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Plant Fibers in Comparison with Other Fining Agents for the Reduction of Pesticide Residues and the Effect on the Volatile Profile of Austrian White and Red Wines

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Abstract: Pesticide residues in Austrian wines have so far been poorly documented. In 250 wines, 33 grape musts and 45 musts in fermentation, no limit values were exceeded, but in some cases high levels (>0.100 mg/L) of single residues were found, meaning that a reduction of these levels before bottling could make sense. In the course of this study, a white and a red wine were spiked with a mix of 23 pesticide residues from the group of fungicides (including botryticides), herbicides and insecticides. The influence of the following treatments on residue concentrations and volatile profiles were investigated: two activated charcoal products, a bentonite clay, two commercial mixed fining agents made of bentonite and charcoal, two yeast cell wall products, and a plant fiber-based novel filter additive. The results of this study show that all the agents tested reduced both residues and volatile compounds in wine, with activated charcoal having the strongest effect and bentonite the weakest. The mixed agents and yeast wall products showed less aroma losses than charcoal products, but also lower residue reduction. Plant fibers showed good reduction of pesticides with moderate aroma damage, but these results need to be confirmed under practical conditions.

Keywords: esters; monoterpenes; bentonite; activated charcoal; yeast wall products; filter additive

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1. Introduction

With a vineyard area of about 48,000 ha, Austria is in the 20th position in terms of vineyard area in the world (about 7,400,000 ha) [1]. However, with an astonishing share of organically cultivated vineyards (15.5%) and 819 organic winegrowers, Austria is considered a pioneer country for organic viticulture [2].

To obtain wine grapes of good quality, the vine has to be protected from both pests and fungal diseases until ripening. The most common vine parasites in Austria are the grape berry moth (Lobesia botrana), downy mildew (Plasmopora viticola), powdery mildew (Erysiphe or Uncinula necator) and gray mold (Botrytis cinerea). To control these, crop protection is used in both conventional and organic farming [3]. In conventional cultivation, approved synthetic insecticides and fungicides can be used, and at harvest time, pesticide residues can stick to the grape skins and pass into the wine during vinification [4]. In Austria must samples, fermenting must samples, and wine samples are randomly inspected for pesticide residues (exclusively botryticide residues) on behalf of the national wine control [5]. However, there are only a few published studies that have looked at concentrations of pesticide residues in Austrian wines. In [5], 791 Austrian samples were analyzed, but this study was limited to botryticide residues. Ref [6] examined a total of 250 wines—including only 4 wines from Austria—by immunoassay and, with the exception of azoxystrobin, this study was limited exclusively to botryticides. Ref [7] examined samples from the entire EU and Switzerland. Pesticide residues in wines from other countries such as Spain [8], Slovenia [9], France [10], Republic of Moldova [11] and Italy [12], to name a few, were already well documented in the early 2000s. More recently, studies on residues in wines with different subjects have also been published [13–19]. Ref [20] summarized in a review the reported levels of residues found in wine grapes, must, and wine in recent years and discussed the impact of these residues on wine quality, in particular on alcoholic fermentation. Simplified, all these studies clearly show that individual pesticide residues of different groups can be detected in sometimes high concentrations (in some cases far above 0.1 mg/L) and that both wine quality and fermentation dynamics of wines can be affected by them.

However, many authors [5,21–24] have shown that when pesticides are used correctly, and the mandatory waiting periods are respected, the levels of pesticide residues do not exceed the legal maximum concentrations (Maximum Residues Limits (MRL's)) for wine grapes. No specific limits for wine have been defined in Austria so far [5], but the concentrations of residues gradually decrease during destemming, pressing, must fining, alcoholic fermentation, wine treatment, filtration and storage [24]. However, since these natural reductions of pesticides are often not enough, some authors [24–45] conducted experiments with different fining agents to investigate their reducing capacity. In summary, these studies have shown that different fining agents can be used to reduce a variety of undesirable compounds. Results using activated carbon to reduce 13 different fungicides (boscalid, cyprodinil, fludioxonil, fenhexamid, pyrimethanil, cyazofamid, dimethomorph, fluopicolide, iprovalicarb, metrafenone, penconazole, spiroxamine, and trifloxystrobin) in grape must showed an average reduction of 30% in must contents [41]. Ref [25,26,28,37,38] also showed a significant reduction when activated carbon was used in must or wine. Ref [46] concluded that fining with bentonite promoted the elimination of about 93% of cyprodinil and 73% of famoxadone, while other pesticides were not significantly reduced by bentonite [38]. Ref [44] showed that 17.3% of the studied pesticides were reduced by the use of polyvinylpolypyrilidone (PVPP). Ref [39] documented the reduction of fungicides by activated carbon and PVPP. Other fining agents have also been studied for their effectiveness at reducing various substances from wines [44,45]. For example, egg albumin significantly reduced the levels of pyrethroids (>50%) and procymidone (about 30%), while casein showed a 35% reduction in the concentration of hexaconazole in wines. In addition, it is reported that a combined fining agent consisting of bentonite, PVPP, potassium caseinate, and diatomaceous earth was more effective than isolated bentonite in reducing concentrations of boscalid in wine [20]. All these studies focus exclusively on the reduction of pesticides but do not really investigate the effect of fining on wine quality and, in particular, on the aroma profiles of the wines. Only [47] studied the combined effect of small amounts of activated charcoal in must on the reduction of pesticides and volatile compounds. The treatment with different activated charcoal significantly reduced the levels of fungicides while the aroma of the wines was not significantly affected. However, this study was limited to some higher alcohols, C6 alcohols, carboxylic acids, and few ester compounds.

A general principle of winemaking asserts that any treatment should only be carried out if it is actually necessary and that using treatments in larger quantities does not automatically lead to better wine quality [48]. Following these guiding principles, wine researchers and, in particular, manufacturers of wine treatment agents, are permanently searching for new products to subtly improve the quality of the wines. Recently, various new fining agents have been launched on the market, including different mixed products based on activated carbon and bentonite, yeast cell wall substances with residue-reducing potential and, last but not least, selective plant fibers [49]. These are used in continuous precoating filtration or as a component of filter sheets.

The aim of the present study is to provide an overview of pesticide residues found in Austrian grape musts, fermenting grape must and wine samples. Furthermore, the potential for a wide range of fining agents to reduce pesticide residues in white and red wine will be investigated. Among the fining agents, new products such as selective plant fibers, yeast cell wall products and mixed agents will be examined for the first time. Finally, the effect of the treatment on various aroma compounds such as free monoterpenes, esters and higher alcohols will be investigated.

2. Materials and Methods

2.1. Samples

For the evaluation of residue contents in Austrian wine samples, a total of 250 different white and red wines of different quality, origin, and production method (conventional (n = 229), organic (n = 18), biodynamic (n = 3)) from the 2012 to 2019 wine harvests, as well as 45 Austrian musts in fermentation (all conventional) and 33 Austrian grape musts (all conventional) of different origins, all 2019 vintage, were collected and analyzed. A complete list of samples can be found in the Supplementary Data: Tables S1 and S2. With the goal of reducing pesticide residues, an Austrian quality white wine (Gruener Veltliner 2018, origin Klosterneuburg, organic production, 12.5% vol, 5.4 g/L titratable acidity calculated as (ca.) tartaric acid, reducing sugars 1.8 g/L, SO_2 free 49 mg/L, total SO_2 108 mg/L) and an Austrian quality wine red (Zweigelt 2018, origin Klosterneuburg, conventional production, 13.0% vol, 4.6 g/L titratable acidity c.a. tartaric acid, reducing sugars n.d., SO_2 free 42 mg/L, SO_2 total 112 mg/L) were used in the fining experiment.

2.2. Materials

A total of 23 pesticide standards were used for analysis. The selection of pesticides was based on an assessment of the frequency of use in Austrian viticulture, the control of popular but no longer registered pesticides, and the use of various active compounds from the groups of insecticides, fungicides and herbicides. Table 1 shows the chemical group, the active substance, the molecular formula, molar mass, common commercial names of the pesticides and the legal limit for wine grapes. Analytical standards were purchased from LGC Standards (Teddington, UK) and Sigma Aldrich (St. Louis, MO, USA). All standards had the highest purity available (purity > 97% (exception: idoxacarb: purity 93.6%)). The internal standard used was triphenyl phosphate, which also was purchased from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile (Sigma Aldrich Company, St. Louis, Missouri, MO, USA) and ethanol (99% (AustrAlco Österreichische Alkoholhandels-GmbH, Spillern, Austria)) were used for the stock and dilution solutions.

Eight fining agents were compared during the course of the experiments. Table 2 shows the product names, substance class and manufacturer. Furthermore, the salts sodium chloride, trisodium citrate dihydrate, disodium citrate sesquihydrate, magnesium sulfate (all Sigma Aldrich, St. Louis, MO, USA), Chromabond Diamino (Macherey-Nagel, Duren, Germany) and the solvents ethyl acetate (99.9% (Fluka, Buchs, St. Gallen, Switzerland, now Sigma Aldrich)), acetone (>99.5%) and formic acid (98 to 100%) (Sigma Aldrich, St. Louis, MO, USA) were used for QuEChERS sample preparation.

Table 1. Pesticide residues examined: chemical formula, molecular weight, Maxiumum Residues Limits (MRL's) for wine grapes, commercial name.

Chemical Group	Active Substance (Investigated Residue)	Application Group	Chemical Formula	Molecular Weight [g/mol]	MRL's [mg/kg] [50]	Commercial Name in Wine Industry
Acetylalanine	Benalaxyl	Fungicide	$C_{20}H_{23}NO_3$	325.40	0.3	Galben M [*1]
Carboxamides	Boscalid	Fungicide/Botryticide	$C_{18}H_{12}C_{12}N_2O$	343.21	5.0	Filan WG, Collis, Cantus, Filan WG
Thiophosphoric acid ester	Chlorpyrifos	Insecticide	$C_9H_{11}C_{13}NO_3PS$	350.58	0.01	Agritox [*2]
Phosphoric acid ester	Chlorpyrifos-methyl	Insecticide/Acaricide	$C_7H_7Cl_3NO_3PS$	322.54	0.01	Reldan 2E [*2], Reldan 22 [*2], Pyrinex M22 [*2]
Amidoximes	Cyflufenamid	Fungicide	$C_{20}H_{17}F_5N_2O_2$	412.36	0.2	Vegas, Star Cyflufenamid, Cidely, Nissovin, Dynali
Pyrethroids	Cypermethrin	Insecticide	$C_{22}H_{19}Cl_2NO_3$	416.30	0.5	Cythrine L, Epigon neu, Cymbigon
Anilino pyrimidines	Cyprodinil	Fungicide/Botryticide	$C_{14}H_{15}N_3$	225.29	3.0	Switch
Phenylpyrroles	Fludioxonil	Fungicide	$C_{12}H_6F_2N_2O_2$	248.19	4.0	Switch
Pyridinyl-ethylbenzamide	Fluopyram	Fungicide	$C_{16}H_{11}ClF_6N_2O$	396.71	1.5	Luna Experience, Luna Max
Phthalimides, sulfenamides and organic chlorine compounds	Folpet	Fungicide	$C_9H_4Cl_3NO_2S$	296.56	20.0	Forum Star, Pergado, Pergado F, VinoStar, Meldody Combi, Vincare, Fantic F, Aktuan 3S
Oxadiazines	Indoxacarb	Insecticide	$C_{22}H_{17}ClF_3N_3O_7$	527.84	2.0	Steward
Strobilurins	Kresoxim-methyl	Fungicide	$C_{18}H_{19}NO_4$	313.35	1.5	Collis
Anilinopyrimidines	Mepanipyrim	Fungicide/Botryticide	$C_{14}H_{13}N_3$	223.27	2.0	Frupica Opti
Acetylalanine	Metalaxyl	Fungicide	$C_{15}H_{21}NO_4$	279.3	1.0	Ridomil Gold MZ, Ridomil Gold MZ Pepite
Triazoles, nitriles and chlorophenyls	Myclobutanil	Fungicide	$C_{15}H_{17}ClN_4$	288.77	1.5	Misha 20EW, Systhane 20EW
Acetamide	Napropamid	Herbicide	$C_{17}H_{21}NO_2$	271.35	0.01	Devrinol
Triazoles	Penconazol	Fungicide	$C_{13}H_{15}Cl_2N_3$	284.18	0.5	Topas, Fatizol 100 EC
Anilino pyrimidines	Pyrimethanil	Fungicide/Botryticide	$C_{12}H_{13}N_3$	199.25	5.0	Scala, Pyrus,
Quinolines	Quinoxyfen	Fungicide	$C_{15}H_8Cl_2FNO$	308.13	1.0	Legend [*3], Arius [*3], Power Arius [*3], System Plus [*3]
Triazoles	Tebuconazol	Fungicide	$C_{16}H_{22}ClN_3O$	307.82	1.0	Flint Max
Strobilurins	Trifloxystrobin	Fungicide	$C_{20}H_{19}F_3N_2O_4$	408.37	3.0	Flint, Flint Max
Monocarboxylic acid amide	Fenhexamid	Fungicide/Botryticide	$C_{14}H_{17}Cl_2NO_2$	302.20	15.0	Teldor WG
Dihydropyrazolones	Fenpyrazamin	Fungicide	$C_{17}H_{21}N_3O_2S$	331.43	3.0	Prolectus

[*1] End-user expiration date: 12 December 2019; [*2] End-user expiration date: 16 April 2020; [*3] End-user expiration date: 27 March 2020.

Table 2. Information regarding fining agent applied.

Commercial Name	Kind of Fining Agent	Producer
Absolut Wein	Yeast Cell wall product	Lallemand Inc. (Montreal, QC, Canada)
Reskue	Yeast Cell wall product	Lallemand Inc. (Montreal, QC, Canada)
NaCalit	bentonite	Erbslöh Geisenheim GmbH (Geisenheim, Germany)
Granucol GE	activated charcoal	Erbslöh Geisenheim GmbH (Geisenheim, Germany)
CarboTec GE	carbon-bentonite mixed products	Erbslöh Geisenheim GmbH (Geisenheim, Germany)
Purity D	carbon-bentonite mixed products	Erbslöh Geisenheim GmbH (Geisenheim, Germany)
Flowpure	Plant fibre	Laffort (Bordeaux, Frankreich)
Grandeco	activated charcoal	DAL CIN (Mailand, Italy)

2.3. Experiments on the Reduction of Residues by Means of Fining Agents

Stock solutions (5000 mg/L) of all standards were prepared in acetonitrile. The stock solutions in acetonitrile were stored at −20 °C until use. Just before use, a mixed solution was prepared in ethanol with a concentration of 50 mg/L of each pesticide.

Before fining, 30 L of the red wine and 30 L of the white wine were spiked with the mixed solution so that the target final concentration of each pesticide was 50 µg/L. Thus, the total concentration of pesticides present in the wine was 1250 µg/L. For comparison, the highest documented value in an Austrian sample was 800 µg/L, but only six botryticide residues were considered in this study [5]. Comparably similar individual or total concentrations of pesticide residues in other reduction studies are well documented [38,40,44]. The wines were homogenized, bottled in 1-L bottles (exact volume (1000 mL) for the reproducibility of the fining tests) and stored at 4 °C until further treatment.

The wines were taken from cold storage and brought to room temperature. Stock solutions of the fining agents were prepared so that the use of 10 mL corresponded to the recommended final concentration. The conditions in the application amount, time of swelling, and water temperature can be seen in Table 3 and were defined after consultation with the manufacturers of the products (average recommended application amount, adequate rehydration).

Table 3. Fining conditions of the used agents.

Finging Agents	Concentration	Time of Swelling	Water Temperature
Absolut Wein	0.6 g/L	6 h	37 °C
Reskue	0.6 g/L	6 h	37 °C
Grandeco	1 g/L	30 min	20 °C
NaCalit	1 g/L	12 h	50 °C
CarboTec GE	1 g/L	30 min	20 °C
Purity D	0.5 g/L	30 min	20 °C
Granucol GE	0.2 g/L	30 min	20 °C
Flowpure	2 g/L	30 min	20 °C

The filtration additive Flowpure was also directly dosed to the wine and 10 mL of water was added to the control sample. The stirring time after the addition of the fining agents was 2 times 15 min for all variants with a break of 1 h between stirring. After fining, the bottles were covered with nitrogen and sealed gas-tight to preserve the aroma. All experiments were performed in triplicate for both red wine and white wine. Analyses for volatile compounds and pesticides were performed directly from the blank supernatant of the samples after 18 h of contact time.

2.4. Analysis of Residue by Means of Quick Easy Cheap Effective Rugged and Safe Gas Chromatography Triple-Quad-Mass Spectroscopy (QuEChERS-GC-TQMS)

All must, must in fermentation, wine, and experimental samples, were analyzed for 23 residues (Table 1) using QuEChERS-GC-TQMS. For this, 6.5 g of extraction mixture (4.0 g magnesium sulfate, 1.0 g sodium chloride, 1.0 g trisodium citrate dihydrate, and 0.5 disodium citrate sesquihydrate), 10 mL of the sample, and 30 µL of the internal standard (triphenyl phosphate, 100 µg/L) were added to a 50 mL centrifuge tube; after doing so, 5 mL ethyl acetate was added. The mixture was then vortexed (Vortex-Genie 2T, Scientific Industries, Bohemia, NY, USA) until the end of heat release and then centrifuged at 4700 rpm for 10 min (Heraeus Megafuge 40 R, Thermo Scientific (now Thermo Fisher Scientific), Waltham, MA, USA). 5 mL of the upper phase were collected into a 15 mL centrifuge tube with 1.05 g clean-up mixture PSA (0.15 g CHROMABOND diamino and 0.9 g magnesium sulfate) and vortexed for 1 min and then centrifuged at 7000 rpm. The supernatant was quantitatively transferred to a 25 mL round bottom flask and acidified with 40 µL formic acid (5% in ethyl acetate) and completely evaporated using a rotary evaporator (Laboxact–Vacuum System Manual VP8VAC, KNF Neuberger GmbH, Freiburg, Germany). The precipitate was dissolved with 0.5 mL of solvent (acetone: ethyl acetate = 1:1). 100 µL of the resulting solution were transferred to a 300 µL GC vial and subsequently analyzed.

The analysis was performed using a gas chromatograph (type 7890 B A, Agilent Technologies, Santa Clara, CA, USA) with an injector, a controller, a CTC Analytics autosampler (Zwingen, Switzerland)), and a triple quad mass spectrometer (TQMS) detector (type, 7010B GC/MS Triple Quad) (Agilent Technologies, Santa Clara, CA, USA). Separation was performed using an HP-5MS column (30 m × 0.25 mm I.D. × 0.25 µm df; Agilent Technologies, Santa Clara, CA, USA) at a column flow rate of 1.02 mL/min. Helium was used as the transport gas. The injection volume was 1 µL, in split mode (50:1), and the injector temperature was 280 °C. The oven temperature gradient started with 60 °C for 1 min, was then increased to 120 °C with increments of 40 °C/min, and then heated up by another 5 °C/min until a temperature of 310 °C was reached. The total run time was 40.5 min, and the temperature in the transfer line and TQMS was 280 °C. The analysis was performed in multiple reaction monitoring (MRM) mode. The retention time, ion transitions, ionization energy, and validation information can be found in the Supplementary Data: Table S3. The calibration was performed with nine different concentration levels from 0.30 µg/L–300.00 µg/L in the unblended zero sample of the white organic experimental wine. The sample preparation for the calibration was carried out under the same conditions as mentioned above. All analyses were performed in duplicate.

The determination of the limit of detection and limit of quantification was performed according to DIN 32645 from the calibration by repeating the calibration six times [51]. The intraday repeatability was calculated by 10-fold repetition of the control variant (with 50 µg/L spiked sample, see chapter "Reduction experiments by finings"). Recovery was also determined from the same sample. Recovery was also determined to estimate the matrix effect in the red zero sample. The EU criteria for pesticide validation were used to evaluate the method [52]. According to these criteria the recovery should be in the range of 70–120%. This was achieved with the exception of pyrimethanil and cyprodinil. The relative standard deviation (RSD) of the repeatability should be less than 20%. This was also achieved for all compounds with the exception of chlorpyrifos-methyl. The limit of quantification (LOQ) of the method was below 15 µg/L for all compounds (except chlorpyrifos and folpet), which corresponded to a reduction of >70% in the fining tests (spiking with 50 µg/L standard) for all residues.

2.5. Analysis of Wine Volatile Compounds by Head-Space Solid-Phase- Microextraction Gas-Chromatography Single-Ion-Mass-Spectroscopy (HS-SPME-GC-SIM-MS)

A total of 48 volatile substances from the chemical groups: esters, higher alcohols, C6 alcohols and free monoterpenes were measured in the test wines to estimate the impairment of the fining of the wine aroma. Two gas chromatographs from Agilent Technologies (Santa

Clara, CA, USA) and a total of three methods were used for the analysis of the different volatile compounds. The first system, consisting of a 6890 N GC system with a 5975 Inert Mass Selective Detector and a CTC Analytics Autosampler (Zwingen, Switzerland), was equipped with a ZB-Wax plus column (length: 60 min, I.D.:0.25 mm, df = 0.25 µm) from Phenomenex (Torrance, CA, USA) and was used to analyze the higher alcohols and C6 alcohols. The second system, consisting of a 7890A GC system with a 5975C Inert MSD with Triple Axis Detector and a CTC Analytics Autosampler (Zwingen, Switzerland), was used for the analysis of major and minor ester compounds and free monoterpenes. This system was equipped with a ZB-5MS column (length: 60 min, I.D.:0.25 mm, df = 0.25 µm) from Phenomenex (Torrance, CA, USA). In this work, the quantification of 33 minor and major ester compounds was performed according to [53]. The determination of 15 free monoterpene compounds and the determination of higher alcohols and C6 alcohols was carried out according to [54]. Information on calibration and validation can be found in the Supplementary Data: Table S4. All analyses were performed in duplicate.

2.6. Statistics

The data was analyzed using SPSS 22.00 from IBM (Armonk, NY, USA) and Microsoft Excel (Redmond, WA, USA). Significant differences between the experimental variants in the levels of pesticide residues and volatile compounds were calculated using ANOVA and Tukey B test. During this process, the data was controlled for the condition of normal distribution and variance homogeneity. In case of non-achievement of the normal distribution a non-parametric test (Kruskall–Wallis test) was performed and in the case of non-achievement of the variance homogeneity, a Tunnet-T3 test was performed.

3. Results

3.1. Residue Contents in Austrian Must and Wine Samples

250 Austrian wines, 45 Austrian musts in fermentation and 33 Austrian grape musts of different origin from 2012–2019 vintages were analyzed for the content of 23 common residues of the fungicides including botryticides, insecticides and herbicides classes. There are no legal limits for pesticide residues in must, fermenting must and wine in Austria. For this reason, the official wine control also uses the limit values for grapes listed in Table 1 for monitoring wine and wine products. These limits were not exceeded in any single sample. Concentrations of more than 0.100 mg/L residue were found in individual samples. With the exception of fluopyram, these exceedances were only related to botryticides (Table 4). In the organically or biodynamically produced samples, no residues were found in any single sample.

Table 4. Percentage of samples with concentrations above 100 µg/L.

	Must (N = 33)		**Fermenting Must (N = 45)**		**Wine (N = 250)**	
	Rel. Share	**Highest Conc.**	**Rel. Share**	**Highest Conc.**	**Rel. Share**	**Highest Conc.**
Pyrimethanil	9.1%	0.229 mg/L			1.2%	0.242 mg/L
Mepanipyrim					0.4%	0.103 mg/L
Fenhexamid	3.0%	0.212 mg/L	2.2%	0.186 mg/L	3.6%	0.192 mg/L
Fenpyrazamin	6.1%	0.204 mg/L	2.2%	0.137 mg/L	0.8%	0.108 mg/L
Boscalid	6.1%	0.562 mg/L	8.9%	0.417 mg/L	0.8%	0.559 mg/L
Fluopyram					0.4%	0.109 mg/L

Figure 1 shows the percentages of samples with detectable residues (>LOD). The residues of the fungicides penconazole, folpet, kresoxim-methyl, cyflufenamid, benalaxyl and quinoxyfen, the herbicide napropamide and the insecticide chlorpyrifos were not detectable in any sample. The fungicides trifloxystrobin and the insecticide cypermethrin were exclusively detectable in a few must samples, and the insecticide indoxacarb was solely detectable in a few must and fermenting must samples. All investigated botryticides, fungicides metalaxyl, fluopyram, myclobutanil and tebuconazole, as well as the insecticide chlorpyrifos-methyl, were detected in some must, fermenting must and wine samples. It is noticeable that botryticides were particularly detectable in many samples. However, depending on the active ingredient, 69 to 100% of the samples were free of detectable residues.

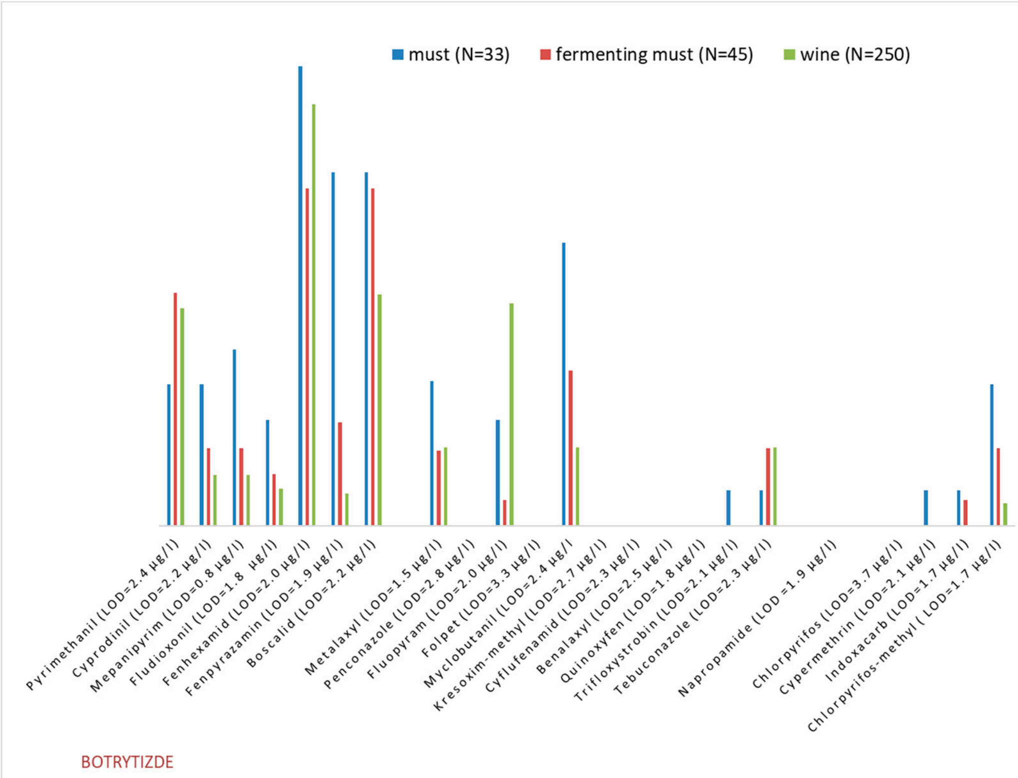


Figure 1. Percentage of samples with detectable concentrations of residues. For identification of the samples Tables S1 and S2.

Figure 2 shows the percentage of samples with quantifiable residues (>LOQ). In addition to the botryicidal active ingredients pyrimethanil, fenhexamid, fenpyrazamine and boscalid, fluopyram and the insecticide chlorpyrifos-methyl were also quantifiable in must, fermenting must and wine samples, while cyprodinil and trifloxystrobin were only quantifiable in must samples. Mepanipyrim and fludioxonil were quantifiable in mustand wine samples, indoxycarb in must and fermenting must samples, and metalaxyl only in fermenting must samples. It is again conspicuous that botryiticides in particular were quantifiable in many samples, yet depending on the residues, 79 to 100% of the samples were free of quantifiable residues. This percentage is comparable to the study of [5] for botryticides, where depending on the residues, 81.4 to 97.6% of the samples showed no measurable residues in 791 tested samples. In both studies, the limits of quantification of the analytical methods were comparable.

3.2. *Reduction of Pesticide Residues through Wine Treatment with Various Fining Agents: The Effect on Botrytices Examined*

One white wine and one red wine were spiked with plant protection active substances (pesticides) and treated by means of different fining agents. Table 5 shows the concentrations of botryticide residues before and after treatment.

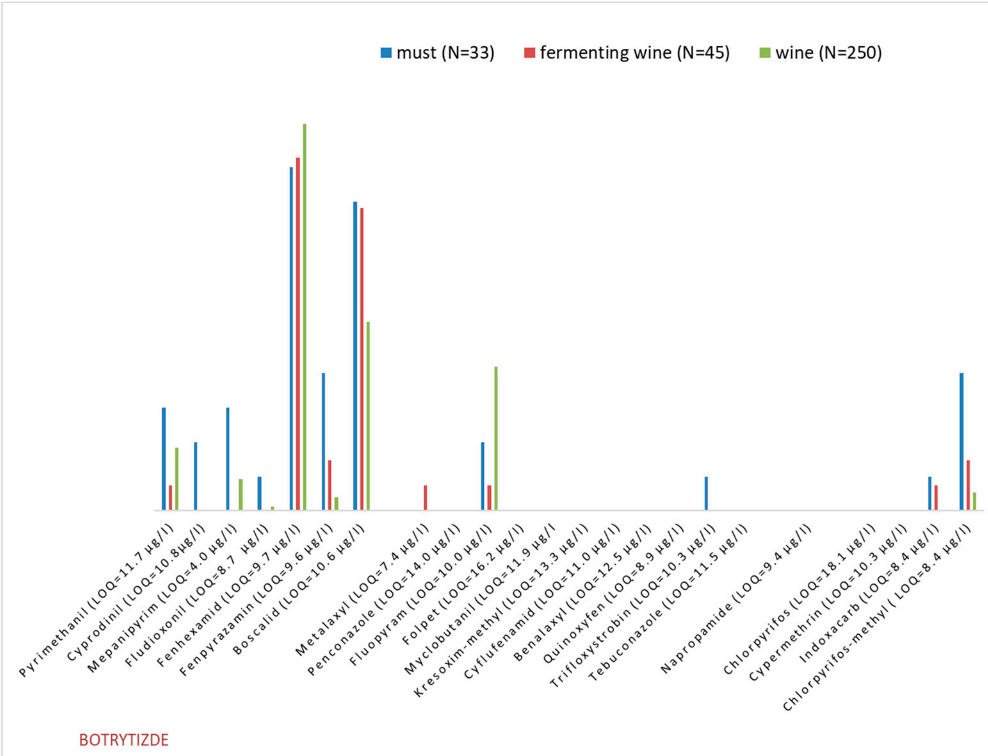


Figure 2. Percentage of samples with quantifiable concentrations of residues: For identification of the samples Tables S1 and S2.

For pyrimethanil, there was a reduction in both white wine and red wine with each of the fining agents used. The reduction was lowest for the bentonite NaCalit and the yeast cell wall products Absolut Wein and Reskue, while the combined products CarboTec GE and Purity D and the plant fibers Flowpure led to medium reductions. The reduction was highest for the two activated charcoal products Granucol GE and especially Grandeco.

For the residue cyprodinil, the reduction was greatest for Flowpure, Purity D, Grandeco and Granucol GE. For these fining agents, the levels of cyprodinil fell below the limit of quantification. The reduction of cyprodinil was lowest from the bentonite fining agent, the two yeast wall products Absolut Wein and Reskue and the combined product CarboTec GE.

The reduction of mepanipyrim, on the other hand, behaved differently from the two previously mentioned botryticides. Concerning white wine, the treatment with all fining agents led to a significant reduction compared to the control. Regarding red wine, the use of CarboTec GE, NaCalit, Flowpure and also the charcoal product Grandeco showed no significant reduction. For mepaniyprim, the two yeast wall products Absolut Wein and Reskue, as well as the combination preparation Purity D, showed very good reductions.

For fludioxonil, fining with bentonite showed no significant reduction of the active substance, while all other treatment agents led to significant reductions of the residue. These were very strong in some cases. In particular, the two charcoal formulations Grandeco and Granucol GE as well as the combined product Purity D produced significant reductions. There was no significant difference between white and red wine.

For the residue fenhexamide, only the two charcoal products Grandeco and Granucol GE led to significant reductions compared to the control.

Regarding fenpyrazamine, the reduction from treatment with charcoal products was also the highest. For both white wine and red wine, treatment with NaCalit and the two yeast cell wall products Absolut Wein and Reskue did not result in any significant reduction compared to the control. While in the white wines the two combined products Purity D and CarboTec GE led to significant reductions compared to the control, this was not found in the red wines. The plant fiber Flowpure showed significant reductions in both red wine and white wine.

Boscalid was significantly reduced by fining with CarboTec GE, Purity D, Flowpure, Grandeco and Granucol GE. The strongest reduction occurred in the treatment with charcoal fining agents Grandeco and Granucol GE. The reduction was similar for white wine and red wine.

Table 5. Concentration of botryticides before and after treatment with different fining agents.

Residues		Fining Product								
		Control	Absolut Wein	Reskue	CarboTec GE	NaCalit	Purity D	Flowpure	Grandeco	Granucol GE
Pyrimethanil (µg/L)	WW RW	31.2 ^{A} 30.1 ^{A}	25.0 ^{B} 24.1 ^{B}	25.8 ^{B} 26.6 ^{B}	18.8 ^{C} 18.1 ^{C}	25.1 ^{B} 24.0 ^{B}	14.5 ^{D} 13.9 ^{D}	15.9 ^{D} 15.1 ^{CD}	<11.7 ^{F} <11.7 ^{E}	12.4 ^{E} 12.8 ^{D}
Cyprodinil (µg/L)	WW RW	33.6 ^{A} 32.4 ^{A}	22.8 ^{B} 21.9 ^{BC}	24.1 ^{B} 23.7 ^{B}	19.3 ^{B} 20.0 ^{C}	25.5 ^{B} 24.8 ^{B}	<10.8 ^{C} <10.8 ^{D}	<10.8 ^{C} <10.8 ^{D}	<10.8 ^{C} <10.8 ^{D}	<10.8 ^{C} <10.8 ^{D}
Mepanipyrim (µg/L)	WW RW	41.3 ^{A} 46.0 ^{A}	8.2 ^{D} 15.6 ^{C}	8.2 ^{D} 13.6 ^{C}	33.2 ^{B} 37.4 ^{AB}	32.6 ^{B} 40.5 ^{AB}	21.9 ^{C} 27.0 ^{BC}	24.8 ^{C} 29.8 ^{ABC}	27.6 ^{BC} 36.2 ^{AB}	6.2 ^{D} 16.1 ^{C}
Fludioxonil (µg/L)	WW RW	42.7 ^{A} 43.4 ^{A}	31.4 ^{C} 32.6 ^{C}	36.5 ^{B} 36.0 ^{B}	28.0 ^{D} 28.2 ^{D}	40.1 ^{A} 40.9 ^{A}	17.9 ^{F} 18.0 ^{F}	22.8 ^{E} 23.0 ^{E}	9.0 ^{G} 9.2 ^{G}	14.4 ^{F} 16.6 ^{F}
Fenhexamid (µg/L)	WW RW	45.6 ^{A} 40.4 ^{A}	35.9 ^{AB} 32.1 ^{AB}	40.2 ^{AB} 35.9 ^{AB}	37.4 ^{AB} 33.2 ^{AB}	43.7 ^{AB} 38.6 ^{A}	39.1 ^{AB} 34.6 ^{AB}	38.1 ^{AB} 34.0 ^{AB}	21.9 ^{C} 19.2 ^{C}	30.0 ^{BC} 26.3 ^{BC}
Fenpyrazamin (µg/L)	WW RW	46.0 ^{A} 47.9 ^{A}	40.4 ^{ABC} 42.2 ^{AB}	44.6 ^{AB} 47.1 ^{A}	36.0 ^{B} 37.8 ^{ABC}	41.4 ^{ABC} 42.9 ^{AB}	37.0 ^{BC} 36.2 ^{ABC}	28.7 ^{C} 30.1 ^{B}	17.3 ^{D} 18.2 ^{D}	26.4 ^{C} 31.5 ^{BC}
Boscalid (µg/L)	WW RW	39.5 ^{A} 41.3 ^{A}	36.3 ^{A} 37.8 ^{A}	38.4 ^{A} 40.9 ^{A}	29.6 ^{B} 31.0 ^{B}	39.3 ^{A} 40.5 ^{A}	19.7 ^{CD} 20.5 ^{C}	20.7 ^{C} 21.7 ^{C}	<10.6 ^{E} <10.6 ^{E}	15.9 ^{D} 18.7 ^{D}

Different letters in a row indicate significant differences with significance level alpha = 0.05, where A represents the significantly largest content and the consecutive letters indicate correspondingly significantly smaller contents. WW: white wine; RW: red wine.

3.3. Reduction of Pesticide Residues through Wine Treatment with Various Fining Agents: The Effect on Fungicides (Without Botryticides) Examined

Table 6 shows the concentrations of fungicides. For metalaxyl, significant reductions were obtained only with the two charcoal products and with the plant fiber Flowpure, with the highest reduction being obtained with the Granucol GE treatment. For penconazole, significant reductions occurred from all fining agents. Grandeco reduced the levels below the limit of quantification for both white and red wine. NaCalit, Absolut Wein and Reskue led to the smallest reduction, while the two mixed fining agents and Flowpure reduced the content of penconazole to a larger extent, and the two charcoal fining agents produced the largest reduction.

Table 6. Concentration of Fungicides (without botryticides) before and after treatment with different fining agents.

Residues		Fining Product								
		Control	Absolut Wein	Reskue	CarboTec GE	NaCalit	Purity D	Flowpure	Grandeco	Granucol GE
Metalaxyl (µg/L)	WW RW	42.9 ^{A} 43.1 ^{A}	35.5 ^{ABCD} 35.8 ^{ABC}	38.8 ^{ABC} 38.5 ^{ABC}	41.3 ^{AB} 41.5 ^{AB}	37.6 ^{ABC} 38.1 ^{ABC}	39.3 ^{ABC} 38.9 ^{ABC}	33.1 ^{CD} 33.2 ^{BC}	33.8 ^{BCD} 33.4 ^{BC}	28.6 ^{D} 32.5 ^{C}
Penconazole (µg/L)	WW RW	49.5 ^{A} 55.3 ^{A}	37.5 ^{C} 41.4 ^{C}	40.1 ^{BC} 45.6 ^{BC}	31.5 ^{D} 35.9 ^{D}	42.4 ^{B} 48.2 ^{B}	20.8 ^{E} 23.2 ^{E}	22.4 ^{E} 24.9 ^{E}	<14.0 ^{G} <14.0 ^{F}	16.5 ^{F} 20.3 ^{E}
Fluopyram (µg/L)	WW RW	48.4 ^{A} 50.5 ^{A}	44.6 ^{AB} 46.1 ^{AB}	48.1 ^{A} 50.4 ^{A}	41.3 ^{ABC} 43.7 ^{AB}	47.1 ^{A} 48.4 ^{A}	38.2 ^{BC} 39.0 ^{BC}	36.1 ^{C} 37.7 ^{BC}	21.8 ^{D} 22.6 ^{D}	25.8 ^{D} 30.9 ^{C}
Folpet (µg/L)	WW RW	41.1 ^{A} 44.2 ^{A}	31.7 ^{B} 34.1 ^{B}	34.4 ^{AB} 36.8 ^{AB}	29.8 ^{B} 32.4 ^{B}	37.5 ^{AB} 43.6 ^{A}	16.9 ^{D} 18.1 ^{C}	20.7 ^{C} 22.1 ^{C}	<16.1 ^{E} <16.2 ^{D}	<16.2 ^{E} 16.2 ^{C}
Myclobutanil (µg/L)	WW	52.5 ^{A}	44.4 ^{ABC}	51.7 ^{A}	41.9 ^{ABC}	48.0 ^{AB}	37.4 ^{BC}	33.1 ^{CD}	19.4 ^{E}	23.0 ^{DE}

Table 6. *Cont.*

Residues		Fining Product								
		Control	**Absolut Wein**	**Reskue**	**CarboTec GE**	**NaCalit**	**Purity D**	**Flowpure**	**Grandeco**	**Granucol GE**
	RW	42.8 A	36.5 B	42.7 A	34.4 BC	38.9 AB	30.9 C D	26.7 D E	15.9 F	23.3 E
Kresoxim_methyl (µg/L)	WW	41.3 A	32.8 BC	36.0 B	32.6 BC	36.2 B	29.1 C	24.5 D	15.4 E	19.8 D
	RW	39.8 A	31.8 BC	34.5 B	31.7 BC	34.7 B	28.0 C	23.5 D	15.0 E	21.1 D
Cyflufenamid (µg/L)	WW	38.2 A	22.3 E	23.7 DE	29.4 BC	33.9 AB	27.8 C D	19.9 E	13.1 F	22.0 E
	RW	39.8 A	23.3 D	24.8 D	30.5 C	35.8 B	29.0 C	20.5 E	13.6 F	20.4 E
Benalaxyl (µg/L)	WW	41.5 A	32.8 B	36.9 AB	31.9 B	37.3 AB	25.5 C	23.7 C	<12.5 E	16.8 D
	RW	36.5 A	18.3 B	18.8 B	<12.5 C	16.5 B	<12.5 C	<12.5 C	<12.5 C	<12.5 C
Quinoxyfen (µg/L)	WW	58.2 A	29.3 B	30.0 B	12.1 D	26.7 C	<8.9 F	<8.9 F	<8.9 F	9.1 E
	RW	59.4 A	47.9 BC	52.4 B	45.4 C	52.7 B	36.5 D	33.9 D	16.7 E	28.4 F
Trifloxystrobin (µg/L)	WW	38.4 A	21.5 D	22.7 CD	25.3 C	34.1 B	22.3 CD	17.3 E	<10.3 G	13.2 F
	RW	42.3 A	23.5 D	24.8 D	27.7 C	37.2 B	24.6 D	19.0 E	<10.3 G	15.8 F
Tebuconazole(µg/L)	WW	43.8 A	38.2 C	39.7 BC	34.0 D	42.5 AB	22.8 EF	26.3 E	11.6G	19.8 F
	RW	36.8 A	32.0 AB	34.1 A	28.5 B	35.3 A	19.3 C	21.9 C	<11.5 D	18.2 C

Different letters in a row indicate significant differences with significance level alpha = 0.05, where A represents the significantly largest content and the consecutive letters indicate correspondingly significantly smaller contents. WW: white wine; RW: red wine.

For Fluopyram, only the two charcoal products, the plant fiber Flowpure and the mixed product Purity D showed significant effects compared to the control. Whereas for Folpet, significant effects were seen for all fining agents except NaCalit and Reskue. The reductions were most pronounced from the charcoal products. Myclobutanil showed a similar picture to Fluopyram, although in the case of red wine, treatments with the mixed preparation CarboTec GE and the yeast bark preparation Absolut Wein also led to significant reductions. Residue concentrations of kresoxim-methyl were significantly reduced by all fining agents, with a similar overall effect. The charcoal products led to the highest reduction, followed by the plant fibers and the two mixed products, then the yeast cell wall products and, finally, bentonite. In the case of cyflufenamide, the two yeast cell wall products were slightly more effective compared to the other fining agents, but here the two charcoal fining agents showed the highest effectiveness too. This was also evident with all other fungicides. Interestingly, for benzalaxyl, the red wine showed a significant reduction below the limit of quantification not only for the charcoal preparations but also for the plant fibers and the two mixed products, while the concentrations of this residue were not so clearly reduced for the white wine. For quinoxyfen, on the other hand, the fining agents showed more reductions, especially for white wine, than for red wine, but again the strongest effects were seen with the use of charcoal, the mixed product Purity D and the plant fibers. Significant reductions also occurred with trifloxystrobin and tebuconazole, although with tebuconazole there was no significant reduction when bentonite was used, and with red wine there was also no significant reduction with the two yeast cell wall products.

3.4. Reduction of Pesticide Residues by Wine Treatment with Different Fining Agents: The Effect on the Herbicide and Insecticides Examined

Table 7 shows the reduction of the herbicide napropamide and the different studied insecticides.

Table 7. Concentration of the herbicides and insecticides examined before and after treatment with different fining agents.

Residues		**Fining Product**								
		Control	**Absolut Wein**	**Reskue**	**CarboTec GE**	**NaCalit**	**Purity D**	**Flowpure**	**Grandeco**	**Granucol GE**
					herbicide					
Napropamide (µg/L)	WW	46.5 ^{A}	35.3 ^{BC}	39.8 ^{B}	32.0 ^{C}	38.5 ^{B}	24.0 ^{D}	25.0 ^{D}	11.5 ^{F}	17.7 ^{E}
	RW	50.9 ^{A}	39.2 ^{BC}	43.8 ^{B}	35.0 ^{C}	42.6 ^{B}	26.4 ^{D}	27.7 ^{D}	12.7 ^{E}	21.7 ^{D}
					insecticides					
Chlorpyrifos (µg/L)	WW	38.0 ^{A}	<18.1 ^{C}	<18.1 ^{BC}	<18.1 ^{C}	33.2 ^{B}	<18.1 ^{C}	<18.1 ^{C}	<18.1 ^{C}	<18.1 ^{C}
	RW	39.3 ^{A}	<18.1 ^{C}	<18.1 ^{C}	<18.1 ^{C}	34.4 ^{B}	<18.1 ^{C}	<18.1 ^{C}	<18.1 ^{C}	<18.1 ^{C}
Cypermethrin_II (µg/L)	WW	36.1 ^{A}	<10.3 ^{C}	<10.3 ^{C}	<10.3 ^{C}	29.4 ^{B}	<10.3 ^{C}	<10.3 ^{C}	<10.3 ^{C}	<10.3 ^{C}
	RW	42.2 ^{A}	<10.3 ^{C}	<10.3 ^{C}	<10.3 ^{C}	33.6 ^{B}	<10.3 ^{C}	<10.3 ^{C}	<10.3 ^{C}	<10.3 ^{C}
Indoxacarb (µg/L)	WW	40.9 ^{A}	17.4 ^{C}	18.1 ^{C}	22.3 ^{B}	39.1 ^{A}	20.7 ^{B}	11.8 ^{E}	<8.4 ^{F}	14.6 ^{D}
	RW	38.7 ^{A}	16.2 ^{CD}	16.9 ^{C}	21.1 ^{B}	37.0 ^{A}	19.8 ^{BC}	11.2 ^{E}	<8.4 ^{F}	12.9 ^{DE}
Chlorpyrifos_methyl (µg/L)	WW	46.4 ^{A}	22.5 ^{E}	18.6 ^{F}	37.3 ^{C}	41.1 ^{B}	30.2 ^{D}	30.8 ^{D}	23.9 ^{E}	15.8 ^{F}
	RW	35.1 ^{A}	17.2 ^{BC}	14.2 ^{C}	28.2 ^{AB}	30.9 ^{A}	22.9 ^{B}	22.9 ^{B}	18.0 ^{B}	12.6 ^{C}
Chlorpyrifos (µg/L)	WW	38.0 ^{A}	<18.1 ^{C}	<18.1 ^{BC}	<18.1 ^{C}	33.2 ^{B}	<18.1 ^{C}	<18.1 ^{C}	<18.1 ^{C}	<18.1 ^{C}
	RW	39.3 ^{A}	<18.1 ^{C}	<18.1 ^{C}	<18.1 ^{C}	34.4 ^{B}	<18.1 ^{C}	<18.1 ^{C}	<18.1 ^{C}	<18.1 ^{C}

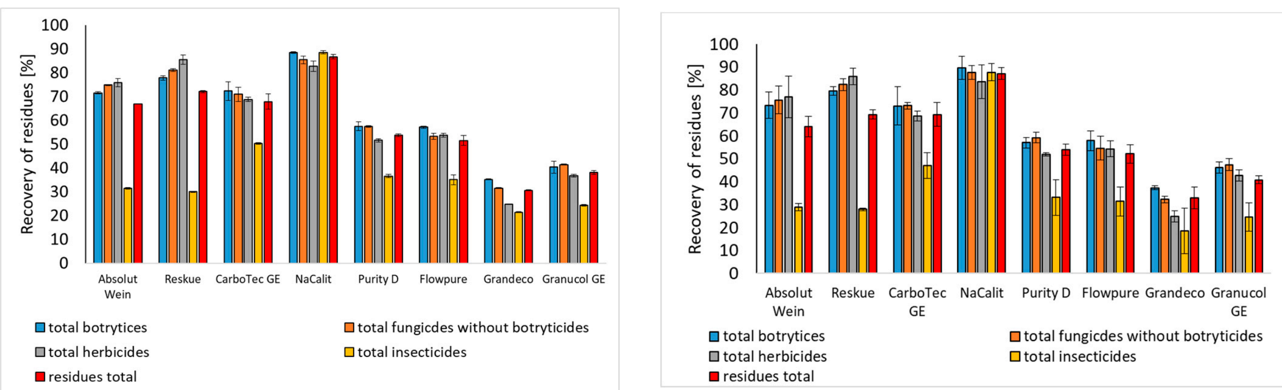
Different letters in a row indicate significant differences with significance level alpha = 0.05, where A represents the significantly largest content and the consecutive letters indicate correspondingly significantly smaller contents. WW: white wine; RW: red wine.

Regarding napropamide significant reductions of all fining agents were found, with the use of carbon, plant fibers and the mixed product Purity D being the most effective.

For the insecticide chlorpyrifos and cypermethrin II, significant reductions below the limit of quantitation were revealed when treated with all fining agents except bentonite, where effects were also observable, but with smaller reductions. For indoxacarb, the reduction was most significant with the charcoal product Grandeco, followed by Fluopyram, Granucol GE, the two yeast cell wall products Absolut Wein and Reskue, and the two mixed products Purity D and CarboTec GE. No significant reduction was detected for NaCalit. There was also a significant reduction in chlorpyrifos_methyl with all treatment agents (except bentonite for red wine). Interestingly, here the two yeast wall products Absolut Wein and Reskue showed a greater reduction than the plant fiber and mixed products and a comparable effect to the two charcoal products Grandeco and Granucol GE.

3.5. Reduction of Pesticide Residues by Wine Treatment with Different Fining Agents: The Overall Effect on the Pesticides Examined

Figure 3 shows the recoveries of the pesticide residue groups botryticides, fungicides (without botryticides), herbicides and insecticides as well as total residues after fining with the various agents for white and red wine.



(**a**) (**b**)

Figure 3. Recovery of pesticide residues after fining; (**a**) white wine; (**b**) red wine.

The greatest reduction was found in the total insecticides studied, where more than 50% of the insecticide content was reduced (recovery less than 50%) in all products except bentonite. The reduction of botryticides, fungicides (excluding botryticides) and one herbicide were about the same. In the case of the fining preparations based on yeast cell wall preparations, the reduction tended to be greater for the botryticides compared to the other residues, while in the case of the other fining products, the herbicide studied was reduced more. The reduction for the carbon products was between 50 and 80% (recovery 50–20%), that for the plant fiber between 40–60% (recovery 60–40%), and that for the two mixed products Purity D and CarboTec GE between 30–60% (recovery 70–40%). Purity D performed better, the two fining agents based on yeast wall products led to a reduction between 15–60% (recovery 85–40%) and bentonite between 10–15% (recovery 90–85%). A similar result was obtained for red wine (Figure 4). The charcoal products reduced the pesticide residues most effectively, followed by the plant fiber Flowpure, the mixed products and the yeast wall products. The reduction was lowest for NaCalit. The insecticides studied were reduced more than the other groups.

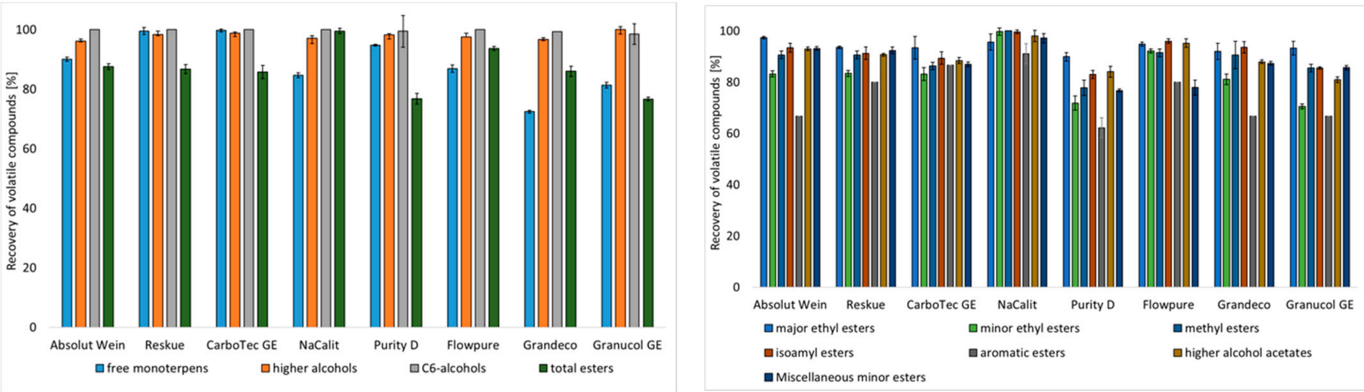
3.6. Influence of Applied Wine Fining on Targeted Volatile Compounds of Wine

Volatile profiles of the experimental wines were determined. Free monoterpenes, higher alcohols, C6 alcohols and ester compounds were analyzed. The results of the individual compounds can be found in the Supplementary Data: Table S5 for white wine and Table S6 for red wine. Table 8 summarizes the individual volatile compounds in sum parameters. Figures 4 and 5 show the recoveries of the respective groups of volatile compounds for white wine and red wine.

Table 8. Concentration of volatile sum parameters before and after treatment with different fining agents.

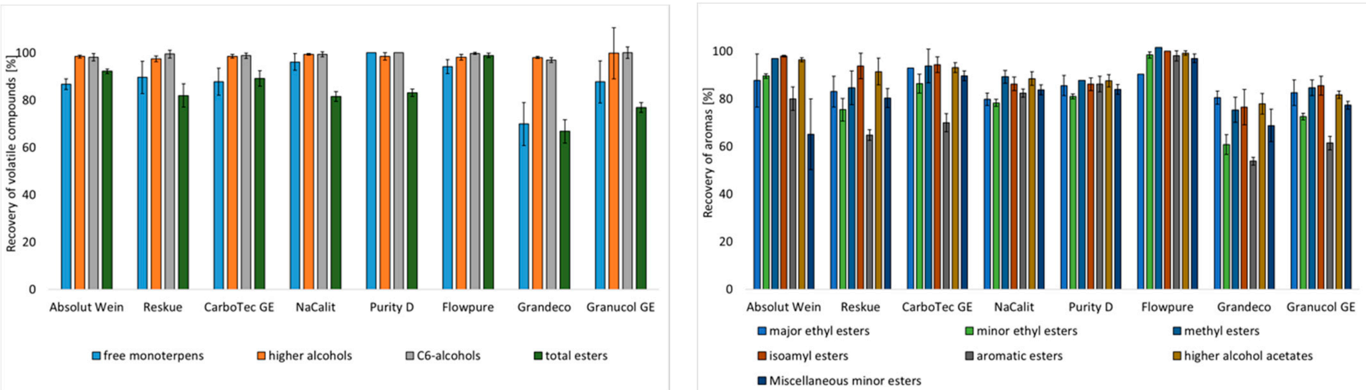
Residues		Fining Product								
		Control	Absolut Wein	Reskue	CarboTec GE	NaCalit	Purity D	Flowpure	Grandeco	Granucol GE
free monoterpens (µg/L)	WW	68.8 ^{A}	62.0 ^{C}	68.5 ^{A}	68.6 ^{A}	58.2 ^{E}	65.2 ^{B}	59.8 ^{D}	49.8 ^{F}	59.6 ^{D}
	RW	42.6 ^{A}	36.9 ^{A}	38.2 ^{A}	37.4 ^{A}	40.9 ^{A}	42.6 ^{A}	40.1 ^{A}	29.8 ^{B}	37.3 ^{A}
higher alcohols (mg/L)	WW	169.57 ^{A}	163.13 ^{A}	166.70 ^{A}	167.43 ^{A}	164.60 ^{A}	166.67 ^{A}	165.33 ^{A}	164.07 ^{A}	170.1 ^{A}
	RW	355.25 ^{A}	349.75 ^{A}	346.36 ^{A}	350.05 ^{A}	352.98 ^{A}	349.76 ^{A}	348.79 ^{A}	348.22 ^{A}	354.67 ^{A}
C6-alcohols (mg/L)	WW	0.51 ^{A}	0.51 ^{A}	0.51 ^{A}	0.51 ^{A}	0.51 ^{A}	0.48 ^{A}	0.51 ^{A}	0.41 ^{B}	0.50 ^{A}
	RW	0.16 ^{A}	0.16 ^{A}	0.15 ^{A}	0.16 ^{A}	0.16 ^{A}	0.16 ^{A}	0.16 ^{A}	0.16 ^{A}	0.16 ^{A}
major ethyl esters (mg/L)	WW	27.13 ^{A}	26.40 ^{AB}	25.40 ^{BC}	25.33 ^{BC}	25.93 ^{AB}	24.40 ^{C}	25.73 ^{BC}	24.97 ^{BC}	25.35 ^{BC}
	RW	288.35 ^{A}	252.80 ^{AB}	239.35 ^{AB}	267.98 ^{AB}	230.22 ^{B}	246.75 ^{AB}	260.43 ^{AB}	232.10 ^{AB}	238.10 ^{AB}
minor ethyl esters (µg/L)	WW	4102.0 ^{A}	3414.1 ^{C}	3421.6 ^{C}	3410.1 ^{C}	4092.0 ^{A}	2947.7 ^{D}	3781.0 ^{B}	3328.9 ^{C}	2722.5 ^{E}
	RW	616.7 ^{A}	552.6 ^{B}	465.1 $^{D E}$	532.8 ^{BC}	483.0 $^{D E}$	499.6 $^{C D}$	607.0 ^{A}	375.0 ^{F}	447.2 ^{E}
methyl esters (µg/L)	WW	3.9 ^{A}	3.5 ^{B}	3.5 ^{B}	3.4 ^{BC}	3.9 ^{A}	3.0 ^{D}	3.6 ^{B}	3.5 ^{B}	3.2 $^{C D}$
	RW	2.2 ^{A}	2.1 ^{ABC}	1.8 $^{D E}$	2.0 ^{ABCD}	1.9 ^{BCD}	1.9 $^{C D}$	2.2 ^{A}	1.6 ^{E}	1.8 $^{D E}$
isoamyl esters (µg/L)	WW	2473.4 ^{A}	2310.6 ^{BC}	2256.9 ^{BC}	2210.5 ^{C}	2464.0 ^{A}	2053.8 ^{D}	2374.5 ^{AB}	2314.5 ^{BC}	2078.3 ^{D}
	RW	261.1 ^{A}	255.8 ^{A}	245.0 ^{AB}	246.3 ^{AB}	225.2 ^{BC}	224.8 ^{BC}	261.1 ^{A}	199.8 ^{C}	223.3 ^{BC}
volatile esters (µg/L)	WW	1.5 ^{A}	1.0 ^{D}	1.2 ^{C}	1.3 ^{BC}	1.4 ^{B}	0.9 ^{D}	1.2 ^{C}	1.0 ^{D}	1.0 ^{D}
	RW	7.0 ^{A}	5.6 ^{B}	4.5 $^{C D}$	4.9 ^{C}	5.8 ^{B}	6.0 ^{B}	6.9 ^{A}	3.8 ^{E}	4.3 $^{D E}$
Higher alcohol acetates (µg/L)	WW	640.7 ^{A}	596.0 $^{C D}$	581.3 $^{D E}$	566.3 ^{E}	628.2 ^{AB}	538.7 ^{F}	610.0 ^{BC}	563.2 ^{E}	502.6 ^{G}
	RW	213.4 ^{A}	205.6 ^{AB}	195.1 ^{AB}	198.7 ^{AB}	188.8 ^{BC}	186.9 ^{BC}	211.7 ^{A}	166.1 ^{D}	174.3 $^{C D}$
Miscellaneous minor esters (µg/L)	WW	42.7 ^{A}	31.4 ^{B}	36.5 ^{B}	28.0 ^{C}	40.1 ^{A}	17.9 ^{D}	22.8 ^{D}	9.0 ^{F}	14.4 ^{E}
	RW	20.0 ^{A}	16.3 ^{BCD}	16.0 ^{BCD}	17.9 ^{ABC}	16.7 ^{ABCD}	16.8 ^{ABCD}	19.4 ^{AB}	13.7 ^{D}	15.4 $^{C D}$
total esters without major ethyl esters (µg/L)	WW	7259.2 ^{A}	6360.3 ^{C}	6299.4 ^{D}	6224.1 ^{D}	7225.9 ^{A}	5573.0 ^{E}	6799.5 ^{B}	6243.9 ^{D}	5339.6 ^{E}
	RW	2000.2 ^{A}	1845.0 ^{AB}	1639.3 $^{C D}$	1783.7 ^{BC}	1631.6 $^{C D}$	1662.3 $^{C D}$	1978.2 ^{A}	1336.4 ^{E}	1538.8 ^{D}

Different letters indicate significant differences with significance level alpha = 0.05.



(**a**) (**b**)

Figure 4. Recovery of volatile substances after fining in white wine; (**a**) main groups: free monoterpenes, higher alcohols, C6-alcohols and total esters; (**b**) specific ester groups: major ethyl esters, minor ethyl esters, methyl esters, isoamyl esters, aromatic esters, higher alcohol acetates, miscellaneous minor esters.



(**a**) (**b**)

Figure 5. Recovery of volatile substances after fining in red wine; (**a**) main groups: free monoterpenes, higher alcohols, C6-alcohols and total esters; (**b**) specific ester groups: major ethyl esters, minor ethyl esters, methyl esters, isoamyl esters, aromatic esters, higher alcohol acetates, miscellaneous minor esters.

Regarding free monoterpenes in white wine, a significant reduction in content was obtained with all fining agents except the mixed product CarboTec GE and the yeast cell wall product Reskue, with the greatest reduction in content achieved with the charcoal product Grandeco (minus 28%). In contrast, no significant reductions were observed in red wine, except for the fining agent Grandeco (minus 30%). For the individual compounds in white wine, it was found that cis- and trans linalool oxide as well as hotrienol and geraniol were strongly reduced in the case of bentonite, while the compounds nerol, citronellol, geraniol and hotrienol (Grandeco only) were strongly affected by the carbon products. In red wine, the reduction of Grandeco was highest for all significant compounds except nerol. For this compound, Granucol GE showed the greatest reduction.

The higher alcohols were not significantly reduced in white or red wine. However, for the individual compounds, there were minor differences in white wine for both 1-propanol and isobutanol, while the difference in red wine was due to the reduction of butanol. While up to 30% of the volatile compounds were reduced from the free monoterpenes, less than 4% were reduced from the higher alcohols (Figures 4a and 5a). There were also no significant effects from C6 alcohols with a maximum reduction of 3% (Grandeco red wine).

Concerning the major ethyl esters, there were significant effects for both the white and red wines, although there were some differences as well. While the two mixed products Purity D and CarboTec GE, the yeast cell wall product Reskue, the two carbon products

Grandeco and Granucol GE, and the plant fiber Flowpure led to significant reductions in major ethyl esters in white wine, that was not the case in red wine. Interestingly, in contrast to the white wine, the use of NaCalit had a significant effect on the sum of the major ethyl esters in the red wine. Looking at the individual compounds, the reductions were mainly seen in ethyl lactate and, in the case of white wine, also in ethyl acetate, although in absolute terms there was only a small impact. The relative reduction in major ethyl esters (Figures 4b and 5b) was classified as a maximum of 10% (Purity D) for white wine, while higher reductions of up to 20% (Purity D) were observed for red wine.

Minor ethyl esters were strongly reduced in white wine by the carbon, the yeast cell wall and the mixed products, while bentonite did not show any effect and plant fibers led to a minor impact on the content of this volatile group. In the case of red wine, only treatment with Flowpure did not lead to any significant reduction in this volatile group. A similar picture emerged for the methyl esters, isoamyl esters and higher alcohol acetates. Purity D, Granucol GE and Grandeco (in the case of red wine) were particularly noticeable here due to strong decrease in these ester compounds. In the case of the other esters, there was an expected reduction in the red wine and white wine due to the carbon products. While in the white wine, though unexpected, the plant fibers reduced this group of esters to a higher extent. On closer examination, only the butyl butanoate compounds were reduced more by the plant fibers in the white wine than in the red wine. Across all volatile groups and thus also in the total ester content, a fairly uniform picture emerged. Purity D reduced the esters most (minus 23% in the total ester content for white wine and minus 33% for red wine), while the bentonite treatment (minus 0.5% in the total ester content for white wine and minus 1% for red wine) hardly affected the ester contents. The two charcoal products, and in particular Granucol GE (minus 23% in total ester content for white wine and red wine), also showed a strong ester reduction. The ester reduction from the plant fibers (minus 6% in the total ester content for white wine and minus 8% for red wine) was lower than the reduction from the yeast wall products.

4. Discussion

4.1. Residues in Austrian Wines

One aim of this work was to give an overview of pesticide residues found in Austrian wine, must and must in fermentation samples. For this purpose, 250 Austrian wines, 45 Austrian musts in fermentation and 33 Austrian grape musts were analyzed regarding the content of 23 common residues. Fifteen of these compounds were detected in individual samples, and 12 compounds were detected in quantifiable concentrations. With the exception of fluopyram (fungicide against oidium with side effect against botrytis), only botryticide residues were found in concentrations above 0.100 mg/L. The highest concentration was detected from boscalid in a must sample at 0.562 mg/L. [20] summarized the observed concentrations of common pesticide residues in grapes, grape must and wine by many authors [15,17–19,46,55,56] in recent years. The results of the present study in respect to botryticide residues are consistent with the results of these studies. Residue concentrations above 0.1 mg/L were obtained from boscalid, fenhexamid, mepanipyrim, and pyrimethanil. However, it is remarkable that the maximum documented residue levels in the studied samples are lower than observed in other studies (in some cases above 1 mg/L) [20]. It is also interesting that the residue metalaxyl was not monitored in larger amounts in the investigated Austrian samples, but in other studies levels above 1 mg/L were found [20]. Fluopyram is not considered in the review [20], while other compounds that were not analyzed in the course of the present study, such as other systemic compounds like iprovalicarb, dithiocarbamates or organic substances like copper and sulfur were discussed in the review [20]. As already mentioned in [5], the Austrian wine control is limited to the monitoring of botryticides. While the results of this present study support this approach, there should be further scientific studies to determine whether the levels of other individual compounds also justify random monitoring in the context of wine control.

Furthermore, these results support the usefulness of studies on the reduction of pesticide residues by means of fining agents.

4.2. The Effects of Bentonite

Due to its capacity to bind proteins, bentonite is mainly used in white must and wine for protein stabilization. However, this process is not selective. Bentonite has been shown to reduce biogenic amines [57,58], cationic anthocyanins [58] and also volatile compounds [59–61]. Another side effect already observed several times is that some residues are reduced by fining with bentonite [24,38,40]. The results of the present study confirm both an aroma reduction and a decrease in pesticide residues. While mainly free monoterpenes were significantly reduced in white wine (>15% in white wine), ester compounds were reduced (>18%) in red wine. The average reduction in pesticide residues was lower than from the other fining agents, at just under 13% for red wine and about 14% for white wine. Botryticides, which are more relevant in practice, were actually reduced by only about 10.5% for red wine and 11.5% for white wine. It is remarkable that the strong reduction of up to 90% cyprodinil as described by [38] could not be observed. In both cases of white and red wine the reduction was about 25% above the average of the other residues, but clearly below the 90% reported. A possible reason for this could be the use of sodium-calicium bentonite, which has been shown to have less absorption power than sodium bentonite [48]. As the results show, due to the loss of aroma, fining with bentonite beyond the necessary level does not make sense and is therefore not recommended.

4.3. Effects of Activated Charcoal

Activated carbon is generally used in winemaking to remove phenolic compounds, pigments and wine defects. It has a high affinity for non-polar compounds and thus also reduces volatile compounds [62]. Activated carbon is known to effectively reduce a number of residues [25,26,28,37,38,41]. The two products investigated in the present study showed residue reduction of about 70% (Grandeco) and about 60% (Granucol GE). The more relevant botryticides were also reduced by more than 60%. Grandeco even reduced the concentrations of the botryticides boscalid, cyprodinil and pyrimethanil to below the analytical limit of quantification, while Granucol GE reduced the concentrations of cyprodinil to below this limit. Compared to activated carbon's strong residue reduction, an adverse effect is a considerable reduction of volatile compounds. Free monoterpenes were reduced by approximately 28% in white wine and 30% in red wine by Grandeco, and by 19% in white wine and 13% in red wine by Granucol GE. Ester compounds were also reduced by Grandeco in white wine by 14% and by Granucol GE by 24%, and by Grandeco in red wine by 33% and by Granucol GE by 23%. Thus, a general charcoal fining in defect-free wine to reduce pesticides cannot be recommended. However, this conclusion can only be reached for wine. Charcoal fining in must is discussed by some authors [28,41,47]. However, a joint consideration of the impact on volatile profiles and the reduction of pesticides in must from charcoal should be performed in future research. This is especially important since the results of [41] suggest that the reducing capacity of charcoal products is lower in must than in wine. In this study, only average reduction values of about 30% were obtained for similar residues. A possible reason for this could be the lack of alcohol and thus the different polarity of the medium [62]. An argument for fining in must is that the volatile compounds were not significantly affected [47]. However, only some fermentation volatile compounds were studied in these analyses. The effect on important primary aroma compounds in must such as monoterpenes was not considered, which is why further studies would be useful.

4.4. Effects of Carbon-Bentonite Mixed Products

In addition to bentonite and charcoal, a number of producers also offer combined products consisting of both fining agents specifically for the reduction of pesticide residues. The two agents CarboTec GE and Purity D were investigated in the course of this study.

The authors are not aware of any publications in which the effect of such combined products on the reduction of pesticide residues and on the volatile profiles of wines was investigated. [20] summarized that combined products of bentonite, PVPP, potassium caseinate, and diatomaceous earth are more effective than isolated bentonite, but did not study the combination of charcoal and bentonite. Both preparations can strongly reduce pesticide residues, although a consistently better result was obtained with the Purity D agent. In general, however, the reductions were significantly lower than with the pure charcoal products (Tables 5–7). Analogous to the reduction of pesticide residues, volatile compounds were unselectively reduced as a negative side effect. Interestingly, the reduction of free monoterpenes was lower compared to the isolated bentonite and charcoal products, whereas esters were reduced to a greater extent, especially in white wine. A general use in white wine and red wine is not recommended, however, the two combination preparations should definitely be tested in the context of must treatment and in the use of grape varieties with high monoterpene contents in further studies.

4.5. Effects of Cell Wall Products

Yeast cell wall products are used to correct stuck fermentations according to [63]. They have the capacity to bind certain fatty acids (octanoic and decanoic acids) that negatively affect the permeability of yeast membranes. Some products have been launched on the market where, according to the producer, it was observed that pesticide residues can also be reduced with these agents. This knowledge is not completely new, ref [24] described the reduction by yeast lees, however it was not clear whether yeast cell wall products also show this effect. In the course of the present study, the agents Reskue and Absolut Wein were tested in wine. It was found that there was a significant reduction in residues. Botryticides were reduced by about 20%, while the total of all pesticide residues examined were reduced up to 35%. Absolut Wein showed a slightly stronger reduction capacity. Disadvantageously, significant flavor damages were also observed when using these treatment agents. However, these were significantly lower than those caused by the charcoal preparations and mixed preparations. Reskue caused more losses of free monoterpenes than Absolut Wein. From both agents, the ester compounds in white wine were reduced by more than 10%, in red wine Reskue showed an ester reduction of almost 20%, while with Absolut Wein the esters were reduced by only about 8%. It would also be interesting to perform experiments with these fining agents in must, especially with Reskue which protects monoterpenes.

4.6. Effects of Plant Fibers

It is known that filtration with cellulose can lead to a reduction of pesticide residues [46,64,65]. Selective plant fibers have been developed as a processing aid to enhance this effect in continuous precoated filtration or as a component of filter sheets [49]. In the course of the present study, however, the use of the market-ready product Flowpure was limited to sedimentation without filtration due to the experimental design. Nevertheless, significant effects were shown in the reduction of pesticide residues (reduction of about 45% of botryticide concentration and almost 50% of total residue concentration) with a slight influence on aroma with the exception of free monoterpenes (14% reduction) in white wine. The effect on ester content was less than with other fining agents in comparison for both red wine and white wine. With the proper use of Flowpure in diatomaceous earth filtration and the resulting short contact times in the wine, the loss of aroma could be eliminated altogether, but it is questionable how the conditions in the filtration vessel affect the reduction of residues. Further studies may be necessary.

5. Conclusions

Pesticide residues are a current issue in Austria despite the high percentage of organically produced wine. In none of the 328 investigated sample pesticide residue values were exceeding limits found; however, partly high contents (>0.100 mg/L) of individual residues were detected. Therefore, a reduction of these contents before filling could make sense in certain cases. The results of this study clearly show that all the agents tested reduced both residues and volatile compounds in the wine, with activated charcoal having the strongest effect and bentonite the weakest on the reduction of residues. The mixed products and yeast cell wall products specifically designed for residue reduction showed less aroma losses than charcoal products but also lower, though still satisfying, residue reduction. With good knowledge of the typical aromas of the respective wines being treated, it can be deduced which of these treatment agents is likely to cause the least amount of damage since not all volatile compounds were damaged to the same extent by all agents. Plant fibers showed a good reduction of pesticide residues with only moderate aroma diminution, but these results must first be confirmed under practical conditions. It would generally make sense to the reduce residues that cannot be avoided in the vineyard before alcoholic fermentation. In this respect, further studies should be carried out that investigate the effects of fining agents in grape juice. It is also not clear whether the aroma damage mentioned here can be perceived at a sensory level at all. The number of aroma-active compounds in wine is high and the non-linear perceptual interactions between these compounds suggest that sensory spaces depend more on relative concentrations than on absolute concentrations in wine. Sensory studies should therefore be included in the future.

Supplementary Materials: The following data is available online at https://www.mdpi.com/article/10.3390/app11125365/s1, Table S1: sample list (wine), Table S2: sample list (must and fermenting must: all conventional), Table S3: Calibration and validation data of the pesticide method, Table S4: Information concerning calibration and validation of the volatile compound, Table S5: volatile profiles of the white wines before and after fining, Table S6: volatile profiles of the red wines before and after fining.

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