




Article

Multi-Analytical Characterisation of an Alcoholic Beverage Obtained by Blending of White Wine and Organic Kiwifruit Wine

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Abstract: While studies on the co-fermentation of fruits and grapes are reported in the literature, blends of wine and fruit wine have never been investigated before. We characterised a blend (80:20 *v/v*) of Trebbiano Abruzzese white wine, organic kiwifruit wine, and the sparkling beverage obtained by its successive refermentation in a bottle. A persimmon/kiwifruit wine (50:50 *v/v*), after and before alcoholic fermentation, was also analysed. The buffer capacity, redox potential, concentration of selected polyphenols, total polyphenol content, antioxidant activity, and volatile profile were evaluated. The addition of kiwifruit wine to Trebbiano Abruzzese confers, to the final beverage, an appreciable improvement in terms of antioxidant activity, related to the content of polyphenols and ascorbic acid, which is substantially preserved in the sparkling product. Appreciable differences in the aroma of the blend compared to pure wine are mainly associated with the increased content of norisoprenoids, terpenes, methyl esters, and sulphur compounds, arising from the kiwifruit wine. The innovative beverages here proposed exhibit distinctive compositional and sensorial attributes that can be appreciated by consumers.

Keywords: kiwi wine; Trebbiano Abruzzese wine; fruit wine blends; polyphenolic profile; aromatic profile; flavour compounds



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1. Introduction

Wine, defined as the beverage obtained from the fermentation of fresh grapes or must of *Vitis vinifera* vine species, is one of the most consumed alcoholic drinks in the world. Winemaking is a biotechnological process known since the dawn of civilization and is currently one of the most commercially prosperous [1]. Nevertheless, alcoholic beverages produced since ancient times by the fermentation of non-grape fruits, called “fruit wines”, have gained the interest of consumers and the food industry in the last years [2,3]. Fruits are commonly known as sources of polyphenols and other bioactive components. In the fermentation process, the fraction of these substances bonded to insoluble plant compounds is transferred into the water–ethanol solution [4] and can be absorbed in the successive consumption [5,6]. This fact, combined with the relatively low alcohol content, confers the character of functional beverages to fruit wines [7,8], which is particularly appreciated

by modern consumers. Moreover, the utilisation of ripe fruits or their juices to produce beverages is considered an attractive means to recover the waste of the food industry and the fruit surplus in the commercial market [3].

While fruit wines obtained from the fermentation of non-grape fruit varieties are widely investigated [3,9–12], only a few studies regarding alcoholic drinks obtained from the co-fermentation of grape must and fruit juices can be found in the literature. The volatile components of a beverage obtained by the co-fermentation of *Lycium ruthenicum* Murray, a genus of Solanaceae mainly distributed in the northwest of China, and wine grapes were determined [12]. The relationship between microflora and the volatile profile of mulberry wine, mulberry–grape wine, and grape wine was investigated [13]. Various alcoholic drinks obtained by the co-fermentation of Cabernet Sauvignon and Chardonnay musts and fruit juices from cherry, kiwifruit, peach, and strawberry were also described [14]. Among the various fruit-based alcoholic drinks considered in this study, the most pleasant was that obtained by co-fermentation using *T. delbrueckii* yeast, Cabernet Sauvignon must, and kiwifruit (*Actinidia deliciosa*) juice in a proportion of 60:40, which was judged acceptable by 67 over 100 consumers.

The post-fermentation blending of grape wines and fruit wines represents a potential alternative to co-fermentation for preparing innovative alcoholic drinks, which, however, has never been described before in the literature, to the best of our knowledge. In an oenological context, significant chemical and sensorial differences between the co-fermentation and blending of Malbec and Merlot wines from the Central Coast of California were recently reported [15]. In light of the above, it is expected that blends of grape and fruit wines exhibit different composition and sensorial properties compared to the drink generated by the co-fermentation of the same source materials. Regarding the production process, post-fermentation blending, compared to co-fermentation, allows for better handling of the differences in the composition, origin, and seasonality of the grapes and fruits. In other terms, fruits and grapes can be separately fermented when each has reached its optimal maturation, which does not require the use of cold storage rooms or cold chains for their preservation. Then, the fermented drinks can be properly stored until the preparation of the blends. In addition, each fermentation process can be individually fine-tuned to exalt the nutraceutical and sensorial characteristics of the grape wine and fruit wine that are expected to be preserved or exalted in the blend. Among the modulable factors, the antioxidant potential of the fruit wine is important, as it can enhance the stability of the blend, leading to reduced use or elimination of the addition of sulphites, which are widely used as stabilizer additives in oenology; however, they act as potential allergens for consumers. Methanol, produced from the hydrolysis of pectins by pectinase enzymes [16,17], which are naturally present in fruits, is also potentially dangerous for consumers because of its neurotoxic action, especially towards the human retina. The legal limits for grape wines (400 mg/L for red wine, 250 mg/L for white and rose wines) [18] are generally assumed also for fruit wines.

Regarding the European marketing rules for fruit wines and blends, Council Regulation (EC) No 491/2009 [19] states that the EU Member States may allow the use of the term “wine” accompanied by the name of a fruit to market beverages obtained by the fermentation of fruits other than grapes. Moreover, according to Regulation EU 1308/2013 [20], regarding the common organisation of the markets in agricultural products, fruit wines are assimilated to “other fermented beverages” (taking the common fruit wines cider and perry as explicit examples) within the category of “other products”, which includes also “mixtures of fermented beverages”.

The main scope of this paper is the characterisation, in terms of both chemical composition and sensorial attributes, of an innovative alcoholic beverage obtained by blending

organic kiwifruit wine and Trebbiano Abruzzese (hereinafter referred as to Trebbiano) wine, a white wine emblematic of the viticulture in the Abruzzo region (central Italy) [21,22]. The Trebbiano wine–kiwifruit wine (80:20 *v/v*) blend was characterised as it is and after being subjected to a sparkling process by refermentation in bottle. A fruit wine obtained by the fermentation of 50:50 *v/v* kiwifruit juice and persimmon (*Diospyros kaki* L.) juice, regarded as a potential future alternative to pure kiwifruit wine in the blend with white wines, was also investigated. The fruit juices and the fermented beverages were characterised using the following analytical approaches: (i) potentiometric determination of pH, buffer capacity, and redox potential; (ii) determination by high-performance liquid chromatography (HPLC) of ascorbic acid and selected polyphenols; (iii) total polyphenolic content and anti-oxidant assays; (iv) volatile profiling by head-space solid phase microextraction (HS-SPME) combined with gas chromatography–mass spectrometry (GC/MS). The novel beverages that could be produced using the surpluses of fruit and grape markets, thanks to the lower alcoholic content compared to grape wines and the greater apport of natural bioactive compounds, can meet the salient attention of consumers towards functional foods and beverages.

2. Materials and Methods

2.1. Preparation of the Beverages

The alcoholic beverages investigated here were obtained through the separate alcoholic fermentation, promoted by commercial *Saccharomyces cerevisiae* yeasts for enological use, of the raw materials in two different years (2022 and 2023). The Trebbiano wine was prepared in September, while the fruit wines were prepared in October. The sequential processing steps of non-grape fruits (kiwifruit or persimmon) subjected to alcoholic fermentation were the following: picking and sorting of fruits, destemming and/or crushing, pressing, and subsequent separation of the juice. The kiwifruit wine (KW) was obtained by alcoholic fermentation without temperature control of the juice (KJ) obtained by pressing organic kiwifruits (*Actinidia deliciosa* cv Hayward) cultivated in Italy. Under the same fermentation conditions, a second fruit wine (KPW) was obtained from a blend (50:50 *v/v*) of persimmon juice and kiwifruit juice (KPJ), prepared by adding, during pressing, the kiwi juice to the persimmon pulp to reduce its viscosity, which is attributable to thickening the pectins of persimmon fruit. The persimmon fruits (*Diospyros kaki* L. cv Kaki Tipo) were cultivated in Italy. The grape wine (TW) was made by alcoholic fermentation at a controlled temperature (15 °C) of Trebbiano Abruzzese grapes (variety number 24748 in the VIVC (*Vitis* International Variety Catalogue [23])). Climatic conditions of vineyards in 2023 were characterised by a rainy spring and an extremely dry summer, with average monthly temperatures between 11 and 22 °C in March–June and between 27 and 22 °C in July–September. The blend of Trebbiano Abruzzese wine and kiwifruit wine (TWKW) was obtained by mixing the two drinks, after their separate fermentation, in the proportion of 80:20 *v/v*. The final amounts of TWKW and KPW produced in 2023 were 150 and 30 L, respectively. In 2022, sulphur dioxide was added as an antioxidant additive during the production process of the individual beverages, maintaining a maximum content of 80 mg/L of total sulphur dioxide until it was bottled. In the beverages prepared in 2023, no sulphur dioxide was added, and for the quantity produced by yeasts, it was estimated that there was less than 40 mg/L of total sulphur dioxide in the analysed samples. The different fermented drinks were stored in 500 mL glass bottles, sealed with a crown cap, while the fruit juices were preserved in a refrigerator. In 2022, a part of the blend made of Trebbiano wine and kiwifruit wine (TWKW) was re-fermented in a bottle by the addition of selected *S. cerevisiae* yeasts, according to the classic champenoise method. In this work, the assays described in the next paragraphs were applied to fruit juices and fermented drinks

prepared in 2023 and to the sparkling Trebbiano wine/kiwi wine blend produced in 2022. The fruit juices and fermented beverages prepared in 2022 were analysed by ISVEA (Istituto per lo Sviluppo Viticolo Enologico e Agroindustriale), an excellence analysis laboratory authorized by the Italian Ministry for Agriculture and Forestry Policies for wine and oil certification and accredited in compliance with UNI CEI EN ISO/IEC 17025:2018 standard by “ACCREDIA”. The most relevant enological parameters are presented in Table 1.

Table 1. Conventional enological parameters of fruit juices and wines prepared in 2022.

Parameter	Beverage ^a					
	KJ	KPJ	KW	KPW	TW	TWKW
Alcoholic strength (% vol. ± 0.19)	-	-	6.52	9.12	12.38	10.82
Sugars (fructose + glucose g/L)	133	181	<1.0	<1.0	<1.0	<1.0
Malic acid (g/L)	3.09	2.91	0.44	3.31	0.49	<0.10
Lactic acid (g/L)	-	-	<0.1	0.1	0.41	0.79
Tartaric acid (g/L)	-	-	0.6	<0.1	1.8	1.0
Citric acid (g/L)	14.3	12.6	14.9	12.2	0.12	<0.10
Succinic acid (g/L)	-	-	0.65	0.39	0.91	0.77
Ascorbic acid (mg/L)	422	254	447	214	<20	<20
Total acidity (g/L eq. tartaric acid ± 0.30)	17.10	14.69	18.98	16.12	4.33	5.78
Volatile acidity (g/L eq. acetic acid ± 0.08)	-	-	0.48	0.25	0.19	0.26
pH (±0.08)	3.26	3.37	3.30	3.42	3.41	3.44
Yeast assimilable nitrogen (YAN) (mg/L)	2	26	-	-	27	9
Methyl alcohol (mg/L)	-	-	511	620	55 ± 19	98 ± 24

^a KJ—kiwifruit juice; KPJ—kiwifruit/persimmon juice; KW—kiwifruit wine; KPW—kiwifruit/persimmon wine; TW—Trebbiano white wine; TWKW—Trebbiano wine/kiwifruit wine blend.

2.2. Chemicals and Materials

Divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 µm fiber was purchased from Supelco (Bellafonte, PA, USA). Standards of the following organic acids and phenolic compounds (purity > 97%) were obtained from Sigma-Aldrich (Saint Louis, MO, USA): ascorbic acid, caffeic acid, catechin, coumaric acid, ellagic acid, epicatechin, ferulic acid, gallic acid, protocatechuic acid, and tyrosol. Folin and Ciocalteu phenol reagent and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent were purchased from Sigma-Aldrich. Sodium chloride (purity > 99.5%), sodium hydroxide 1 N RPE-Normex, and sodium hydrogen carbonate (purity > 99%) were obtained from Carlo Erba reagents (Milano, Italy). HPLC-grade acetonitrile and methanol were purchased from Chromasolv TM (Riedel-de HaënTM, Seelze, Germany). Double-deionized water was obtained from a milli-Q water filtration/purification system (Millipore, Bedford, MA, USA). Absolute ethanol (purity > 99.7%) was obtained from VWR Chemicals (Radnor, PA, USA).

2.3. DPPH Radical Scavenging Assay

The free radical scavenging activity of the beverages was assessed by the DPPH method, adapting the protocol recommended by Makris et al. [24]. An amount of 75 µL of wine or 15 µL of fruit wine was mixed with 4.35 mL of DPPH solution (prepared by dissolving 10 mg of DPPH powder in 20 mL of methanol for the stock solution, then diluted 1:20 with methanol for the working solution). The volume was adjusted to 5 mL with methanol, and the mixture was vortexed. The final DPPH concentration was 21.75 mg/L. After 60 min, the absorbance of the residual DPPH was measured at 516 nm by an ONDA UV-30 SCAN UV (Onda, Carpi, Italy) spectrophotometer using quartz cells with 1 cm optical path length and methanol as a blank reference. Antioxidant activity was calculated as the difference between the initial absorbance of the sample in the DPPH solution and the absorbance after 60 min of reaction with the DPPH solution. A calibration curve ($R^2 =$

0.95) using standard solutions of gallic acid was established, and the results were expressed as mg/L gallic acid equivalent (mg GAE/L).

2.4. Total Phenolic Content Assay

The total polyphenol content was determined according to the Folin–Ciocalteu method [25]. The measurement was conducted by mixing 330 μ L of the sample previously diluted (from 1:2 to 1:5) with a water–ethanol solution (ethanol 12% *v/v*), 330 μ L of Folin–Ciocalteu reagent, and, after 6 min, 3.33 mL of a 7% sodium bicarbonate solution. Water was added instead of the test solution in the control sample. The samples were mixed and incubated for 1.5 h at 25 °C in the dark, and the absorbance was measured at 760 nm using water as a blank reference. A calibration curve with standard solutions of gallic acid ($R^2 = 0.98$) was built, and the results were expressed as mg/L gallic acid equivalent (mg GAE/L).

2.5. pH, Buffer Capacity, and Redox Potential

pH and redox potential [26] were determined by an XS pH8+ DHS potentiometer (BioScientifica snc, Anzio, Italy) coupled with an XS Polymer electrode or an XS Standard Orp electrode, respectively. Buffer capacity was evaluated by measuring pH before and after the addition of 200 μ L of NaOH 1N solution to 10 mL samples.

2.6. HPLC Analysis of Selected Polyphenols and Ascorbic Acid

The analysis of ascorbic acid and the selected polyphenols (gallic acid, protocatechuic acid, tyrosol, catechin, caffeic acid, epicatechin, *p*-coumaric acid, ferulic acid, and ellagic acid) was performed on an HPLC system (Waters, Milford, MA, USA) equipped with a Kinetex C18 column (Phenomenex, Torrance, CA, USA), a Model 600 pump, a 600-pump controller module, a 717 Plus autosampler, and a Photodiode Array (PDA) detector. The system was controlled by Empower software Version Pro (Waters, Milford, MA, USA). The mobile phase was degassed by an Agilent 1200 degasser (Agilent Technologies, Waldbronn, Germany) and delivered at a constant flow rate of 1 mL/min and in a gradient mode by mixing solution A (water acidified with 0.1% H_3PO_4) and solution B (pure acetonitrile). The gradient program was the following [27]: solution B was increased from 2% to 20% *v/v* in 30 min, then from 20% to 50% *v/v* in 20 min, and finally decreased from 50% to 2% *v/v* in 10 min. Before injection (10 μ L), the samples were filtered using 0.22 μ m cellulose acetate filters.

2.7. SPME-GC/MS Volatile Profiling

The volatile profiles were obtained by Headspace Solid-Phase Micro-Extraction (HS-SPME) on a DVB/CAR/PDMS sorbent, following a previously optimised procedure [28]. Gas-chromatographic separation of the volatile compounds was conducted on a Varian Saturn 2000 (Varian, Inc., Walnut Creek, CA, USA) GC-MS system, comprising a Star GC 3400 CX (Varian, Inc., Walnut Creek, CA, USA) gas chromatograph coupled to an ion-trap mass spectrometer. The instrument was equipped with a 1078 split/splitless injector fitted with an SPME liner, and all the analyses were conducted in split mode with a 50:1 split ratio. A Varian Factor-Four™ VF5-ms (Varian, Inc., Walnut Creek, CA, USA) capillary column (30 m \times 0.25 mm \times 0.25 μ m film thickness) was used, with helium as the carrier gas, delivered at a flow rate of 1.0 mL/min. The oven temperature program began at 35 °C (held for 5 min), followed by an increase at 5 °C/min until it reached 150 °C (held for 1 min), then it was ramped to 280 °C at 10 °C/min, and this temperature was held for 5 min. A 5 mL wine sample mixed with 30% *w/v* NaCl was placed in a 10 mL vial sealed with a PTFE-silicone septum. The sample was maintained under magnetic stirring at a temperature of 30 ± 1 °C, controlled by a Vertex probe. The SPME fiber was preconditioned at 270 °C, exposed to the sample headspace for 30 min, and then inserted

in the injection port of the gas chromatograph, where desorption of the analytes occurred at 270 °C for 5 min.

2.8. Statistical Analysis

Unless differently specified, all the assays were performed in triplicates, and the results were expressed as mean values and standard errors. To establish the statistical differences between the samples, one-way analysis of variance (ANOVA) with the post-hoc Tukey HSD (honestly significant difference) test was carried out using the freely available online web calculator Astasa [29]. Throughout the text, the term significant means p values less than 0.05 ($p < 0.05$) for the statistical differences.

3. Results and Discussion

3.1. Potentiometric Analysis and Ascorbic Acid Content

Table 2 summarizes the results of potentiometric (pH, buffer capacity, and redox potential) analyses conducted on the fruit juices and fermented beverages prepared in 2023. The sparkling beverage STWKW obtained by secondary fermentation of the blend made of kiwifruit wine and Trebbiano wine, prepared in 2022, was also analysed, and the concentration of ascorbic acid in the samples, determined by HPLC–PDA, was also reported

Table 2. Results of the potentiometric analyses of beverages and ascorbic acid content. Mean values and standard errors ($n = 3$) are reported.

Parameter	Beverage							ANOVA #
	KJ	KPJ	KW	KPW	TW	TWKW	STWKW	
pH	3.47 ± 0.02 ^a	3.66 ± 0.03 ^{ab}	3.48 ± 0.03 ^a	3.58 ± 0.01 ^{ab}	3.00 ± 0.05 ^c	3.17 ± 0.07 ^c	3.76 ± 0.01 ^{ab}	***
Buffer capacity (mmol/L/pH)	18.31 ± 0.91 ^a	90.03 ± 10.08 ^{bc}	64.10 ± 12.82 ^{ab}	126.98 ± 15.87 ^c	25.89 ± 1.50 ^{ab}	46.70 ± 13.91 ^{ab}	36.18 ± 2.28 ^{ab}	**
Redox potential (mV)	94.53 ± 1.51 ^a	78.50 ± 3.50 ^{ab}	132.51 ± 7.48 ^c	58.50 ± 0.50 ^{bc}	165.47 ± 7.49 ^d	152.01 ± 6.03 ^{cd}	46.52 ± 0.49 ^e	***
Ascorbic acid (mg/L)	158.45 ± 0.72 ^{ab}	311.62 ± 13.24 ^c	293.55 ± 4.63 ^c	122.66 ± 6.42 ^a	n.d. [§]	79.22 ± 4.37 ^d	164.60 ± 3.05 ^b	***

KJ—kiwifruit juice; KPJ—kiwifruit/persimmon juice; KW—kiwifruit wine; KPW—kiwifruit/persimmon wine; TW—Trebbiano white wine; TWKW—Trebbiano wine/kiwifruit wine blend; STWKW—sparkling Trebbiano wine/kiwifruit wine blend. [§] Not detected. # Statistically significant differences among samples at $p < 0.001$ (***) or $p < 0.01$ (**). Different letters in the same row indicate statistically significant differences ($p < 0.05$) according to the Tukey HSD test.

It is known that acidity, mainly associated with the content of organic acids, influences its stability and organoleptic properties of wines [30]. Moreover, the existing equilibria between organic acids and their salts confer a buffer capacity to wines, connected with the organic acid content. For a single or prevalent organic acid, buffer capacity is maximal at a pH value close to its pKa. However, in the case of weak acid mixtures, the contributions to the buffer capacity of the various acid/base systems are not additive [31]. Therefore, the significant differences of pH and buffer capacity among the various beverages must be attributed to a different content of the organic acids. A previous investigation regarding organic acid mixtures [31] has also revealed that the increase of the content of ethanol can produce a decrease of the buffer capacity, which could explain the significantly greater buffer capacity of the fruit wines KW and KPW compared to the respective fruit juices (KJ and KPW). Despite the fact that the pH of the fruit juices and fruit wines is significantly higher than that of Trebbiano wine, the pH of TWKW is not significantly different from that of TW alone. The buffer capacity of wine is also preserved in the blend with kiwifruit wine and in the sparkling beverage STWKW. These findings suggest that the alteration of acidity due to the addition of kiwifruit to wine does not negatively influence the stability and organoleptic properties of the final beverage.

Monitoring the redox potential can give information on complex oxidoreductive reactions in wine involving many organic and inorganic redox couples [32]. The redox

potential of grape must, typically around a few hundred mV, decreases to negative values, reaching the minima at the end of alcoholic and malolactic fermentation [26]. After the end of alcoholic fermentation and throughout wine aging, an increase of redox potential, inversely related with the concentration of dissolved oxygen, occurs. In the specific case of Trebbiano Toscano grapes [33], a redox potential of 240 mV was measured in the must, while after racking and a successive three-month aging on lees, a redox potential of about 150 mV was observed. Moreover, values of redox potential in the range of 120–180 mV were recommended for high-quality bottled white wines [26]. Based on the above, the observed redox potentials of fruit juices and fermented juices are therefore diagnostic of a low oxidation state of these beverages. In this regard, it must be stressed that the natural presence of ascorbic acid in kiwifruit and persimmon can help to keep the potential low in the fruit juices and fruit wines. However, the addition of KW to TW in the proportion 20:80 *v/v* is not sufficient enough to produce a significant decrease of the redox potential in the blend. STWKW, despite being aged one year, exhibits a significantly lower redox potential compared to TWKW, which is the consequence of the consumption of free oxygen by wine yeast cells during secondary fermentation that occurred in a bottle combined with the reductive action of glutathione released by yeasts [34]. It must be also remarked that STWKW was obtained from the TWKW drink prepared in 2022, which was subjected to light sulphitation. Therefore, the residual sulphur dioxide in this beverage can cause a lowering of redox potential [26], contributing to an increase in the difference between STWKW and TWKW, produced in 2023 with no sulphite addition.

3.2. Polyphenols and Antioxidant Activity

The concentrations of selected polyphenols, determined by HPLC, in the various beverages are reported in Table 3, while Figure 1 displays the total polyphenol content and the results of the DPPH radical scavenging assay.

Table 3. Concentration (mg/L) of selected phenolics in the fruit juices and fermented beverages. Mean values and standard errors (n = 3) are reported.

Phenolic	Beverage							ANOVA #
	KJ	KPJ	KW	KPW	TW	TWKW	STWKW	
Gallic acid	1.23 ± 0.04 ^a	15.99 ± 2.05 ^b	19.40 ± 1.35 ^b	19.30 ± 1.61 ^b	n.d. [§]	0.79 ± 0.00 ^a	4.36 ± 0.21 ^a	***
Protocatechuic acid	n.d.	n.d.	1.08 ± 0.08	n.d.	n.d.	n.d.	n.d.	
Tyrosol	7.55 ± 0.03 ^a	n.d.	34.86 ± 1.58 ^b	19.19 ± 2.79 ^c	27.30 ± 0.64 ^b	25.39 ± 1.04 ^{bc}	21.22 ± 0.42 ^{bc}	***
Catechin	n.d.	5.30 ± 0.11 ^a	n.d.	n.d.	3.31 ± 0.01 ^b	2.73 ± 0.04 ^c	7.27 ± 0.02 ^d	***
Caffeic acid	n.d.	n.d.	4.76 ± 0.61 ^a	2.36 ± 0.49 ^a	3.77 ± 0.05 ^a	4.68 ± 0.52 ^a	12.49 ± 0.11 ^b	***
Epicatechin	2.21 ± 0.10 ^{ab}	5.15 ± 0.20 ^c	4.26 ± 0.22 ^c	3.09 ± 0.36 ^a	1.48 ± 0.00 ^b	2.41 ± 0.05 ^{ab}	3.23 ± 0.01 ^{ab}	***
<i>p</i> -Coumaric acid	n.d.	0.41 ± 0.01 ^a	0.87 ± 0.07 ^b	n.d.	1.50 ± 0.00 ^c	1.43 ± 0.01 ^c	1.44 ± 0.00 ^c	***
Ferulic acid	n.d.	0.38 ± 0.00 ^a	0.49 ± 0.04 ^b	n.d.	0.49 ± 0.00 ^{ab}	0.54 ± 0.03 ^b	0.59 ± 0.00 ^b	**
Ellagic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.53 ± 0.01	

KJ—kiwifruit juice; KPJ—kiwifruit/persimmon juice; KW—kiwifruit wine; KPW—kiwifruit/persimmon wine; TW—Trebbiano white wine; TWKW—Trebbiano wine/kiwifruit wine blend; STWKW—sparkling Trebbiano wine/kiwifruit wine blend. [§] Not detected. [#] Statistically significant differences among samples at $p < 0.001$ (***) or $p < 0.01$ (**). Different letters in the same row indicate statistically significant differences ($p < 0.05$) according to Tukey's HSD test.

Table 3 reveals that the concentrations of all the selected phenolics in KW are significantly higher compared to their content in KJ. This can be associated with the more efficient solubilization of phenolics in hydroalcoholic media compared to aqueous media and the increase of the alcohol content in KW up to 6–7% (Table 1), which can favor their extraction from the lees. The blending (50:50 *v/v*) of kiwifruit juice and persimmon juice to give KPJ determines a significant increase in the content of all the selected polyphenols, except for tyrosol. The fermentation of KPJ produces a significantly further increase in the concentration of some polyphenols (tyrosol and caffeic acid) but a significant decrease in the content of others (catechin, epicatechin, *p*-coumaric acid, and ferulic acid). This

behaviour, which disagrees with the general trend observed in pure kiwifruit beverages, is an evident consequence of the presence of persimmon juice. In this regard, it is known that persimmon contains significant amounts (1–2%) of pectin [35], a family of polysaccharides formed by the degradation of cellulose during the fruit softening, with a marked tendency to interact with polyphenols [35–37]. It is also known that the addition of alcohol disrupts the interaction pectin–water and promotes the precipitation of pectin molecules, this effect being exalted at pH 3.5, when the carboxylic groups of the pectin units are uncharged [38]. Based on the above findings, we can hypothesize that, as the alcohol content increases during the fermentation, a part of the polyphenols is transferred to the lees through the precipitation of pectin.

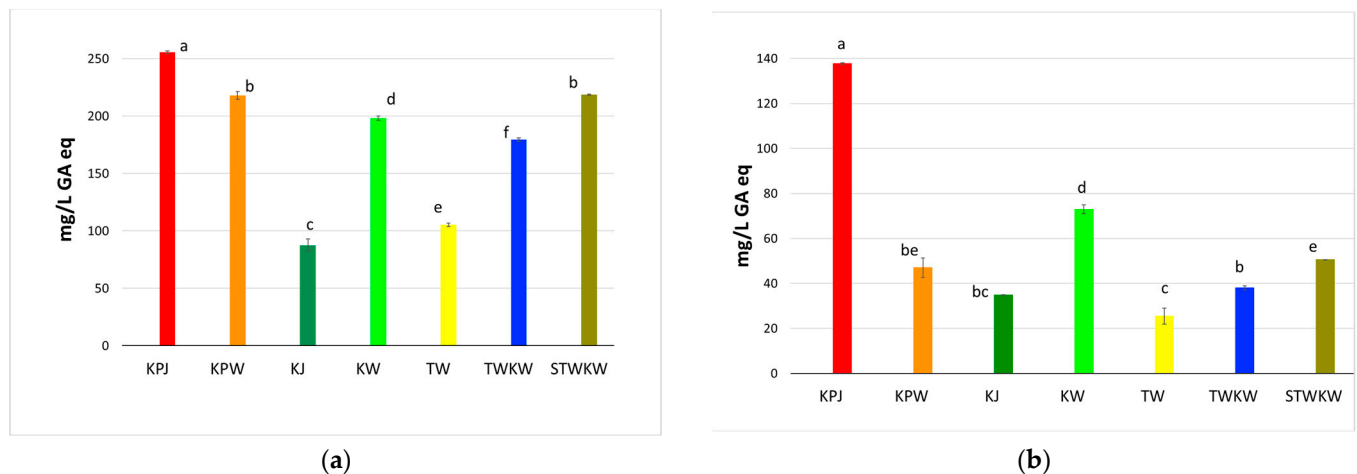


Figure 1. (a) Total polyphenol content and (b) antioxidant activity of fruit juices and fermented beverages. Mean values and standard errors ($n = 3$) are reported. Different letters indicate statistically significant differences ($p < 0.05$) according to Tukey's HSD test.

The evaluation of the content of the selected polyphenols in KW and TW reveals that the addition of kiwifruit wine to Trebbiano wine does not produce a significant variation in the concentration of most of them, with their concentration in KW and TW being comparable. In the case of gallic acid, whose concentration is below the detection limit in TW and close to 20 mg/L in KW, the contribution due to KW in the blend can be instead appreciated.

The trend of the total polyphenol content, shown in Figure 1a, qualitatively reflects that observed for most of the specific polyphenols analysed by HPLC and previously discussed. In particular, the increase of alcohol content in KW seems to favor the extraction of polyphenols from the lees. On the other hand, despite the fact that the addition of persimmon juice to kiwi juice significantly increases the total content of polyphenols in KPJ compared to KJ, a fraction of these compounds is no longer detected in the fermented drink. As argued previously, this effect can be attributed to the growth of alcoholic strength that causes the precipitation of persimmon pectin, promoting the transfer of polyphenols from the hydro-alcoholic medium to the lees. In regards to TWKW, the apport due to KW causes a highly significant increase in the total polyphenol content, even larger than expected assuming a simple additive effect. It can be supposed that the increase of the alcoholic content in this beverage (about 11%) compared to KW (about 6.5%) is responsible for the more efficient extraction of polyphenols from the lees.

The variation of antioxidant activity within the investigated beverages (Figure 1b) is qualitatively similar to that of the total phenolic content (Figure 1a). The correlation coefficient between these variables is in fact 0.716, which is consistent with the predominant influence of phenolic compounds to the antioxidant activity observed in wines [24,39–41]. It

must be remarked, however, that ascorbic acid (Table 2), being a DPPH scavenging agent of medium strength [42], also contributes to the observed antioxidant activity of the samples, especially KPJ and KW. Therefore, the addition of KW to TW causes a significant increase in the antioxidant activity of the blend compared to the grape wine alone. Regarding the significantly higher polyphenol content and antioxidant activity of STWKW with respect to TWKW, it must be considered that the interference of sulphites in STWKW leads to an overestimation of both quantities [43], which can justify the greater value of the two parameters in STWKW compared to TWKW. An additional positive effect in the case of STWKW can be attributed to the reductive action of glutathione released by yeasts in the refermentation in the bottle [34].

3.3. Volatile Profiles

Figure 2 displays the volatile profiles of the fruit juices and fermented beverages acquired by HS-SPME/GC-MS using a DVB/CAR/PDMS fiber. The data are reported in terms of relative (%) areas of the most intense gas-chromatographic peaks and are displayed in Table S1 of the Supplementary Materials, together with the ANOVA results. Ethyl butanoate and 1-hexanol are the volatile compounds that dominate the gas chromatogram of kiwi and persimmon/kiwi juices. Moreover, the addition of persimmon juice to kiwifruit juice causes a significant increase in the relative peak area of ethyl acetate and hexyl acetate. The peak of ethyl benzoate, despite its relatively small contribution to the chromatograms, is significantly more intense in the fruit juices compared to the alcoholic beverages. Among the fermented drinks, KPW exhibits the highest relative peak area of 3-methyl-1-butanol and 2-methyl-1-butanol. The chromatographic peak of 2-methyl-1-propanol, although less intense, is also significantly greater in KPW compared to the other beverages. The relative contribution to the total chromatogram of KW of these three compounds, produced by amino acid metabolism of yeasts [44], is instead significantly lower, which suggests an important apportion of their precursors due to persimmon. Ethyl octanoate, followed by ethyl hexanoate, and then ethyl decanoate are the volatile ethyl esters dominating the gas chromatogram of the alcoholic drinks, especially the beverages based on grape wine, while a lower content of these volatiles is observed in the fruit wines. Significantly higher contents of ethyl and hexyl acetates are instead detected in KW compared to TW. Ethyl esters of fatty acids and acetates of superior alcohols, formed during the alcoholic fermentation, are responsible for fruity and floral aromas [45].

As a general trend, a significant reinforcement or decrease of the relative contribution of given volatiles in TWKW is observed depending on their significantly greater or lower content in the aromatic profile of KW compared to that of TW. A comparison between volatile profiles of TWKW and STWKW, keeping in mind that the two beverages differ in the production year (2023 and 2022, respectively) and, therefore, aging time, reveals some interesting differences. Except for ethyl acetate and ethyl 9-decenoate, a general significant decrease of contributions of fatty acid esters and acetates can be noted in the sparkling beverage compared to its precursor, while a significantly more intense peak of diethyl succinate is detected in STWKW. The decrease of ethyl esters of linear fatty acids in the STWKW (produced in 2022) compared to TWKW (produced in 2023), rather than to the sparkling process, must be attributed to the aging-dependent shifting of the equilibrium between the esters and the corresponding acids [46]. Diethyl succinate, which is formed by the activity of lactic acid bacteria during malolactic fermentation [47], could be developed in the sparkling process in the bottle, increasing the amount generated in the fermentation of the must.

A more detailed picture of the effect of blending kiwifruit wine and Trebbiano wine on the aroma profile of the final product is provided by the analyses conducted in March 2023

by ISVEA on the TW, KW, and TWKW drinks prepared in 2022, through the application of HS-SPME/GC-MS protocols based on the use of multiple internal standards [48]. The concentration of the detected volatiles in the three beverages is displayed in Table S2 of the Supplementary Material. The odour threshold (OT) values assessed on wine, wine–model solution, fruit wine, other alcoholic beverages, or water are available in the literature [45,49–54] and are also reported in Table S2. The odour activity value (OAV) of the aromatic compounds can be evaluated for each volatile compound by dividing its concentration by the OT value [50]. Cumulative OAV (Σ OAV) quantities for specific volatile classes, obtained as the sum of the OAVs associated with the members of each class and determined for each of the three beverages, are graphically displayed in Figure 3. The list of odourants with OAV > 1 in KW is reported in Table 4, in the decreasing OAV order, together with the values observed in TW and TWKW.

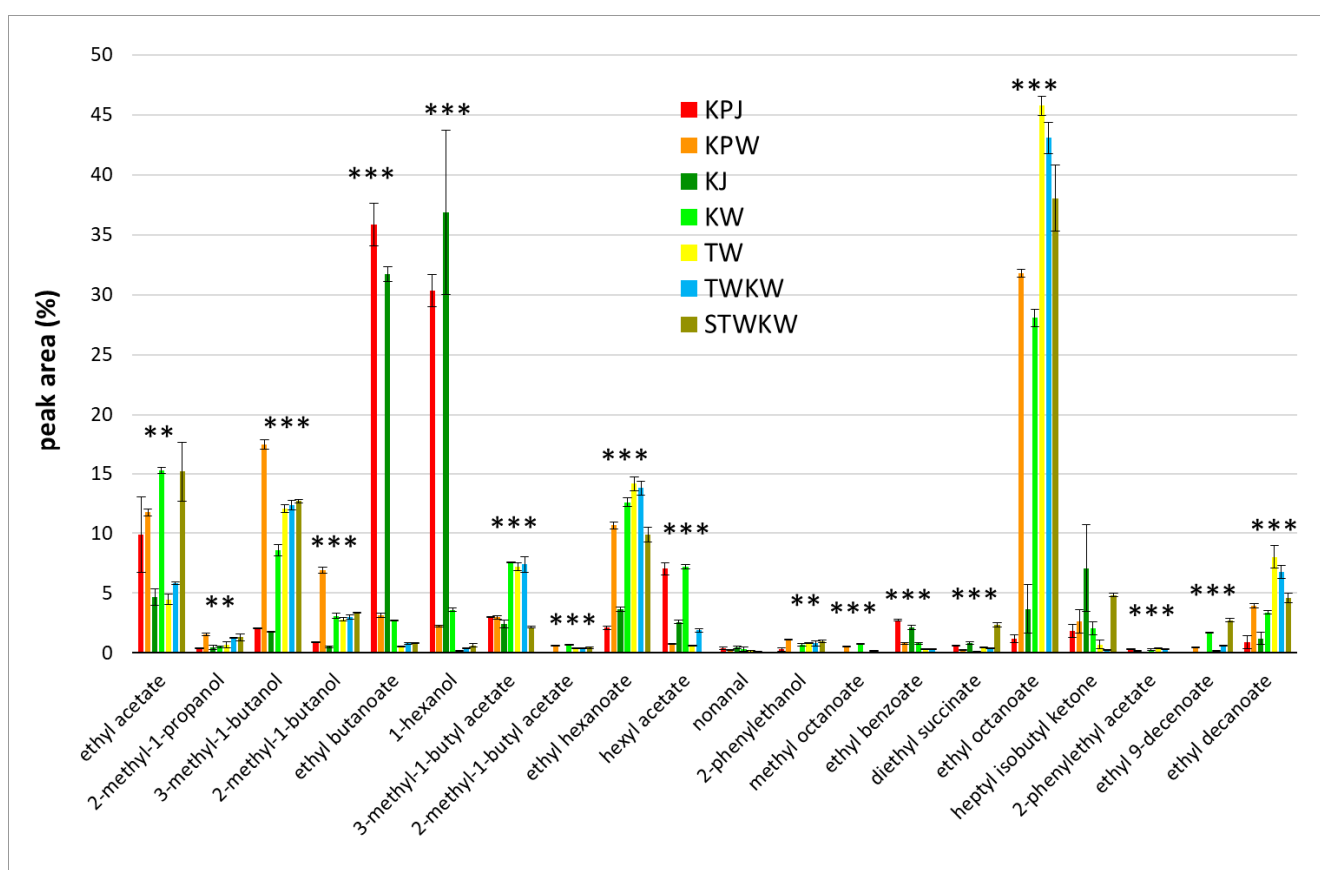


Figure 2. Peak area (%) of the detected volatiles by means of HS-SPME/GC-MS on DVB/CAR/PDMS sorbent. Statistically significant differences among samples at $p < 0.001$ (***) or $p < 0.01$ (**), according to one-way ANOVA, are displayed.

The volatile compounds belonging to the class of norisoprenoids (β -damascenone and β -ionone) and terpenoids (hoptrienol, linalool, and geraniol) contribute to the aroma of KW, TW, and TWKW with fruity and floral notes [50]. These odourants are intrinsic of the fruit variety (primary or varietal compounds). Norisoprenoids are in fact formed by the oxidative degradation of carotenoids during fruit crushing and other pre-fermentation practices, while the terpenoids are directly extracted from grapes or fruits [55]. Isoamyl acetate, contributing a “banana” smell to the aroma of the three beverages, dominates the OAV of the class of acetates. Regarding esters, different OAV values of methyl hexanoate and the other methyl esters (Figure 3) in KW compared to TW seem to reflect, as reported in Table 1, the different concentration of methyl alcohol, which is the precursor alcohol in the esterification reactions generating the above volatiles [45,55]. Among volatile phenols, 4-vinylphenol

and 4-vinylguaiacol, although usually associated with aging in a barrel, originated from the phenolic acids naturally present in the grapes or fruits and are responsible for negative odours [45,47]. Some fatty acids, possessing unpleasant aromas (rancid, pungent, fatty, or cheese-like) [44], have been determined. In this regard, it was reported that C6–C10 fatty acids, despite being associated with negative odours, in the total concentration range 4–10 mg/L, as is also the case for the beverages investigated here, are very important for the aromatic equilibrium of wine [47]. Moreover, the action of aroma enhancers of fruity notes exerted by branched and linear acids has been described [56].

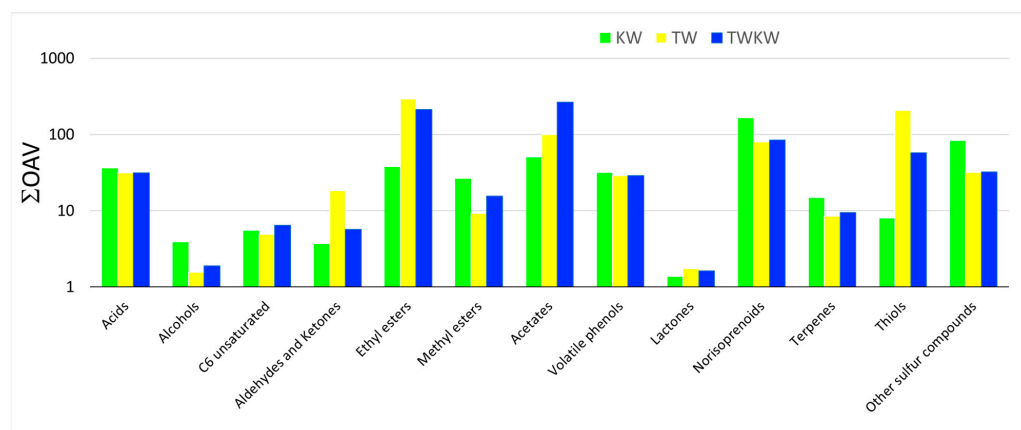


Figure 3. Sum of odour active values (Σ OAV) of volatile compounds grouped according to the chemical class.

Table 4. Odour active value (OAV) of volatile compounds with OAV > 1 in KW and a comparison with OAVs observed in TW and TWKW.

Compound	KW	TW	TWKW	Odour Descriptor [44,47,48,50,52,54,57]
β -Damascenone	158.4	77.8	81.8	stewed fruit, apple, peach
Methanethiol	77.0	27.6	27.8	cooked cabbage, intense onion
Isoamyl acetate	50.3	98.3	264.3	banana
Ethyl Propanoate	31.8	7.2	30.8	sweet, ethereal, fruity
Furaneol	27.4	10.0	9.4	cotton candy
Methyl Hexanoate	25.3	8.8	14.7	fruity
4-Vinylguaiacol	20.9	22.3	19.6	phenolic, smokey
Isovaleric acid	17.9	12.9	14.2	cheese
Butanoic Acid	8.3	4.1	4.7	rancid, cheese, sweat
4-Vinylphenol	6.6	6.3	8.7	spicy, pharmaceutical
Hotrienol	6.0	1.4	2.6	fresh, sweet, floral, lemon-like
3-Mercaptoheptan-1-ol	5.9	204.3	56.3	grape fruit
β -Ionone	5.4	1.2	1.8	violets
Linalool	5.1	4.9	5.1	fruit, citrus
2-Methyl-3-Furanthiol	4.3	2.3	3.1	meat
Octanoic Acid	3.3	5.2	4.8	animal, spicy, cheese
Hexanoic Acid	3.2	4.2	3.9	rancid, pungent, green
Geraniol	2.7	1.6	1.4	rose, geranium
2-Hexen-1-ol	2.7	3.0	3.1	fruity, slightly, green
Eugenol	2.4	0.1	0.3	clove, honey
Ethyl Hexanoate	2.3	3.2	6.1	apple peel, fruit
(E)-2-Hexenal	2.2	1.9	3.2	green, herbal
2-Furanmethanethiol	2.0	0.9	1.6	roasted coffee
Acetaldehyde	1.8	3.4	2.8	pungent, ether (bruised apple)
1-Hexanol	1.7	0.7	0.9	resin, flower, green (cut grass)
Ethyl isobutyrate	1.4	0.5	1.3	fruity, strawberry
Decanoic Acid	1.4	2.6	1.9	fatty acid
Phenylacetaldehyde	1.3	14.0	2.2	flowery, rose
Acetic Acid	1.3	0.9	0.8	vinegar
γ -Nonalactone	1.1	1.5	1.4	peach, coconut
(E)-Isoeugenol	1.1	0.0	0.1	floral
3-Mercaptohexan-1-ol	1.1	0.8	0.9	grape fruit, passion fruit
1-Octen-3-ol	1.0	0.1	0.1	mushroom, fishy

2-Hexen-1-ol, 1-hexanol, and (E)-2-hexenal, belonging to the so-called C6-family, are derived from the enzymatic oxidation of fatty acids during must processing and are responsible for herbaceous notes [57]. 1-octen-3-ol, which is developed in the fermentation of grapes affected by fungal attacks, is responsible for unpleasant (mushroom, fishy) scents [57]. Furanol (4-hydroxy-2,5-dimethyl-3(2H)-furanone) is a primary volatile recognised as one of the key odourants of aromatic grapes [57]. Methanethiol, responsible for off-flavours, is formed in bottled wines from the degradation of methionine during anoxic aging [58]. 3-mercaptoheptan-1-ol and mercaptohexan-1-ol are thiols of varietal origin [59]. The non varietal thiols 2-methyl-3-furanthiol and 2-furanmethanethiol, formed during aging [45,60], are responsible for meat and toasty odours, respectively. Both acetaldehyde and phenyl acetaldehyde, together with other volatile aldehydes, are considered chemical indicators of the state of wine oxidation [61]. γ -Nonalactone is a well-known wine component included in the jammy odour class [57].

The OAV and Σ OAV patterns suggest that the kiwifruit wine can provide an appreciable contribution to the aroma of the blend with Trebbiano wine through the increase of the content of some norisoprenoids, terpenoids, and methyl esters, while the apport in terms of fruity and floral notes, attributed to the esters, predominantly comes from Trebbiano wine. Some sulphur-containing volatiles, because of their low OT, have been identified as important odourants in the blend, providing positive or negative effects with respect to Trebbiano wine alone, depending on the associated odour and the relative concentrations in the precursor wines.

4. Conclusions

The kiwifruit wine prepared here, considering the observed differences/similarities in terms of chemical composition with respect to that of a white wine obtained from Trebbiano Abruzzese grapes, was found to be a good candidate to be used in blend with this wine for the preparation of innovative still and sparkling alcoholic beverages. The beverages were obtained through the independent alcoholic fermentation of grapes and fruits, which allows for managing the different seasonality of the source materials. The addition of kiwifruit wine to white wine leads to a significant increase in the content of polyphenols and ascorbic acid and influences its aroma through the increased apport of specific volatiles. Because of dilution, nevertheless, the methanol content in the blends is well below the legal limits. It is not clear whether the significant apport of ascorbic acid and other antioxidants due to kiwifruit or persimmon could lead to the reduction or elimination of sulphites in the beverages. To clarify this point, further investigation is in progress to evaluate the effect of aging on the chemical composition, stability, and organoleptic properties of the fruit wines alone and in their blend with Trebbiano white wine.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/beverages11020048/s1>, Table S1: Mean ($n = 3$) peak areas (%) and related standard errors of volatile compounds detected by HS-SPME/GC-MS on DVB/CAR/PDMS sorbent. Statistically significant differences among samples at $p < 0.001$ (***), $p < 0.01$ (**) or $p < 0.05$ (*), according to ANOVA. Different letters in the same row indicate statistically significant differences ($p < 0.05$) according to Tukey's HSD test; Table S2: Concentration (mg/L) of volatile compounds in kiwifruit wine (KW), Trebbiano wine (TW) and their blend (TWKW), and odour threshold (OT) of the volatiles, taken from the literature [31,35–40].

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Conflicts of Interest: G.P. (Pescara Vini di Guerino Pescara) prepared the drinks investigated in this study. These beverages were produced on a pilot scale and are not being marketed. The scientific collaboration between Pescara Vini di Guerino Pescara and Dipartimento di Fisica e Chimica, Università degli Studi dell'Aquila, was authorized and approved by the Department Board in June 2023 and is perfectly in line with the current research interest of the members of the university partner, devoted to the characterisation of typical food products, including wines. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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