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Aroma Profile of Monovarietal *Pét-Nat* Ciders: The Role of Croatian Traditional Apple Varieties

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Abstract: The aromatic and sensory profiles of monovarietal sparkling ciders made according to the modified *Méthode Ancestrale* or *Pétillant Naturel* (*Pét-Nat)* method were established. Three Croatian traditional apple varieties ('Božićnica', 'Bobovac', and 'Crvenka') were basic raw materials for *Pét-Nat* ciders in this study. The basic apple must and cider parameters were determined by applying OIV methods and nitrogenous compounds, total phenols, and color parameters were analyzed by spectrophotometer. Volatile compounds in final *Pét-Nat* ciders were determined by SPME-Arrow-GC/MS method and Odor Active Values (OAV) were calculated. The results show that variety significantly altered the pH value, color, aromatic and sensory profile of *Pét-Nat* ciders. The main contributors (OAV > 1) to the aroma of all *Pét-Nat* ciders were 1-hexanol, 1-propanol, (6Z)-nonen-1-ol, 1-dodecanol, hexanoic, octanoic and isovaleric acid, citronellol, ethyl hexanoate, ethyl butanoate, ethyl-9-decenoate and isoamyl acetate, eugenol and methionol. 'Božićnica' *Pét-Nat* was differentiated by a high concentration of 1-decanol and 4-ethylphenol, 'Bobovac' by 4-vinyl guaiacol and 'Crvenka' by 4-ethyl guaiacol. Sensory analysis showed that the highest rated overall quality was attributed to 'Crvenka' *Pét-Nat* cider, with the high-quality color, fruity odor ('apple','apple juice/compote', 'pineapple', and 'buttery') and well-balanced taste. This research demonstrates the possibilities in the production of natural sparkling cider from traditional Croatian apple varieties by analyzing the composition and quality of the final product for the first time.

Keywords: cider technology; OAV; sparkling cider; spontaneous fermentation; volatile compounds; sensory attributes

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1. Introduction

The consumption and production of cider, a fermented beverage made from modern and traditional apple varieties (*Malus domestica* Borkh.), is an important part of the global market which together with other fruit wines have the fastest growing rates among all alcoholic beverages despite of a decline in total alcoholic beverages revenue growth [1,2]. According to Fior Markets [3] ciders make less than 1% of the total alcoholic beverages market with global sales estimated at $4.34 billion in 2018 and projected rising to $5.43 billion by 2026. Cider, or apple wine, is low-alcoholic beverage abundant with nutrients [4] that is encountered in many countries around the world, especially in those without agro-climatic conditions required for grapevine cultivation. The impressive statistics, together with the high yield and plentiful varieties of apples in the world make it a very promising segment of the fruit industry [5,6]. According to The European Cider and Fruit Wine Association (AICV) [2] ciders and sparkling ciders are still predominantly European. Within Europe, the main cider-making countries based largely on farmhouse production are the United Kingdom (cider), Spain (*sidra*), France (Normandy and Brittany—*cidre*), Germany (*apfelwein*) and Ireland, followed by Poland, Finland, Ukraine, Czech Republic, etc. [7,8]. Among the fastest growing European cider markets during the 5-years period of 2015–2020, Croatia was in the 6th place with production of 39,160 hL of cider in 2020, after Moldova, Macedonia, Slovenia, Ukraine and the Czech Republic [9].

The Croatian cider market is characterized by the production of industrial ciders, mostly flavored ones, with some individual enthusiastic attempting of making craft ciders from old Croatian apple varieties that were never analyzed in this context. Although pushed out by commercial apples, traditional varieties of apples with a long tradition of production, mostly collected and described in the Plant genetic resources bank [10] are a valuable resource and natural heritage in Croatia, receiving more scientific attention recently [11–13]. According to previous research, apple variety has the primary attribute influencing the volatile composition of cider [14], together with control of the ripening stage [13], which opens the wide area of analyzing the potential and role of traditional Croatian apple varieties in the growing cider market.

The term cider is usually a synonym for cyder, but in some countries cyder can mean apple juice or non-alcoholic beverage, in the USA, which has undergone growth in its production, fermented apple juice is known as hard cider [7]. As an alcoholic beverage, cider can be obtained by complete or partial alcoholic fermentation of fresh apple juice, diluted apple juice concentrate, or a mixture of both [2,15]. The alcohol content, in Europe, is within the range of 1.2% to 8.5% alcohol by volume (ABV) and the adding of distilled alcohol is forbidden. Depending on the ABV, ciders can be classified as soft ciders (1–5%) or hard ciders (6–7%) [16]. Reduced-alcohol ciders on the European market are classified as alcohol-free ciders (<0.5% ABV) or low-alcohol ciders (0.5–1.2% ABV) [2]. Furthermore, ciders can be dry (<20 $g \cdot L^{-1}$), semidry (30–50 $g \cdot L^{-1}$) or sweet (>50 $g \cdot L^{-1}$). While sweet ones contain residual sugar from fermentation, or are sweetened after, dry ciders do not contain sugar, with 6–7% ABV. Still ciders have a low sugar content and do not contain carbon dioxide (CO_2), while sparkling ciders have a low sugar content and an alcohol content higher than 3.5% ABV, with partial retention of the CO_2 formed during fermentation or carbonation [7].

Sparkling cider can be made by traditional or *champenoise* method characterized by secondary fermentation in the bottle. Additional fermentation of still, base apple wine (5–6% ABV) is mixed with a combination of yeast strains, sugar liqueur and bentonite resulting in sparkling wine with 9–10% ABV. Considering that autolysis of yeasts takes place during maturation on lees, the sensory and foaming properties of the cider are significantly modified [17,18].

As part of the general trend toward natural wines, sparkling ciders can be obtained by *Méthode Ancestrale* or *Pétillant Naturel* (*Pét-Nat)* as one of the oldest methods of natural sparkling wine production. *Pét-Nat* involves authentic craftsmanship, with production in mostly small quantities, and the natural handling of wine. In *Pét-Nat* production, the natural (spontaneous) fermentation driven by indigenous flora present on apples, equipment, and in the cellar, is interrupted as the wine is moved from a vat into individual bottles while it is still fermenting, and then sealed under a crown cap. The traditional production of cider in France is characterized by spontaneous fermentation with indigenous microorganisms, and bottling when its density is between 1.009 and 1.029, depending on the desired level of sweetness, and ADY can be added before bottling to obtain sparkling cider [19]. Modification of this method can be achieved by finishing the fermentation in isobaric tank and bottling of sparkling cider after ageing on the lees, an isobaric filler. *Pét-Nat* ciders are usually completely fermented and dry, and the pressure in the bottle is usually 2.5–3 bar. *Pétillant naturel* wines are commonly unfiltered, which leaves them cloudy, with some lees sediment at the bottom of the bottle.

The sensory profile of *Pét-Nat* ciders is significantly associated with raw material, production parameters and microbial activity [19]. Cider microbiota are strongly influenced by variety, and picking apples from the ground brings more bacterial diversity [20]. Due to the variations in the proportions of a 'resident' and 'transitory' microflora the cider yeast population fluctuates from vintage to vintage [21]. The microbial ecology of ciders is complex, and includes several genera, species and strains of yeasts and bacteria [22,23]. Yeast diversity is higher in cider musts than in bottled ciders and among many species [24], the main yeasts found in cider are *Saccharomyces* yeasts [19,25]. According to Coton et al. [24] and Suarez Valles et al. [26], in French and Spanish ciders, *Saccharomyces bayanus* is the predominant species from the beginning to the middle steps of the fermentation process, whereas *Saccharomyces cerevisiae* takes over the process in the final stages of fermentation.

The cider-making process typically involves three main stages: apple crushing and pressing out the juice, followed by the most important stage of elaboration, fermentation. This includes the classical alcoholic fermentation of sugars into ethanol performed by yeast strains and malolactic fermentation (MLF), and processed by lactic acid bacteria (LAB) that can occur during the maturation [19]. In the late 1980s, modern ciders were born: ciders containing juice and flavorings began to be produced, alongside traditional ciders. The industry calls these 'flavored ciders', and they can contain ingredients such as juice of other fruits, extracts, flavorings, etc. [2]. Since trends over the last few years have resulted in diverse, natural, and locally produced beverages, cider reappeared in a food markets, restaurants and bars [6] as a fermented beverage, for which the recognition of *terroir* is important for appreciation [19]. With increased cider consumption, cider makers were looking for ways to diversify their products. The newest trend in cider production is natural craft ciders, using whole juice from local apple varieties without any addition of preservatives, sugar, or active dry yeast (ADY) to distinguish them from industrial ciders made from concentrate, water, and additives. In addition to the varietal choice and maturity of the apples [27], the cider typicity is significantly associated with microbial activities and indigenous microorganisms [7,12,14]. In Asturias, traditional cider is made by spontaneous fermentation (alcoholic and malolactic) of apple musts, followed by an optional step of maturation on lees, which is claimed to bring about positive sensory characteristics, such as improved foaming properties and aroma complexity [28], although lees can retain undesirable components, such as sulfurous compounds and volatile phenols [29,30].

Since there are only two studies on apple wines from Croatia [31,32], and with the recent increased interest in traditional apple varieties [11–13], the aim of this study was to obtain the missing knowledge about traditional apple varieties, namely their byproducts and composition. To the best of our knowledge, there is no information available on the sparkling cider composition produced by the *Pét-Nat* method, which is a novelty of the Croatian cider market. In the present study, three natural monovarietal *Pét-Nat* ciders were produced to investigate the effect of traditional apple varieties ('Božićnica', 'Bobovac', and 'Crvenka') on their basic composition, aromatic, and sensory profiles.

2. Materials and Methods

2.1. Plant Material, Location and Climatic Conditions

Apple fruits for this research were harvested in a private ecological collection orchard of traditional apple varieties in the estate of Perenci in Požega-Slavonia County (45°23′45.0″ N, 17°34′06.3″ E). Characteristic of the orchard area is the continental humid or semi-humid climate with moderately warm/hot summers and warm autumns, with moderate amounts of precipitation and occasional periods of drought. The altitude of the orchard is 260 m, with favorable microclimate for the growth of fruit trees and the ripening and quality of apple fruit. The soil is deep and has a good structure and suitable organic and nutrient composition for apple cultivation. The orchard was established in 2000 on an area of about 3.5 ha. The planting distance is 7 × 7 m, and the rootstock for apple seedlings is determined according to the traditional method of apple cultivation in this area.

The research was conducted during the 2020 harvest on the varieties 'Bobovac', 'Božićnica' and 'Crvenka' (Figure 1). The fruits were harvested at the optimal harvest time in October and delivered to the laboratory of the Department of Pomology University of Zagreb, Faculty of Agriculture, where they were photographed, analyzed, and forwarded for further processing. The fruits of the examined varieties had a well-developed complementary skin color (different intensity of red color), were sweet and sour in taste, with average soluble solids content between 14° and 15° Brix, total acidity about 0.7% and pH of 3.0 to 3.4, which makes them bittersweet to sharp varieties according to the English classification of cider apple varieties [33].

Figure 1. Fruits of the investigated apple varieties (source: Skendrović Babojelić, 2020).

2.2. *Apple Fruit Processing and Fermentation*

In the harvest 2020, 700 kg of apples per variety were used for the experiment. In total, 70% were picked from the ground, and 30% were manually harvested from the trees. The apple fruits were washed and crushed by heavy rotor and then shredded through the cutting screen in centrifugal mill (Voran RM 2.2), and then pressed with a packing press with two moveable juice basins (sliding carriage) and a hydraulic power unit (Voran packpress 100 P2), with an initial pressure of 2 bar.

The juice of each variety obtained after pressing was directly transferred into three 90 L-isobaric stainless steel unitanks for spontaneous alcoholic fermentation, without the addition of sulfites and commercial yeasts. Fermentations started after three days at 18 °C without temperature control during the fermentation. The fermentation lasted for 21 days, with daily control of dynamics and sensory properties; the maximum temperature during that period reached 24 °C. After alcoholic fermentation, the stirring of sediment was conducted every 48 h during the next four months by keeping the wine pressured with CO_2 on 2.5 bar inside the tanks. After the ageing period, the transfer of cider into 750 mL bottles was performed using an isobaric filler, without losing the carbonation. Sparkling ciders were stored for one month in a dark and cool room until sensory evaluation and analysis.

2.3. *Physicochemical Analysis*

The basic apple must and wine parameters, including alcohol content (%, *v/v*), pH values, total and volatile acidity, were quantified by applying methods recommended by the International Organization of Vine and Wine [34].

2.4. *Malic Acid Analysis*

The analysis of individual acids (malic acid) was carried out by an HPLC system Agilent Series 1100 equipped with a diode array detector (Agilent, Palo Alto, Santa Clara, CA, USA). In brief, the determination was performed isocratically with a flow rate set to 0.6 mL·min^{-1} with 0.065% phosphoric acid (p.a. Merck, Darmstadt, Germany) as a mobile phase. The Column Aminex HPX-87H 300 mm × 7.8 mm i.d (Bio-Rad Laboratories, Hercules, Newark, CA, USA) was heated at 65 °C, while the detector was set to 210 nm. Quantification was performed by calibration curve obtained by standard. The results were expressed in g·L^{-1}.

2.5. Spectrofotometrical Analysis

2.5.1. Nitrogenous Compounds Analysis

Free-amino nitrogen (FAN) and ammonium (NH^{4+}) were determined by spectrophotometer Lambda XLS+, (PerkinElmer, Waltham, MA, USA), using the method proposed by Dukes and Butzke (1998) [35]. Yeast assimilable nitrogen (YAN) represents the sum of FAN and NH^{4+}.

2.5.2. Total Phenols

Total phenols (TP) were determined spectrophotometrically using the method by Singelton and Rossi [36] based on the color reaction of the phenolic compounds with the Folin-Ciocalteu reagent. The average of three measurements was used as the final absorbance value. The results were expressed in gallic acid equivalents (mg·L^{-1} of gallic acid).

2.5.3. Color Parameters

The color intensity, hue/tint/tonality and pigments were analyzed by the direct measurement of wine absorbance at 420, 520, and 620 nm by using a Lambda XLS+ spectrophotometer (PerkinElmer, Waltham, MA, USA). The color intensity (CI) and color tint/tonality/hue (T) were calculated as follows: CI = Abs 420 + Abs 520 + Abs 620 [37].

2.6. Volatile Compounds Determination

The SPME-Arrow extraction was performed by RSH Triplus autosampler (Thermo Fisher Scientific Inc., Brookfield, WI, USA). The 5 mL of sample and 2 g of NaCl was put in 20 mL headspace screw-top vials sealed with PTFE/silicone septum containing caps. The sorption conditions were as follows: the sample was incubated at 60 °C for 20 min and then SPME-Arrow fiber DVB/CWR/PDMS (120 µm × 20 mm; Thermo Fisher Scientific Inc., Brookfield, WI, USA) was exposed for 49 min. Then, the fiber was inserted into GC injector port operating in splitless mode and desorbed at 250 °C for 10 min.

Sample analysis was conducted on TRACETM 1300 Gas Chromatographer coupled to ISQ 7000 TriPlus quadrupole mass spectrometer (Thermo Fisher Scientific Inc., USA) equipped with TG-WAXMS A capillary column (60 m × 0.25 mm × 0.25 µm film thickness; Thermo Fisher Scientific, USA). The volatile compounds injected into the inlet were delivered to the column at a splitless mode and helium was used as a carrier gas at a constant flow rate 1 mL/min. The oven temperature program was as follows: initial temperature of 40 °C was maintained for 5 min, increase 2 °C/min to 210 °C and hold for 10 min. The MS spectra were recorded in the electron impact ionization mode (EI) at an ionization energy 70 eV. The mass spectrometer was performed in full scan mode in the range 30–300 *m/z*. The data obtained were processed using ChromeleonTM Data System (Thermo Fisher Scientific Inc., USA). Identification was done by comparing retention times, retention index and mass spectra with those of standards and with the data available in Wiley Registry 12th Edition/NIST Spectral Library. Quantification was done by calibration curves. The curves (based on quantification ions) were constructed with Chromeleon™ Chromatography Data System (CDS) software. For all available standards, six different concentrations were prepared while for the other compounds semi-quantitative analysis was performed. Their concentrations were expressed in equivalents of similar compounds, with the assumption that a response factor was equal to one.

2.7. Determination of Odor Activity Values

Each chemical substance can have a specific influence on the wine aroma. It can be presented by the odor activity value (OAV) which remains an essential concept in flavor research. OAV is calculated as the quotient of its concentration (c) and corresponding odor detection threshold (t) reported in the literature [38]. Volatile aroma substances with an OAV $\geq$ 1 can have a direct impact on aroma, and they are usually marked as one of the most significant volatile substances or the most active odors [39]. Volatiles with OAVs < 1 can also positively influence the wine aroma complexity, and aromatic intensity of other compounds through synergistic effects [40].

2.8. Sensory Analysis

Sensory analysis of *Pét-Nat* ciders was carried out by seven experts (four females and three males), members of the Committee for Organoleptic Evaluation of Wine and Fruit Wines appointed by the Ministry of Agriculture. The panelists were specialists in the field and well-experienced based on the evaluations in the Croatian Agency for Agriculture and Food, accredited according to the HRN EN ISO/IEC 17065 standard for the implementation of the procedure for placing wines with PDO, i.e., certification of wines with a label of origin, on the market. Panelist did not receive compensation for the evaluation that was approved and performed in a Laboratory for Sensory Analysis of Agricultural and Food Products, University of Zagreb Faculty of Agriculture, under standardized conditions. The *Pét-Nat* ciders were kept and served at 10 °C in standard wine tasting glasses (ISO 3591:1977). Blind tasting by Quantitative Descriptive Analysis (QDA) of coded *Pét-Nat* ciders made from different apple varieties was performed by three replicates in the experiment randomly. A total of 20 sparkling wine and cider attributes [41] for color, foamability, odor, taste and overall quality (Figure 3) were selected by the research group and further developed and evaluated by panelists. Quantification was performed a six-point scale, on a paper sheet, as follows: 0–1 weak, 2–3 medium and 4–5 strongly pronounced attribute. Sample differences and significance were graphically presented by radar graph based on the analysis of one-way variance (ANOVA) of the evaluation data.

2.9. Statistical Analysis

One-way analysis of variance (ANOVA) was used to test the significance of the effect of the apple varieties for all measured parameters. In the case of significant effect obtained by ANOVA, the means were compared using Duncan's multiple range test. To evaluate the variability in aromatic profiles of *Pét-Nat* ciders from three varieties, principle component analysis (PCA) was performed using the average value of aromatic compounds with OAV > 1, and variables and observation scores for the first two canonical factors were used to create scatter plots to explain multivariate differences among samples. All of the analyses were carried out using XLSTAT software v.2020.3.1. (Addinsoft, New York, NY, USA).

3. Results and Discussion

3.1. Apple juice Analysis

After pressing apples of each variety, a samples of juice were analyzed. All measurements important for the cider production were done in triplicate (Table 1). Cider musts, and generally, are known to be of much lower density, acidity and nitrogen content in comparison to wine musts [42]. There was no difference between juices density between the three apple varieties; however, the highest acidity and malic acid concentration, together with the lowest pH value was observed in 'Crvenka' juice. Both apple juices and ciders contain mainly malic acid (≅5 $g \cdot L^{-1}$) generating pH value ranging from 3.0–3.5, which usually prevents the growth of spoilage flora [19]. In modern fermentation, a high concentration (4.8 $g \cdot L^{-1}$) of malic acid is observed due to acidic apples, whereas in traditional method of fermentation, malic acid is low (3–3.8 $g \cdot L^{-1}$) [7]. The process of cider making usually maintains the TA (as malic acid) between 4.5–7.5 $g \cdot L^{-1}$ with lower levels in English cider from bittersweet apples, and the higher levels in North American sparkling cider [14]. The same juice was highest in yeast assimilable nitrogen (YAN), which is an essential nutrient absorbed and utilized by yeast and essential in the synthesis of aromatic compounds [43]. All concentrations of YAN in apple juices were lower than typical recommended amount of 150–200 $mg \cdot L^{-1}$, which is the minimum level necessary to prevent problems in fermentation kinetics [44]. The lowest level of YAN in 'Bobovac' juice resulted in *Pét-Nat* cider with residual sugar or stuck fermentation, while the fermentation in the other two ciders was smooth and complete (Table 2).

Table 1. Quality parameters of apple juices before fermentation.

Parameter	'Božićnica'	'Bobovac'	'Crvenka'
Density (Oe°)	56 ± 2	57 ± 1	55 ± 1
pH	3.38 ± 0.3 a	3.33 ± 0.2 b	3.24 ± 0.3 c
Total acidity, TA ($g \cdot L^{-1}$) *	5.6 ± 0.05 c	6.1 ± 0.1 b	8.0 ± 0.05 a
Malic acid ($g \cdot L^{-1}$)	4.96 ± 0.05 c	5.18 ± 0.03 b	6.41 ± 0.02 a
FAN ($mg \cdot L^{-1}$)	16.7 ± 0.09 a	12.7 ± 0.08 c	14.72 ± 0.1 b
NH^{4+} ($mg \cdot L^{-1}$)	97.2 ± 1.7 b	94.6 ± 1.6 c	124 ± 1.9 a
YAN ($mg \cdot L^{-1}$)	113.9 ± 1.0 b	107.3 ± 1.5 c	138.7 ± 1.1 a

* as malic acid; results are means of three measurements; means with different letters differ significantly among varieties ($p \leq 0.05$) based on the Duncan's multiple rang test.

Table 2. Basic enological parameters of *Pét-Nat* ciders.

Parameter	'Božićnica'	'Bobovac'	'Crvenka'
Specific gravity (20/20 °C)	0.9989 ± 0.002 c	1.0006 ± 0.003 a	1.0001 ± 0.001 b
Alcohol (*v/v*, %)	7.3 ± 0.06 a	7.1 ± 0.03 b	7.0 ± 0.03 b
Dry extract ($g \cdot L^{-1}$)	23.2 ± 0.03 b	27.1 ± 0.03 a	27.1 ± 0.02 a
Residual sugar ($g \cdot L^{-1}$)	1.4 ± 0.03 b	5.8 ± 0.06 a	<1.0 ± 0.03 c
Total acidity [1] ($g \cdot L^{-1}$)	3.5 ± 0.03 c	5.2 ± 0.06 b	8.6 ± 0.05 a
Volatile acidity [2] ($g \cdot L^{-1}$)	0.43 ± 0.01 a	0.35 ± 0.02 b	0.26 ± 0.01 c
pH	3.89 ± 0.05 a	3.63 ± 0.01 b	3.38 ± 0.01 c
Ash ($g \cdot L^{-1}$)	2.51 ± 0.01 c	2.56 ± 0.01 b	2.60 ± 0.01 a

[1] as malic acid; [2] as acetic acid; means (n = 3) with different letters differ significantly among varieties ($p \leq 0.05$) based on the Duncan's multiple rang test.

3.2. *Pét-Nat Ciders Basic Analysis*

The final product characteristics are given in Tables 2 and 3. The basic parameters on *Pét-Nat* ciders showed the average alcohol content of 7.13% (*v/v*) with no significant differences between the varieties (Table 2). An overall increase in pH value was observed (0.1–0.5), with significant differences between samples, the highest being observed in 'Božićnica'. The highest drop in total acidity, accordingly, was noted in 'Božićnica', while in 'Crvenka' cider, an increase, in comparison to juice, was observed. The fluctuation in the metabolism of acids is influenced by many factors, such as temperature, pH, and oxygenation, the microorganisms involved, and the chemical composition of the medium (nitrogen compounds and SO_2) [45]. The content of polyphenols affects the quality of ciders through organoleptic characteristics such as color, bitterness, astringency and aroma [7] and their fluctuations can be affected by the adsorption and desorption ability of yeast [45]. The total phenols (TP) content (Table 3) of *Pét-Nat* ciders was within the range 416–539 $mg \cdot L^{-1}$, with significant differences between the varieties. The TP content was thus lower than the range in apple wines of 734–2463 [46] and 792–3399 $mg \cdot L^{-1}$ depending on the variety. According to other authors, traditional varieties have the same groups of polyphenols as commercial but in higher or similar amounts [12,47,48]. The investigation of twenty-five traditional apple varieties, in higher or similar polyphenol concentration in comparison to a commercial variety 'Idared', mostly grown in Croatia [12]. In the available literature, one study reported 660 $mg \cdot L^{-1}$ of TP in sparkling cider, which decreased after the secondary fermentation [49] due to the adsorption ability of yeast. The highest concentration of TP was determined in 'Bobovac' sparkling cider (Table 2), although 'Božićnica' and 'Crvenka' apples were determined as richer in polyphenols according to Jakobek et al. (2020) [12]. The TP concentration of 721.6 $mg \cdot L^{-1}$ in cider from 'Fuji' apples reported by Ye et al. (2014) [45] was 1–3 times higher than in ciders obtained from 'Šampion', 'Idared' and 'Gloster' apples varieties. Since the higher concentration of certain phenolic compounds are usually responsible for higher color intensity and browning of wine through enzymatic or non-enzymatic pathways [5], the color intensity (CI) of analyzed sparkling ciders was, as expected, accordant with TP amount (Table 3) and visual evaluation of wines during sensory evaluation.

Table 3. Total phenols and color parameters of sparkling ciders.

Parameter	'Božićnica'	'Bobovac'	'Crvenka'
Total phenols ($mg \cdot L^{-1}$) *	474 ± 2.64 b	539 ± 4.58 a	416 ± 4.36 c
A 420 nm	0.646 ± 0.001 b	0.778 ± 0.002 a	0.415 ± 0.003 c
A 520 nm	0.238 ± 0.003 b	0.323 ± 0.003 a	0.130 ± 0.003 c
A 620 nm	0.123 ± 0.001 b	0.184 ± 0.003 a	0.055 ± 0.002 c
Color intensity (CI)	1.007 ± 0.001 b	1.285 ± 0.002 a	0.600 ± 0.002 c

* as gallic acid equivalents (GAE); means (n = 3) with different letters differ significantly among varieties ($p \leq 0.05$) based on the Duncan's multiple rang test.

3.3. Volatile Compounds in Sparkling Pét-Nat Ciders

The composition of the volatile fraction in fermented products is determined by the volatilome of the starting material, and yeast metabolism depending on the nutrients available [50]. GC-MS analysis of sparkling ciders allowed the identification of 101 volatile compounds (VCs) shown in Table 4, and presented as a heat map, for a rapid visual assessment of the differences between the apple varieties. Higher alcohols (HAs) and fatty acids (FAs) were the main volatiles, followed by esters, terpenes, volatile phenols (VPs), C13 norisoprenoids, and carbonyls.

Table 4. Concentration of aromatic compounds in *Pét-Nat* ciders ($\mu g \cdot L^{-1}$).

	'Božićnica'		'Bobovac'		'Crvenka'		ANOVA	
Compounds	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F**	***p***
(6Z)-Nonen-1-ol	7.31 ab	0.10	7.70 a	0.11	7.44 b	0.11	7.01	0.07
1-Butanol	3143.91 a *	44.91	2212.90 c	31.61	2402.54 b	34.32	346.29	0.00
Isoamyl alcohol	12,825.40 a	183.21	11,629.49 b	166.13	11,360.83 b	162.29	41.68	0.01
1-Decanol	5119.46 a	73.13	1542.46 b	22.03	1246.84 c	17.81	4532.56	<0.0001
1-Dodecanol	5648.33 a	80.69	1965.21 c	28.07	2224.13 b	31.77	3052.26	<0.0001
1-Hexanol	8133.26 b	116.18	9894.79 a	141.35	7595.85 c	108.51	191.75	0.00
1-Octanol	96.27 a	1.38	24.64 c	0.35	28.60 b	0.41	4457.47	<0.0001
1-Octen-3-ol	37.87 b	0.54	39.74 a	0.57	33.04 c	0.47	85.50	0.00
1-Pentanol	1936.05 b	27.66	2317.47 a	33.10	1811.43 c	25.88	164.83	0.00
1-Propanol	5136.21 a	107.35	3679.34 c	76.90	4466.41 b	93.35	2311.56	<0.0001
3-ethoxy-1-propanol	1088.43 b	22.75	1280.10 a	26.75	1018.51 b	21.29	65.27	0.00
[S-(R *,R *)]-2.3-butanediol	36.62 b	0.77	40.22 a	0.84	25.78 c	0.54	214.14	0.00
2.3-Dehydro-4-oxo-*β*-ionol	7.69 b	0.16	9.35 a	0.20	6.36 c	0.13	164.72	0.00
2-Furanmethanol	101.82 c	2.13	218.51 a	4.57	121.92 b	2.55	732.48	<0.0001
2-Heptanol	17.19 a	0.36	5.80 c	0.12	11.35 b	0.24	972.62	<0.0001
(E)-2-Hexen-1-ol	43.00 b	0.90	51.85 a	1.08	44.67 b	0.93	46.42	0.01
2-Nonanol	9.61 a	0.20	9.04 a	0.18	8.22 b	0.17	27.62	0.01
(E)-2-Octen-1-ol	39.36 b	0.82	44.95 a	0.94	33.58 c	0.70	94.55	0.00
3-Ethyl-4-methylpentan-1-ol	1799.54 a	37.61	1202.27 b	25.13	896.37 c	18.73	528.20	0.00
(E)-3-Hexen-1-ol	456.87 a	9.55	258.10 c	5.39	429.29 b	8.97	346.54	0.00
(Z)-3-Hexen-1-ol	218.66 b	4.57	267.90 a	5.60	153.54 c	3.21	315.73	0.00
3-Methylpentan-1-ol	980.43 a	27.19	816.09 b	22.63	840.54 b	23.31	26.29	0.01
3-Octanol	43.30 a	1.20	38.79 b	1.08	32.68 c	0.91	49.80	0.00
6-Methyl-5-hepten-2-ol	9.57 b	0.27	9.15 b	0.25	11.99 a	0.33	57.62	0.00
(Z)-3-Octen-1-ol	66.42 b	1.84	151.95 a	4.21	53.96 c	1.50	730.13	<0.0001
3-Methyl-3-pentanol	79.90 c	2.22	92.06 b	2.55	123.05 a	3.41	128.78	0.00
5-Hexen-1-ol	108.89 a	3.02	81.97 b	2.27	50.47 c	1.40	315.84	0.00
(E)-5-Octen-1-ol	90.21 a	2.50	85.33 a	2.37	67.98 b	1.89	53.12	0.00
(Z)-5-Octen-1-ol	29.53 a	0.82	30.96 a	0.86	29.58 a	0.82	1.90	0.29
Benzyl alcohol	118.64 a	1.66	83.26 b	1.17	81.95 b	1.15	478.29	0.00
Chavicol	7.93 a	0.17	6.47 b	0.14	6.81 b	0.15	49.75	0.01
cis,cis-4.6-Octadienol	72.74 a	1.57	74.68 a	1.61	66.41 b	1.43	15.83	0.03
Isobutanol	86.49 a	2.50	68.91 b	1.99	89.60 a	2.59	44.29	0.01
Phenylethyl Alcohol	2236.08 a	48.16	2082.41 b	44.85	2123.85 ab	45.74	5.91	0.09
Z-*a*-*trans*-Bergamotol	6.13 a	0.09	6.07 a	0.08	5.55 b	0.08	29.58	0.01
Total higher alcohols	**49,839.15 a**	**352.24**	**36,650.59 b**	**363.63**	**37,511.14 b**	**245.87**	**1031.30**	**<0.0001**
Isovaleric acid	1020.80 a	28.31	1020.36 a	28.29	849.29 b	23.55	27.21	0.01
Butanoic acid	344.40 b	4.82	384.76 a	5.39	283.79 c	3.97	227.67	0.00
Decanoic acid	875.43 a	18.85	788.30 b	16.98	695.97 c	14.99	55.65	0.00

Table 4. *Cont.*

	'Božićnica'		'Bobovac'		'Crvenka'		ANOVA	
Compounds	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F**	***p***
Dodecanoic acid	18.92 b	0.41	22.34 a	0.48	19.69 b	0.42	33.40	0.01
Ethyl *trans*-cinnamic acid	61.99 a	1.34	61.78 ab	1.33	57.96 b	1.25	6.06	0.09
Heptanoic acid	43.29 b	1.25	56.46 a	1.63	47.00 b	1.36	45.68	0.01
Hexanoic acid	5872.22 a	169.48	5761.55 ab	166.29	5307.94 b	153.20	6.72	0.08
2-ethyl-Hexanoic acid	103.37 b	2.98	114.44 a	3.30	120.97 a	3.49	14.83	0.03
Nonanoic acid	67.24 b	1.94	75.77 a	2.19	64.67 b	1.87	16.83	0.02
Octanoic acid	3843.53 a	110.93	2722.36 b	78.57	2966.22 b	85.61	80.83	0.00
Propanoic acid	73.99 b	1.59	81.39 a	1.75	74.62 b	1.61	12.31	0.04
2-Methyl-propanoic acid	322.07 b	6.94	555.02 a	11.95	249.58 c	5.38	695.00	<0.0001
Undecanoic acid	8.52 b	0.18	10.04 a	0.22	8.81 b	0.19	33.40	0.01
Total fatty acids	**12,655.79 a**	**282.77**	**11,654.57 b**	**251.01**	**10,746.50 c**	**241.83**	**27.17**	**0.01**
3-Octanone	0.63 b	0.02	3.94 a	0.11	0.47 b	0.01	1859.03	<0.0001
1-Octen-3-one	n.d.		5.28 a	0.08	3.16 b	0.05	5483.41	<0.0001
Nonanal	8.61 a	0.25	8.37 b	0.24	8.45 ab	0.24	0.52	0.64
Total carbonyls	**9.25 c**	**0.23**	**17.59 a**	**0.21**	**12.08 b**	**0.28**	**625.37**	**0.00**
allo-Ocimene	n.d.		13.76 a	0.19	n.d.		10,201.00	<0.0001
a-Ocimene	n.d.		11.42 a	0.16	n.d.		10,201.00	<0.0001
a-Terpinene	8.31 a	0.12	n.d.		n.d.		10,201.00	<0.0001
Menthol	6.42 b	0.19	25.79 a	0.74	6.10 b	0.18	1232.21	<0.0001
cis-*a*-Bisabolene	5.31 a	0.11	5.16 a	0.11	5.13 a	0.11	1.42	0.37
cis-*ß*-Farnesene	n.d.		8.99 a	0.19	n.d.		4312.11	<0.0001
Isocaryophyllene	10.53 a	0.30	9.64 ab	0.28	8.78 b	0.25	19.63	0.02
(E,Z)-2.6-dimethyl-2.4,6-octatriene	9.08 a	0.19	4.52 b	0.09	4.92 b	0.10	688.92	0.00
Citronellol	59.81 a	1.29	45.62 b	0.98	32.55 c	0.70	358.00	0.00
D-Limonene	5.95 b	0.13	9.52 a	0.20	6.19 b	0.13	312.58	0.00
Linalool	5.49 a	0.16	5.45 a	0.16	5.46 a	0.16	0.04	0.96
a-Terpineol	5.40 a	0.12	5.22 a	0.11	5.34 a	0.12	1.34	0.38
Linalyl butyrate	n.d.		6.28 a	0.18	n.d.		2401.00	<0.0001
Linalyl isobutyrate	5.32 a	0.15	n.d.		n.d.		2401.00	<0.0001
Geranyl formate	33.82 a	0.73	15.13 b	0.33	3.85 c	0.08	2136.64	<0.0001
Total terpenes	**155.45 b**	**2.87**	**166.51 a**	**2.84**	**78.33 c**	**1.63**	**729.52**	**<0.0001**
Vitispirane A	7.48 b	0.16	8.25 a	0.18	5.31 c	0.11	196.98	0.00
Vitispirane B	14.68 b	0.32	9.85 c	0.21	25.23 a	0.54	843.29	<0.0001
4-Hydroxy-*ß*-ionone	5.08 b	0.14	5.28 a	0.15	5.04 b	0.14	1.65	0.33
Total C13 norisoprenoids	**27.25 b**	**0.34**	**23.38 c**	**0.24**	**35.58 a**	**0.52**	**530.38**	**0.00**
Ethyl hexadecanoate	37.95 a	1.10	37.97 a	1.10	37.55 a	1.08	0.09	0.91
Ethyl hexanoate	1231.26 b	35.54	1355.90 a	39.13	1036.20 c	29.91	42.24	0.01
3-Methylbutyl octanoate	19.63 a	0.57	13.65 b	0.39	11.06 c	0.32	200.78	0.00
Ethyl octanoate	392.03 a	11.31	404.22 a	11.67	344.43 b	9.94	16.50	0.02
Methyl octanoate	17.89 b	0.52	21.79 a	0.63	14.10 c	0.41	107.28	0.00
Isopentyl decanoate	9.00 a	0.26	7.49 b	0.22	5.45 c	0.16	137.35	0.00
Ehyl pentadecanoate	6.30 b	0.18	7.43 a	0.21	5.92 b	0.17	34.33	0.01
Ethyl nonanoate	48.66 a	1.40	49.12 a	1.42	48.65 a	1.40	0.07	0.93
Butyl ethyl succinate	51.91 a	1.12	46.77 b	1.01	44.11 b	0.95	29.78	0.01
2-Phenylethyl acetate	167.75 b	4.65	207.59 a	5.76	93.13 c	2.58	329.68	0.00
Phenylethyl acetate	49.42 b	0.69	57.46 a	0.80	27.82 c	0.39	1103.41	<0.0001
Diethyl succinate	948.24 a	13.28	295.61 c	4.14	492.43 b	6.90	2790.58	<0.0001
2-Methylpentyl butanoate	22.18 c	0.31	29.04 a	0.41	25.79 b	0.36	179.83	0.00
Butyl 3-hydroxybutanoate	96.04 b	1.34	150.03 a	2.10	70.24 c	0.98	1383.47	<0.0001
Ethyl butanoate	124.91 a	1.75	53.82 c	0.75	91.19 b	1.28	1443.30	<0.0001
Diethyl malate	63.87 c	1.33	72.96 b	1.52	79.98 a	1.67	56.73	0.00
Ethyl 4-hydroxybutanoate	58.40 a	1.26	50.58 c	1.09	54.46 b	1.17	22.12	0.02
Ethyl 9-decenoate	135.81 a	2.92	126.11 b	2.72	126.65 b	2.73	7.63	0.07
Ethyl 9-hexadecanoate	5.02 a	0.11	3.28 b	0.07	2.96 c	0.06	355.12	0.00
Ethyl hydrogen succinate	468.59 a	10.09	171.52 b	3.69	164.40 b	3.54	1412.49	<0.0001
3-Methylbutyl decanoate	9.64 a	0.27	7.40 b	0.21	7.36 b	0.20	65.66	0.00
Isoamyl acetate	460.65 a	6.58	211.68 b	3.02	163.66 c	2.34	2633.37	<0.0001
Isoamyl propionate	n.d.		24.61 a	0.35	n.d.		9801.00	<0.0001
Total esters	**4581.28 a**	**52.80**	**3528.43 b**	**53.77**	**3010.30 c**	**41.27**	**520.81**	**0.00**
4-Ethyl-phenol	3151.76 a	87.40	n.d.		126.62 b	3.51	2496.68	<0.0001
Eugenol	48.68 a	1.05	47.62 a	1.03	48.31 a	1.04	0.53	0.63
4-Vinylguaiacol	25.28 b	0.70	87.23 a	2.42	18.13 c	0.50	1313.64	<0.0001
4-Ethylguaiacol	n.d.		n.d.		25.50 a	0.55	4312.11	<0.0001
Total volatile phenols	**3225.73 a**	**87.05**	**134.85 b**	**1.39**	**218.56 b**	**2.42**	**2452.52**	**<0.0001**
Methionol	4.74 a	0.10	1.92 c	0.04	3.51 b	0.07	711.76	<0.0001

* Means (n = 3) with different letters differ significantly among varieties ($p \leq 0.05$) based on the Duncan's multiple rang test; n.d. = not detected.

Higher alcohols (HAs) formation is an important criterion to determine the quality of any fermented beverage as the most abundant group of VCs [50]. Their concentrations can vary depending on yeast strain, apple cultivar used, and fermentation conditions employed [51]. According to recent studies, HAs were the most abundant VCs in ciders and sparkling ciders [5,15,18,45,50,52]. Generally, they are directly synthesized by yeast fermentation of sugars and the yeast metabolism of amino acids [53]. Rapp and Mandery, in 1987 [54], found the total HAs in wine in the range 80–540 mg·L^{-1} with concentrations up to 300 mg/L, contribute to the pleasant aroma, but concentrations above 400 mg·L^{-1} produced unpleasant flavor and harsh taste. According to Antón-Díaz et al. [28] there are no differences in dominant alcohols (2-methyl-1-butanol, 1-propanol, isobutanol, and isoamyl alcohol) between ciders fermented by *Saccharomyces* sp. or wild yeast, but treatment based on lees contact significantly increases HA levels [28]. In this study the highest concentration with a significant difference was noticed in 'Božićnica' *Pét-Nat* cider. The most abundant compound in all three varieties was isoamyl alcohol with the content ranging from 11,360 to 12,825 mg·L^{-1}, which is in accordance with published results [50,52,55]. The following HAs were 1-hexanol, 1-dodecanol, 1-propanol, 1-decanol, 1-butanol and phenylethyl alcohol. The same HAs including (6Z)-nonen-1-ol are listed in Table 5 as compounds with OAV > 1 that actively contribute to the aroma characteristics of wines [39]. The highest values were reported for melon/green and ripe fruit/alcoholic odors.

Fatty acids (FAs) are fermentation aromas produced by yeast and bacteria, their profile being related to apple cultivars and harvest [56]. In low concentrations they can have an impact on the complexity of the sensory characteristics, with fruity, cheese, fatty, and rancid aromas [57], while they have a negative effect when above their thresholds [58]. Among 13 detected compounds, hexanoic, octanoic, and isovaleric acid exceeded their thresholds, with the highest significant concentrations in 'Božićnica' *Pét-Nat* cider (Table 5), with a goat cheese nuance. As mentioned, fatty acids, together with decanoic acid, were the main acids in ciders that agrees with previous researches [45,52,59]. Anton-Diaz et al. [28] reported an increase in hexanoic and octanoic acids in ciders undergoing the fermentation lees contact. It is significant to note that, despite conducting spontaneous fermentation and lees contact, the concentrations of total FAs were lower than 20 mg·L^{-1} in all variants, as it has been reported that higher concentrations have a negative impact on the final wine aroma [60].

Terpenes mainly derive directly from fruits and contribute to the primary or varietal aroma of the wine. Because of their relatively low thresholds, they contribute to the floral and fruity aromas, either directly or through synergistic effects [61]. There was a significant difference in total terpenes between the ciders, with the highest level in 'Bobovac' sparkling cider, mainly due to the compounds not detected in other varieties (*allo*-ocimene, *a*-ocimene, *cis-ß*-farnesene and linalyl butyrate). Among the 13 terpenes analyzed, only citronellol exceeded its odor threshold in 'Božićnica' and 'Bobovac', (Table 5), contributing to the cider aroma with a rose odor [58]. *a*-Terpinene and linalyl isobutyrate were compounds found only in 'Božićnica' cider, while significant differences between ciders were noticed only in menthol and geranyl formate concentrations. In comparison to research on apple wines with different treatments [5], the concentration of citronellol was more than 10-folds higher, and linalool was within the published ranges [5,50]. Ruppert et al. [50] detected only linalool oxide, linalool and α-farnesene in 'Ilzer rose' cider, with small but significant differences in concentrations regarding different fermentation strategies.

C13-norisoprenoids are a diverse class of aromatic compounds, originating from carotenoids, that contribute to the varietal character of many grapes and wines. They are detectable at very low levels, sensory thresholds have been reported as low as 2 ng·L^{-1}, although it is more likely that they begin to impact wine character at levels between 2 and 10 µg·L^{-1}. β-Ionone plays an important role in the floral, violet character of some red wines and vitispirane has an aroma that has been described as floral, fruity, woody, or reminiscent of eucalyptus. Microbial biotransformation of α- and β-ionones to a number of hydroxy and keto derivatives has been reported for several fungi [62]. In our case, the highest significant

C13-norisoprenoids concentration was detected in 'Crvenka' cider, strongly influencing its sensory profile.

Esters are one of the most important volatile byproducts of alcoholic fermentation in cider, after ethanol [63]. Most esters are responsible for fruity, floral and sweet notes [64]. Although some of the esters are present in apples juice, they are primarily synthetized by yeast and lactic bacteria, or by chemical esterification during ageing or lees contact. Esters are subjected to continuous changes regarding the complexity of cider [59], and the smallest variation in concentration may have a significant impact on wine quality [65]. Modifications in ester profiles are influenced by ageing temperature, and pH, as well as by the alcoholic level of the final products [66]. In this study, total esters concentration was, significantly, the highest in 'Božićnica' cider. In total, 27 esters were detected, while ethyl hexanoate was the most representative compound, with the highest concentration in 'Bobovac' cider, with 1231 $\mu g \cdot L^{-1}$, which is above the range reported in monovarietal 'Ilzer rose' ciders (275–980 $\mu g \cdot L^{-1}$) [50]. According to the high OAV value, it significantly contributed 'fruity', 'green apple' and 'banana' scents to the cider aroma, together with other esters with OAV > 1 (Table 5). Previous research noted that ethyl hexanoate and ethyl octanoate are influenced by the time of contact with lees and are often referred to as the "apple esters" [18,28], while acetate esters production is influenced by non-*Saccharomyces* activity [67].

Volatile phenols (VPs) contribute characteristic odors to various foods and fermenting alcoholic beverages, including ciders. They impart odors reminiscent of smoke, medicinal/cleaning products, vanilla, spice and leather [44] or organoleptic defects (´Brett character´) associated with animal (e.g., horse-like) or phenolic aromatic notes [68]. The ´Brett character´ occurs in ciders rich in polyphenols, caused by some types of *Brettanomyces* yeast and *Lactobacillus* bacteria. In this study, four VPs were detected, eugenol, 4-ethylphenol (4-EP), 4-vinylguaicol, and 4-ethylguaiacol. Only eugenol was detected in all ciders with OAV > 1 (cinnamon, clove). The lowest total VPs were detected in sparkling cider 'Crvenka' (Table 2). The most the most abundant compound was 4-EP (126–3151 $\mu g \cdot L^{-1}$), with the the highest significant concentration and OAV > 1 found in 'Božićnica' sparkling cider (Table 5). According to published data, the 4-EP detection threshold is influenced by product complexity (0.6–4.0 $mg \cdot L^{-1}$) [68]. 4-Ethylcatechol (4-EC) and 4-EP are the main volatile phenols in French ciders and phenolic off-flavor defects were found in ciders containing up to 12 $mg \cdot L^{-1}$ 4-EC and 3.0 $mg \cdot L^{-1}$ 4-EP [69]. The 4-EP concentration in Spanish cider presenting a significant ´Brett character´reaching up to 0.7 $mg \cdot L^{-1}$ [70]. According to Antón-Díaz et al. [28], levels of VPs in Asturias cider range between 100 $\mu g \cdot L^{-1}$ and 14 $mg \cdot L^{-1}$, similar to French ciders, with a significant increase in 4-EP after the lees contact. Antón et al. [59] suggest revising VPs as markers of sensory defects, since despite the high contents of VAs found in ciders, none of them presented this defective character. Clear phenolic off- flavor was not perceived under 11.8 $mg \cdot L^{-1}$ of 4-EC. 4-Vinylguaicol characterized by a spicy and smoky, clove-like odor was detected with OAV > 1 only in 'Bobovac' sparkling cider, and 4-ethylguaiacol (spice, clove, smoke) was detected in 'Crvenka' above its threshold (Table 5).

As the only representative of *sulfurous compounds*, methionol was detected in concentration above its threshold in all *Pét-Nat* ciders. Fermentation aroma derived from the sulfur amino acid methionine, which occurs in many fruits, beer, and malt whisky, and presents an important constituent in naturally flavored products. According to Peng et al. [71], it is one of the key aroma compounds of apple wines.

Principal component analysis (PCA) was used to analyze the main aromatic compounds with OAV > 1 in *Pét-Nat* ciders produced from different apple varieties. It can be observed from Figure 2 that PC1 explains 62% and PC2 38% of the of total variability among varieties. *Pét-Nat* ciders from different varieties were clearly separated along PC1 and PC2 on the scatter plot. The bi-plot shows that apple variety influenced the aromatic profile of the final sparkling wines. 'Božićnica' *Pét-Nat* cider (on the right side of the bi-plot) is specific for the higher level of the 13 compounds from different groups of primary and secondary aromas. The ‘Crvenka’ *Pét-Nat* cider located on the left negative side of PC1 is characterized for 4-ethylguaiacol and total C13 norisoprenoids content. In contrast, ‘Bobovac’ *Pét-Nat* cider, on the positive side of PC2 is associated with 1-hexanol and ethyl hexanoate, (6Z)-nonen-1-ol and 4-vinylguaicol. The PCA results show that, based on compounds with highest odor active values, the apple variety under conditions of natural fermentation plays an important role in the cider sensory profile.

Table 5. Volatile compounds and odor descriptors of *Pét-Nat* ciders with OAV > 1.

	Odor Active Value (OAV)			
Aromatic Compound	**‘Božićnica’**	**‘Bobovac’**	**‘Crvenka’**	**Odor Descriptors**
1-Hexanol	3.25	3.96	3.04	Cutted grass [44]
1-Decanol	1.02	0.31	0.25	Pear, waxy, violet [72]
1-Propanol	6.19	4.43	5.38	Ripe fruit, alcohol [73]
(6Z)-Nonen-1-ol	7.31	7.70	7.44	Melon, green [74]
1-Dodecanol	5.64	1.96	2.22	Waxy, coconut [75]
Hexanoic acid	13.98	13.72	12.64	Goaty, cheesy, oily [44]
Octanoic acid	7.69	5.44	5.93	Goaty, oily [44]
Isovaleric acid	4.08	4.08	3.40	Sweaty, cheesy [44]
Citronellol	1.50	1.14	0.81	Rose, citrus [44]
Ethyl hexanoate	87.95	96.85	74.01	Fruity, green apple [44]
Ethyl butanoate	6.24	2.69	4.56	Apple, fruity [44]
Ethyl 9-decenoate	2.26	2.10	2.11	Fruity, fatty [76]
Isoamyl acetate	15.35	7.06	5.45	Banana [44]
4-Ethylguaiacol			3.69	Phenolic, smoke, clove [44]
Eugenol	8.11	7.94	8.05	Cinnamon, clove [44]
4-Vinylguaiacol	0.63	2.18	0.45	Clove, curry, smoke [44]
4-Ethyl-phenol	7.16		0.28	Leather, horse stable [44]
Methionol	23.75	9.60	17.55	Potato, cauliflower [44]

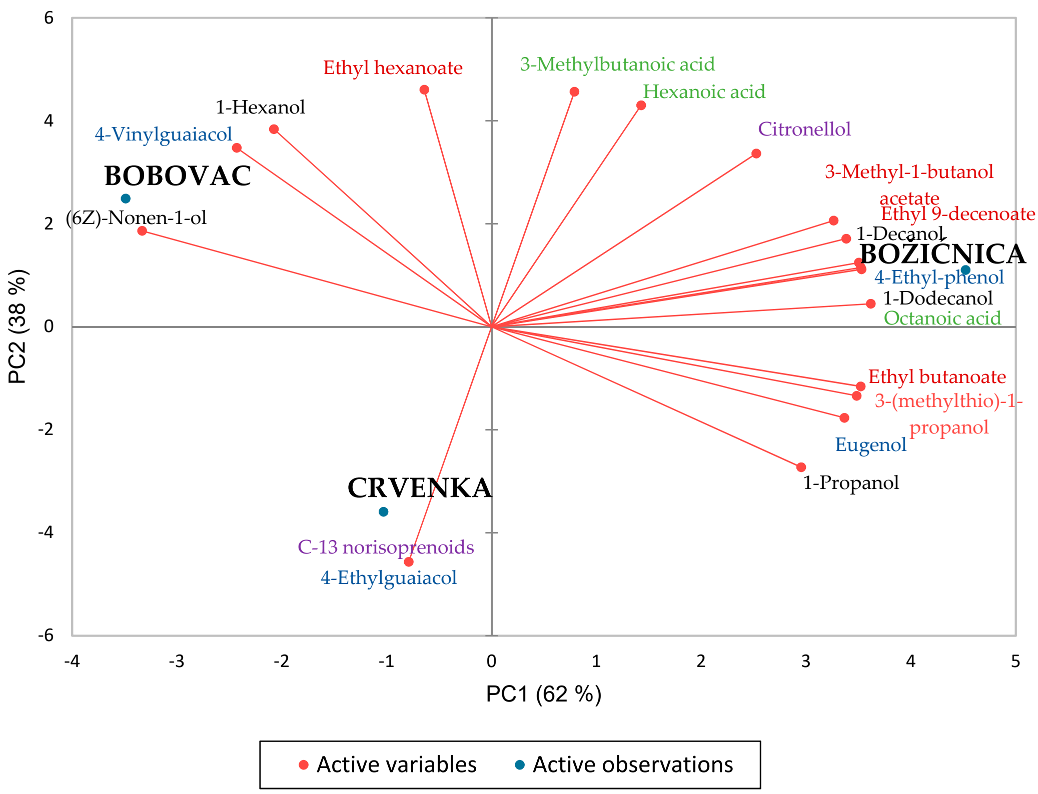

Figure 2. Principle component analysis (PCA): Distribution of *Pét-Nat* ciders from three apple varieties in two-dimensional coordinate system defined by the first two principal components (PC1 and PC2) according to aromatic compounds contents with OAV > 1.

4. Sensory Analysis

Expert panelists detected significant differences in sensory properties among the *Pét-Nat* ciders. The color of cider is determined by the extent of juice oxidation or degradation i.e., water-white, high-tannin cider can be produced if oxidation is completely inhibited [77]. The most intensive color was noticed in 'Bobovac' cider, which was confirmed by spectrophotometric analysis of color parameters, as opposed to 'Crvenka', with a medium intensity straw-yellow color. Foamability is one of the most important properties of sparkling cider and its typical visual attribute, dependent on proteins, polysaccharides and fatty acids content [17]. The foaming properties, such as initial foam (expressed in mm) and foam stability (expressed in sec), were the highest in 'Crvenka' and the lowest in 'Božićnica' *Pét-Nat* cider (results shown in total as foamability score). The odor attributes, together with the highest significant attributes, are displayed in Figure 3. The cleanest and the most intense fruity odor was attributed to 'Crvenka' *Pét-Nat* cider, associated with 'apple', 'apple juice/compote', 'pineapple', and 'buttery' descriptors. 'Božićnica' *Pét-Nat* cider was characterized as complex with intense 'apple', and medium intense 'vegetal', 'grassy' and 'vinous' profile. The flavors connected to 'phenolic', 'earthy/moldy' and 'dry leaves' descriptors were noticed only in 'Bobovac' *Pét-Nat* cider. The acidity was highly scored in 'Crvenka', together with medium astringency and body/mouthfeel perception. The other ciders were evaluated as low acidic. The sweetness was observed only in 'Bobovac' *Pét-Nat* cider with higher residual sugar. The highest scored overall quality was attributed to 'Crvenka' *Pét-Nat* cider, with the most quality color, fruity odor, and well-balanced taste.

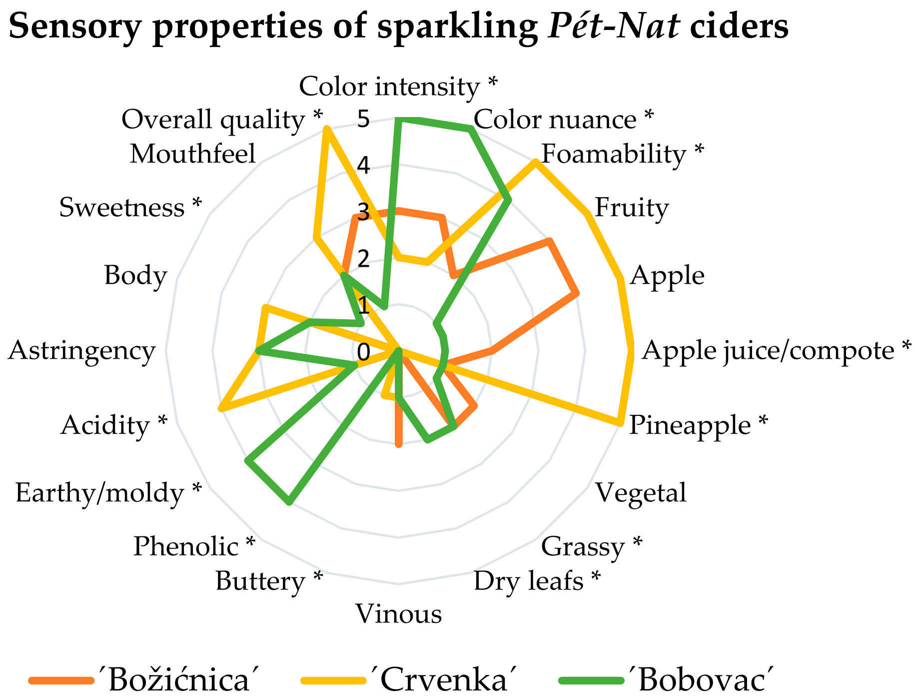

Figure 3. Sensory evaluation of *Pét-Nat* ciders; * represents significant differences ($p \leq 0.05$) between the *Pét-Nat* ciders.

5. Conclusions

According to the general trend towards natural, low-alcoholic, and sparkling wines, there is a growing awareness about traditional apple varieties that can be a valuable resource of raw material, with optimal basic composition and sensory properties for natural sparkling cider production. In this study, evaluation of three Croatian monovarietal *Pét-Nat* ciders produced from traditional apple varieties 'Božićnica', 'Bobovac' and 'Crvenka', via modified *Méthode Ancestrale* or *Pétillant Naturel* was obtained. In addition to the significant differences in aromatic composition between ciders produced by spontaneous fermentation, the highest overall quality was referred to 'Crvenka' *Pét-Nat* cider. Regarding the results of the composition and sensory analysis, the varietal effect was significant, which is in accordance to the results in other similar research studies. The other differences between the *Pét-Nat* ciders could be associated with initial nitrogen content and pH values, which affect fermentation kinetics, microbial activities, and aromatic compounds synthesis. The obtained results will have a practical value in increasing the knowledge about the large compositional and aromatic diversity of traditional apple varieties and appropriate apple variety selection, since the general principle of cider making is blending of varieties to ensure a satisfactory odor and taste balance. Additional microbiological monitoring of apples and ciders during fermentation should be conducted to avoid the limitations of spontaneous fermentation, and for the optimization of applied technology aiming at producing a high-standard natural beverage. The renaissance of the cider production around the world in the past decade provides new opportunities for current and new apple growers and small-scale cider producers. The future research should be integrative, recognizing opportunities and limitations in the economic, horticultural, and enological aspects of cider and sparkling cider production, which will support participation of Croatian natural products in the growing cider market in the coming decades.

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