunaPhenyl-Hexyl (4.6 × 250 mm; 5 µm particle; Phenomenex, Torrance, CA, USA). The solvents were water: phosphoric acid (99.5:0.5, v/v, eluent A), and acetonitrile: water: phosphoric acid (50:49.5:0.5, $v/v/v$, eluent B). The flavan-3-ols were detected by FLD detector at λex = 225 nm and λem = 320 nm and by DAD detector at 280 nm. The individual compounds in the berry seeds' extracts were identified by matching the retention time of each chromatographic peak with external standards. The individual peaks of the flavan-3-ols were quantified using a calibration curve of the corresponding standard compound which was based on the peak area. All of the used standard compounds were obtained from Extrasynthese (Genay, France) and were analytical standard grade with a purity greater than 98%. The results are expressed in mg/kg.

2.4. Statistical Analysis

The individual polyphenolic compounds from the grape seeds were analyzed, using one-way ANOVA and the differences between the given means were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$). The data reported in all of the tables are the average of triplicate observations. The mean values were used for principal component analysis (PCA). The statistical analysis was carried out using XLSTAT program (Addinsoft, 2020, New York, NY, USA).

3. Results and Discussion

3.1. Seed Content

The varieties differed significantly in all of the analyzed parameters, the most important ones being the seed share per berry and per bunch. The seed share per berry and per bunch were similar and ranged 1.9–7.4%. The highest seed content both in the berry and in the bunch was recorded for Tribidrag and Malvasija dubrovacka, followed by Plavina and Vranac. The varieties Cetinka, Dobricic, Zilavka, Debit had a seed content of around 3%. The other varieties had a seed content around 2%. Although there was no statistically significant differences, the Grk variety had the smallest seed content both in berry (1.92%) and in bunch (0.57%). It is worth noting that Grk is the only analyzed variety with a female flower, resulting in two types of berries in the bunch, fully developed seeded and undersized seedless [27]. Thus, this is probably one of the reasons why the seed content in the bunches was under 1%.

3.2. Flavan-3-Ols in Grape Seeds

The flavan-3-ols are one of the main classes of polyphenolic compounds, influencing the sensory properties of the wine, contributing to the overall astringency, bitterness, and the perception of dryness in the wine [28,29]. However, due to their positive biological activities, these compounds have become increasingly popular, confirmed by the number of functional foods combining them in various formulations [30]. Moreover, grape seeds contain 60–70% of the extractable phenols present in grapes [5], making them a valuable source of polyphenolic compounds. In Table 3 the mean values of the analyzed polyphenolic compounds for all of the cultivars are shown. A total of eight individual compounds of flavan-3-ols were identified and quantified in the grape seeds, and those were catechin, epicatechin, epigallocatechin, epicatechin gallate, gallocatechin, and procyanidins B1, B2, B4.

The analyzed grape cultivars differed significantly in the content of the flavan-3-ols. The total content of the flavan-3-ols ranged from 1723.66 mg/kg to 17,966.52 mg/kg. The most abundant compound was catechin, comprising 35.25% of the total flavan-3-ols, and followed by epicatechin (30.89%), procyanidins B2 (16.19%), B4 (9.92%), and B1 (3.70%). The other compounds were represented with less than 1% in the total content of flavan-3-ols.

Table 3. Content of flavan-3-ols (mg/kg) in analyzed red and white cultivars. Means with different letters in the same column differ significantly ($p \leq 0.05$).

Variety	Color	Catechin	Epicatechin	Epigallocatechin	Epicatechin Gallate	Gallocatechin	Procyanidin B1	Procyanidin B4	Procyanidin B2	Total Flavan-3-Ols
Babica	Red	6233.13 ± 2548.51 b	4257.02 ± 850.86 b	38.31 ± 10.24 b	164.31 ± 79.00 a	155.07 ± 105.49 a	401.80 ± 95.91 a	1400.60 ± 433.39 a	2084.89 ± 576.48 ab	14,953.29 ± 4697.78 b
Babic	Red	3491.26 ± 506.59 de	3967.44 ± 513.66 bc	0.00 ± 0.00 cd	157.19 ± 45.86 ab	53.38 ± 10.10 cde	343.56 ± 77.40 abc	951.16 ± 167.56 bcd	1901.80 ± 181.63 abc	11,095.50 ± 1276.97 c
Dobricic	Red	4206.28 ± 1783.25 cd	3623.46 ± 1657.45 bcd	6.64 ± 16.26 cd	130.01 ± 69.21 ab	50.91 ± 4.12 cde	287.62 ± 112.17 bcdef	1062.37 ± 392.24 bc	1620.28 ± 707.27 cde	11,127.40 ± 4754.21 c
Lasina	Red	3049.24 ± 726.43 de	3342.35 ± 835.11 bcd	12.11 ± 20.96 c	100.70 ± 89.94 bc	54.31 ± 6.52 cde	328.80 ± 38.86 abcd	814.38 ± 242.17 cde	1660.74 ± 360.22 bcde	9607.69 ± 2237.02 cd
Ljutun	Red	4135.25 ± 329.79 cd	3745.28 ± 705.22 bc	0.00 ± 0.00 d	147.77 ± 55.75 ab	42.67 ± 9.44 de	358.59 ± 34.98 abc	1052.81 ± 139.98 bc	1789.39 ± 172.40 abcd	11,455.87 ± 871.41 c
Nincusa	Red	833.80 ± 462.01 gh	1296.58 ± 516.26 hij	0.00 ± 0.00 d	48.36 ± 14.35 cdef	80.72 ± 19.73 bcd	234.07 ± 86.44 efgh	452.04 ± 117.72 ghi	930.35 ± 261.51 gh	4024.24 ± 1459.56 fgh
Plavac mali	Red	8124.22 ± 221.37 a	5224.38 ± 100.15 a	53.68 ± 1.19 a	138.37 ± 8.12 ab	52.63 ± 1.41 cde	392.91 ± 10.73 a	1539.43 ± 26.51 a	2207.81 ± 37.12 a	17,966.52 ± 399.24 a
Plavina	Red	4945.50 ± 566.45 c	3144.57 ± 651.87 cde	0.00 ± 0.00 d	137.08 ± 57.03 ab	66.85 ± 3.33 cde	373.59 ± 56.79 ab	1162.97 ± 216.21 b	1447.45 ± 233.43 def	11,473.41 ± 1772.70 c
Tribidrag	Red	1417.59 ± 684.75 fgh	1718.51 ± 793.86 ghij	0.00 ± 0.00 d	14.85 ± 20.64 ef	72.55 ± 5.68 cde	234.38 ± 98.56 efgh	649.95 ± 248.69 defg	1003.64 ± 371.61 fgh	5271.73 ± 2282.51 efg
Vranac	Red	1757.09 ± 603.27 fg	1654.93 ± 509.09 ghij	7.22 ± 17.67 cd	49.09 ± 25.73 cdef	88.08 ± 34.51 bc	358.15 ± 46.27 abc	885.29 ± 147.25 cd	1156.38 ± 247.52 fg	6162.14 ± 1543.89 ef
Bogdanusa	White	1653.10 ± 1151.04 fg	1051.29 ± 605.32 ijk	0.00 ± 0.00 d	19.87 ± 5.92 def	64.99 ± 15.80 cde	242.63 ± 78.26 defgh	486.21 ± 180.56 fghi	856.21 ± 399.74 ghi	4476.81 ± 2415.95 fg
Cetinka	White	3476.75 ± 277.26 de	2245.50 ± 537.78 efgh	0.00 ± 0.00 d	59.74 ± 25.00 cde	37.19 ± 21.60 e	320.24 ± 43.37 abcde	724.26 ± 99.56 def	851.51 ± 89.24 ghi	7885.48 ± 478.24 de
Debit	White	858.36 ± 336.54 gh	866.17 ± 227.03 jk	0.00 ± 0.00 d	37.59 ± 11.69 def	78.0809 ± 11.82 cd	191.75 ± 38.79 gh	315.29 ± 51.31 hi	602.18 ± 76.58 hi	3069.75 ± 662.19 gh
Gegic	White	263.24 ± 178.50 h	277.53 ± 71.11 k	0.00 ± 0.00 d	0.00 ± 0.00 f	78.40 ± 9.49 cd	278.44 ± 49.90 cdefg	276.68 ± 31.48 i	420.18 ± 53.71 i	1723.67 ± 308.68 h
Grk	White	2326.48 ± 660.31 ef	2741.60 ± 757.16 def	0.00 ± 0.00 d	68.70 ± 39.71 cd	116.41 ± 23.17 b	221.24 ± 23.17 fgh	720.97 ± 101.00 def	1166.30 ± 250.64 fg	7538.83 ± 1793.52 de
M. dubrovacka	White	1209.34 ± 465.01 fgh	1116.76 ± 824.79 ijk	0.00 ± 0.00 d	11.84 ± 28.08 ef	57.63 ± 8.23 cde	253.30 ± 8.23 defgh	433.70 ± 108.80 ghi	842.20 ± 330.70 ghi	4037.02 ± 1689.63 fgh
Posip	White	1609.79 ± 739.19 fg	2322.77 ± 1076.25 efg	0.00 ± 0.00 d	41.87 ± 37.06 cdef	67.05 ± 6.45 cde	203.98 ± 6.45 fgh	507.88 ± 159.19 fghi	1278.19 ± 473.46 efg	6143.39 ± 2437.25 ef
Prc	White	1809.78 ± 482.62 fg	1394.38 ± 68.95 ghij	0.00 ± 0.00 d	14.27 ± 3.82 ef	69.59 ± 28.87 cde	197.25 ± 28.87 gh	671.14 ± 53.84 defg	915.02 ± 46.13 gh	5207.20 ± 575.94 efg
Zilavka	White	869.76 ± 136.38 gh	1155.83 ± 154.08 ijk	0.00 ± 0.00 d	18.06 ± 9.38 def	81.01 ± 18.65 bcd	255.84 ± 18.65 defg	536.72 ± 112.31 efgh	930.76 ± 151.64 gh	3938.97 ± 596.29 fgh
Pr > F		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significant		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

The red cultivars had significantly higher content of all of the flavan-3-ols than the white cultivars (Figure 1). The exception was gallocatechin, the content of which was similar between the red and white cultivars. Furthermore, the epigallocatechin was not found in the white cultivars, as well as in some red cultivars. The red cultivars in which epigallocatechin was identified (Table 3) were Plavac mali (53.68 mg/kg), Babica (38.31), Lasina (12.11 mg/kg), and Vranac (7.21 mg/kg). Pantelic et al. [31] also identified epigallocatechin in the red varieties, Cabernet Sauvignon, Merlot, Cabernet Franc, Sangiovese, Pinot noir, and Prokupac, ranging from 3.73 mg/kg up to 39.29 mg/kg. Furthermore, the authors reported the presence of epigallocatechin in the white varieties, Sauvignon Blanc, Welschriesling, and Chardonnay. Among the red cultivars, the highest content of total flavan-3-ols (Table 3) was found in Plavac mali and Babica, while the lowest content was found in Nincusa, Tribidrag, and Vranac. Among the white cultivars, the highest amount of total flavan-3-ols was recorded for cultivars Cetinka, Grk, and Posip, while the lowest amount was recorded for Gegic and Debit.

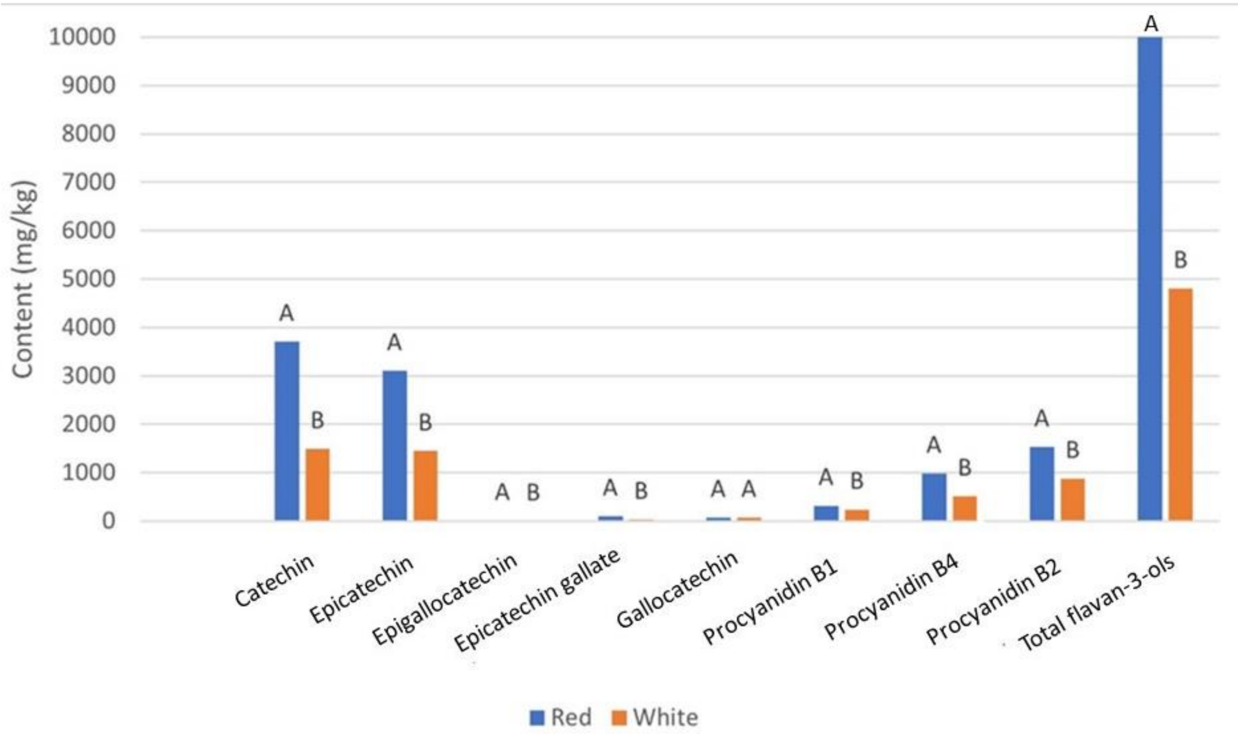

Figure 1. Mean values of total content of flavan-3-ols (mg/kg) in red and white cultivars. Different letters above individual compounds represent statistically significant difference ($p \leq 0.05$).

Regarding the most abundant compound, catechin, the highest content among the red cultivars was found in Plavac mali and Babica, while Nincusa, Vranac, and Tribidrag showed the lowest content. However, Nincusa and Tribidrag had an epicatechin content higher than catechin. In the case of Nincusa, the epicatechin content was almost two-fold higher than catechin. The results reported for Touriga Nacional and Touriga Francesa also showed a higher content of epicatechin than catechin [32]. Among the white varieties, the highest content of catechin was recorded for Cetinka and Grk. An epicatechin content higher than catechin was observed for Posip and Zilavka.

Among the analyzed procyanidins, the most abundant was B2, comprising 15.32% and 18.01% of the total flavan-3-ols in the red and white varieties. Even though the red varieties had a higher content of procyanidin B2, the white varieties had a higher share of this compound in the total content of flavan-3-ols (18.01% for the white and 15.32% for the red varieties). The highest content was recorded for the varieties Plavac mali, Babica and Babic for red, and Posip and Grk for white varieties.

The main constituents of the seed flavan-3-ols in both the red and the white varieties were catechin and epicatechin, while epicatechin gallate occurred in small amounts. This is in accordance with the reported results in Babic, Plavac mali [13], Pinot noir, Teroldego, and Cabernet Sauvignon [33] for the red varieties, and in Chardonnay, Sauvignon Blanc, Pinot Blanc, Riesling, Viognier, and Veltliner [34,35] for the white varieties. Regarding the differences between the red and white varieties, in our study, the red varieties had considerably higher content of all of the flavan-3-ols, which is in accordance with the results reported by Popov et al. [35]. On the other hand, in a study by Montealegre et al. [34] the white varieties had a higher content of flavan-3-ols than the red varieties. However, the author highlighted that the white varieties were less ripe than the red, which can influence the content of polyphenols in seeds. After catechin and epicatechin, the most abundant compound was procyanidin B2, in both the red and white cultivars, followed by procyanidin B4. Our results are in accordance with results reported in Merlot, Cabernet Sauvignon [36,37], Gamay, Chardonnay [37], Ugni Blanc, and Semillon [38].

3.3. Prediction of Flavan-3-Ols Yield in Conventional Production

The amount of grape pomace, which includes seeds, generated from winemaking is dependent on the grape cultivar and the pressing process, as well as the fermentation steps [39]. Traditionally, the grape pomace has been used as a fertilizer, animal feed, or to produce distillates. However, over the last decade, other approaches have been proposed for the production and utilization of grape pomace derivatives [40], mainly dietary fibers and polyphenolic compounds. Since there is an increasing demand for healthy and natural food preservation substances that can replace synthetic antioxidants and food preservation substances [41], the grape seed polyphenols are becoming an ingredient in novel quality products with health benefits.

In Table 4, the expected yields of flavan-3-ols are shown, if the grape yield is 10 t/ha. If the grape yield is 10 t/ha, the yield of flavan-3-ols, depending on the variety, would range from 0.18 kg/ha up to 73.98 kg/ha. The highest yield of total flavan-3-ols would come from Lasina, Plavina, and Plavac mali for the red varieties, and M. dubrovacka and Cetinka for the white varieties. Catechin, as the most abundant compound in grape seeds, would range 0.06–25.81 kg/ha. The highest yield would come from the varieties Plavina, Lasina, and Plavac mali. The smallest catechin yield would come from Grk and Gegic. After catechin, the highest yield would be epicatechin, ranging 0.07–25.74 kg/ha. The highest yield would come from the varieties Lasina, Plavina, and Tribidrag. The procyanidins, namely B1, B2, and B4, have a positive impact on human health. They possess antioxidant, anti-inflammatory, anticancerogenic, and antibacterial activity [3]. The procyanidins make up 56.35% (Gegic) of the total flavan-3-ol yield, while the total procyanidin yield ranged from 0.18 (Grk) to 73.98 (Lasina) kg/ha. The most abundant procyanidin was procyanidin B2, with a yield of 21.59 kg/ha (Lasina), followed by procyanidin B4 with 6.67 kg/ha (Lasina). In general, the red grape varieties had significantly higher procyanidin yields than the white grape varieties. Besides Lasina, among the red varieties, Plavina (59.45 kg/ha) and Plavac mali (37.73 kg/ha) had the highest yield of procyanidins, while among the white varieties, the highest yield was found in M. dubrovacka (26.58 kg/ha), followed by Cetina (24.6 kg/ha), and Posip (16.28 kg/ha).

In the Dalmatia subregion, the area under vineyards is 5 978 hectares, representing 31% of the total vineyard area in Croatia. Moreover, the grape seed extracts' global market value was estimated to be 392.1 million USD in 2015 [42], thus making grape seeds a source of added value.

Table 4. Prediction of flavan-3-ols yield (kg/ha) when grape yield is 10 t/ha. Means with different letters in the same column differ significantly ($p \leq 0.05$).

Variety	Color	Catechin	Epicatechin	Epigallocatechin	Epicatechin Gallate	Gallocatechin	Procyanidin B1	Procyanidin B4	Procyanidin B2	Total Flavan-3-Ols
Babica	Red	11.12 ± 1.92 c	7.96 ± 0.58 de	0.07 ± 0.01 ab	0.30 ± 0.14 b	0.26 ± 0.12 cd	0.75 ± 0.09 def	2.56 ± 0.18 cde	3.83 ± 0.18 fg	27.26 ± 2.03 def
Babic	Red	6.63 ± 0.96 de	7.54 ± 0.97 de	0.00 ± 0.00 c	0.30 ± 0.08 bc	0.10 ± 0.01 efg	0.65 ± 0.14 def	1.81 ± 0.31 ef	3.61 ± 0.34 fg	21.08 ± 2.42 fg
Dobricic	Red	10.15 ± 0.53 cd	8.64 ± 0.75 cde	0.01 ± 0.03 c	0.31 ± 0.13 b	0.14 ± 0.05 ef	0.71 ± 0.16 def	2.62 ± 0.21 cde	3.89 ± 0.27 fg	26.828 ± 1.56 def
Lasina	Red	23.479 ± 5.59 a	25.736 ± 6.43 a	0.09.16 a	0.775 ± 0.69 a	0.418 ± 0.05 ab	2.532 ± 0.29 a	6.27 ± 1.86 a	12.79 ± 2.77 a	73.98 ± 17.22 a
Ljutun	Red	10.08 ± 3.44 cd	8.61 ± 0.81 cde	0.00 ± 0.00 c	0.32 ± 0.03 b	0.10 ± 0.19 fg	0.85 ± 0.18 de	2.47 ± 0.62 cde	4.22 ± 0.90 ef	27.11 ± 6.01 def
Nincusa	Red	1.90 ± 1.35 fg	2.86 ± 1.67 f	0.00 ± 0.00 c	0.10 ± 0.02 cd	0.16 ± 0.05 def	0.51 ± 0.28 ef	0.97 ± 0.45 fgh	2.00 ± 0.96 hij	8.82 ± 4.90 hij
Plavac mali	Red	17.06 ± 0.46 b	10.97 ± 0.21 cd	0.11 ± 0.01 a	0.29 ± 0.02 bc	0.11 ± 0.00 efg	0.83 ± 0.02 def	3.23 ± 0.05 c	4.64 ± 0.07 def	37.73 ± 0.83 c
Plavina	Red	25.81 ± 2.69 a	16.15 ± 0.87 b	0.00 ± 0.00 c	0.68 ± 0.16 a	0.36 ± 0.07 bc	1.94 ± 0.11 b	6.00 ± 0.39 a	7.49 ± 0.46 b	59.45 ± 3.97 b
Tribidrag	Red	9.18 ± 2.51 cd	11.17 ± 2.79 c	0.00 ± 0.00 c	0.08 ± 0.11 cd	0.52 ± 0.14 a	1.54 ± 0.35 c	4.30 ± 0.71 b	6.66 ± 0.99 bc	34.50 ± 7.53 cd
Vranac	Red	8.85 ± 2.69 cd	8.35 ± 2.23 de	0.04 ± 0.08 bc	0.25 ± 0.12 bc	0.46 ± 0.19 ab	1.82 ± 0.17 bc	4.49 ± 0.56 b	5.85 ± 1.02 cd	31.15 ± 6.54 cde
Bogdanusa	White	3.174 ± 1.56 efg	2.09 ± 0.70 f	0.00 ± 0.00 c	0.05 ± 0.02 d	0.16 ± 0.78 def	0.52 ± 0.08 ef	1.02 ± 0.10 fgh	1.75 ± 0.35 ij	8.98 ± 2.61 hij
Cetinka	White	10.64 ± 1.57 c	7.24 ± 3.12 e	0.00 ± 0.00 c	0.17 ± 0.03 bcd	0.13 ± 0.08 efg	1.00 ± 0.30 d	2.20 ± 0.24 de	2.68 ± 0.79 ghi	24.60 ± 6.20 ef
Debit	White	2.38 ± 1.21 fg	2.36 ± 0.92 f	0.00 ± 0.00 c	0.10 ± 0.01 cd	0.21 ± 0.06 def	0.52 ± 0.15 ef	0.84 ± 0.18 gh	1.60 ± 0.33 ij	8.32 ± 2.83 ij
Gegic	White	0.56 ± 0.42 g	0.59 ± 0.19 f	0.00 ± 0.00 c	0.00 ± 0.00 d	0.16 ± 0.02 def	0.58 ± 0.05 ef	0.58 ± 0.08 hi	0.88 ± 0.15 jk	3.62 ± 0.81 ij
Grk	White	0.06 ± 0.01 g	0.07 ± 0.01 f	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 g	0.01 ± 0.00 g	0.02 ± 0.00 i	0.03 ± 0.00 k	0.18 ± 0.02 j
M. dubrovacka	White	8.27 ± 4.54 cd	7.1337 ± 4.29 e	0.00 ± 0.00 c	0.06 ± 0.13 d	0.36 ± 0.08 b	1.70 ± 0.76 bc	2.84 ± 1.13 cd	5.51 ± 2.45 cde	26.58 ± 12.62 ef
Posip	White	4.41 ± 2.71 ef	6.13 ± 3.43 e	0.00± 0.00 c	0.09 ± 0.05 cd	0.17 ± 0.04 def	0.52 ± 0.23 ef	1.31 ± 0.58 fg	3.37 ± 1.72 fgh	16.28 ± 8.68 gh
Prc	White	3.93 ± 1.61 ef	2.94 ± 0.56 f	0.00 ± 0.00 c	0.03 ± 0.01 d	0.14 ± 0.04 ef	0.41 ± 0.09 f	1.42 ± 0.31 fg	1.92 ± 0.29 hij	11.08 ± 2.87 hi
Zilavka	White	2.42 ± 0.60 fg	3.21 ± 0.71 f	0.00 ± 0.00 c	0.05 ± 0.03 d	0.22 ± 0.05 de	0.71 ± 0.13 def	1.50 ± 0.45 fg	2.59 ± 0.65 ghi	10.95 ± 2.66 hi
Pr > F		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significant		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

3.4. PCA Analysis

To evaluate the variability in the seed flavan-3-ols of 20 varieties, principal component analysis (PCA) was performed. The results of the PCA are shown in Figure 2, which represents the distribution of the varieties in the space defined with the first two canonical factors, which explained 87.36% of variability (75.17% for F1 and 12.19% for F2). The Figure also shows the vectors which explain the direction of the variables in the same space.

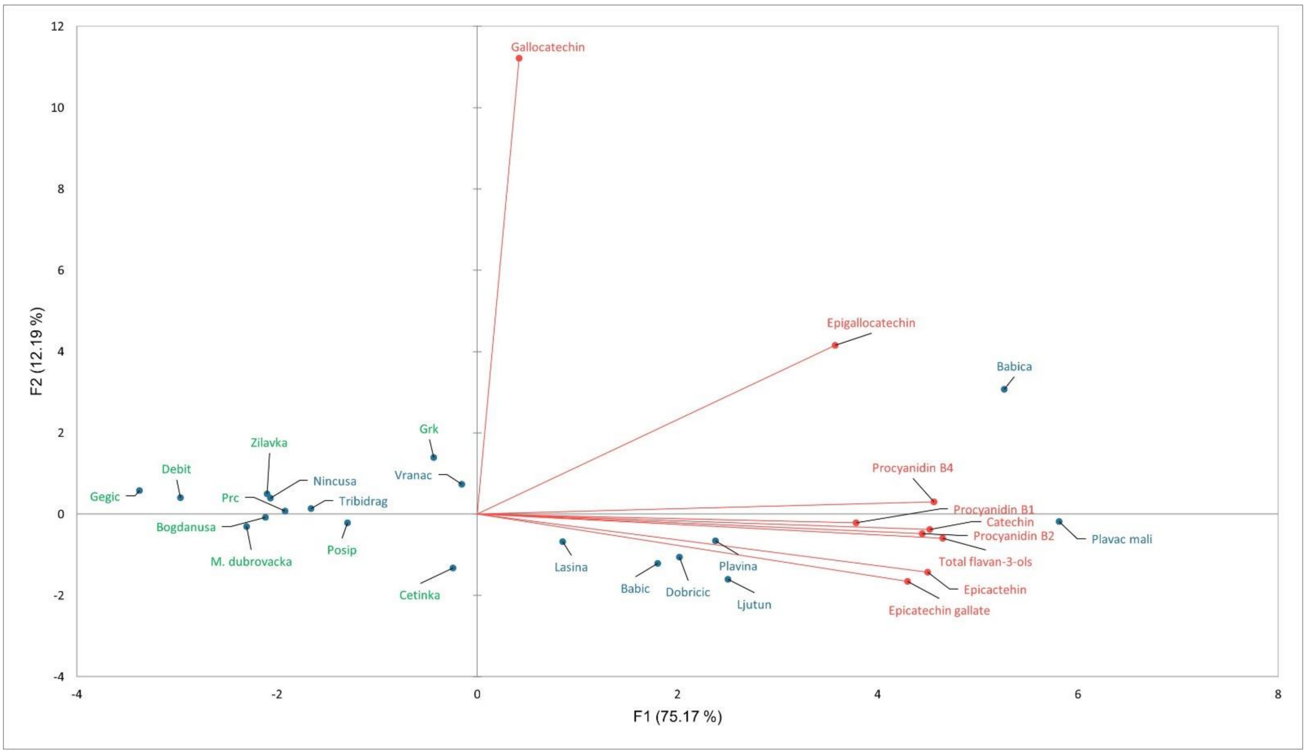

Figure 2. The scatter plot of PCA analysis. Blue shows the red varieties, green shows the white varieties.

Half of the varieties are located near the x axis in the quadrants II and III, and most of these varieties are white varieties. A few of the red varieties are situated near white varieties, which are Nincusa, Tribidrag, and Vranac. When looking at their flavan-3-ol profile, these varieties have a similar profile to the white varieties. This means a low content of total flavan-3-ols comparing to the analyzed red varieties. The rest of the red varieties are situated in the IV quadrant. Two more varieties that are separated from the rest of the red varieties are Plavac mali and Babica. When looking at the vectors, these varieties have a higher content of all of the individual compounds, as well as the total flavan-3-ols. These results confirm the overall profile given in Table 3.

4. Conclusions

This research gives insight into the phenolic profiles of the seeds from Croatian grapevine varieties. The flavan-3-ol profiles varied among the varieties, with a higher content recorded for the red varieties. The most abundant compounds were catechin, epicatechin, and procyanidin B2. Based on the analyzed profiles, the potential yield of flavan-3-ols was calculated. Depending on the variety, the potential yield of flavan-3-ols grown in the coastal region could reach up to 73.97 kg/ha. This shows that the usage of grape seeds after vinification could bring an added value to the winemaking industry.

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