Fungicide Residues in Strawberry Processing

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The fate of three fungicides (dichlofluanid, procymidone, and iprodione) applied under field conditions was studied during strawberry processing to juice, wine, and jam. An untreated control was compared to raw material treated with fungicides according to recommended doses and to a sample with 6-fold higher application rates. The highest residue values were found in the pomace after pressing. Residue values in readily produced juices and fruit wines were very low and did not exceed legally required maximum residue levels. Generally, processing steps such as pressing and clarification diminished fungicide residues from 50 to 100%. If the whole fruit is processed, as in fruit preparations or jam, the residue levels remain higher due to missing processing steps.

Keywords: *Residues; fungicides; strawberry; juice; wine; jam*

INTRODUCTION

Strawberries are a popular raw material for the food industry. If the fruits are not commercialized on the fresh market or used in jam production, the fruit juice industry processes them into fruit preparations and clear juices or concentrates. Fruit preparations are used in the dairy industry as ingredients for yogurts, curds, milk shakes, and ice cream or in the candy industry. Juices or concentrates are mainly used in multi-fruit-based juices or beverages and for the production of liqueurs, fruit wines, and sparkling fruit wines.

To cultivate strawberries with reasonable yields and in line with real market conditions, it is necessary to apply pesticides. They are effective against a number of fungal diseases and pests. The major disease is *Botrytis cinerea*. Infections occur during the flowering stage, so that fungicide treatments have to start early in the vegetation period. There is only a short time between flowering and harvesting. Additionally pesticide application is limited by legally required preharvest intervals between the last spray and harvest. In Germany, the legally fixed preharvest intervals for the active ingredients (ai) used in this study are between 7 and 12 days. In 1997, four active ingredients were registered for *Botrytis* control in strawberries under the German crop protection. The goal of the present study was to monitor residues of the fungicides dichlofluanid (Euparen WG, Bayer, Germany), procymidone (Sumisclex WG, Bayer), and iprodione (Rovral, Rhône-Poulenc, Germany) during processing of strawberries to clear juice, fruit wine, and jam.

MATERIALS AND METHODS

Field Trial. The field trial was carried out at the Department of Fruit Growing in Geisenheim at the experimental site "Sand". Strawberry plants, cv. Elsanta, were set in 1996 in 3 × 60 m^2 plots, each with 200 plants: plot A, control, no fungicide application; plot B, standard, treatment according to recommended dose; plot C, high rate, 6-fold application of fungicides as standard. The fungicides were applied sequentially over three applications using a mounted hose and spraying gun device. Table 1 summarizes dates and application doses of the treatments with the fungicide formulations. All fungicides were applied during the flowering stage. The plots were not irrigated. From the first application until harvesting there were 90 mm of rain and 300 h of sunshine. Both values were in the range of the annual mean of this site.

Processing of Strawberries, Sampling, and Storage. *Juices.* Fruits were harvested during a 3 week period from May 23 to June 13 and transported to the Department of Wine Analysis and Beverage Research. They were kept frozen at −22 °C. For processing, they were thawed and crushed in a corundum disk mill; 300 mg/kg ascorbic acid was added. The mash was heated to 50 °C in a tube heater and enzyme treated with 50 mg/kg Fructozym MB for 1 h. Afterward, the mash was pressed on a rack and frame press; the juice was again enzyme treated with 50 mg/kg Fructozym BE (both preparations from Erbsloeh, Geisenheim, Germany) until the negative pectin test. The average values for total acid were 9 g/L at 10 °Brix. Juices were fined with 150 g/hL bentonite, sheet filtered, bottled, and pasteurized. Four samples were taken from the mash, the pomace after pressing, the juice after pectinase treatment, and the readily bottled product. Samples taken directly during processing were frozen in portions of 200 g and kept frozen until analysis.

Fruit Wine. Strawberry wine was manufactured using the traditional mash fermentation. The fruits were crushed, and 200 mg/kg ascorbic acid and 50 mg/kg Fructozym MB were added. To prevent action of lactic acid bacteria during fermentation, 50 mg/kg SO_2 was added. A rapid fermentation was started by adding 20 g/hL dry yeast preparation (Oenoferm, Erbsloeh) and 40 g/hL ammonium phosphate at the mash stage. After 3 days of fermentation, the mash was pressed on a rack and frame press, and the resulting wine was adjusted to a total acidity of 6.5 g/L and 10% vol alcohol by the addition of corresponding amounts of water and sugar. After complete fermentation, the wine was racked with a separator (Westfalia SA-1) and fined with 15 g/hL gelatin, 75 mL/hL silica sol, and 150 g/hL bentonite. The strawberry wine was adjusted to 40 g/L sugar and 50 mg/L free SO_2, sheet filtered, and bottled. Samples were taken from the pomace after mash fermentation, the young wine after separation, and the bottled wine.

Jam. The industrial method of jam production was simulated. Strawberries were washed under running tap water, and

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Table 1. Fungicide Treatments in the Field Trial

		application dose (kg of ai/ha)	
date	formulation, ai	standard	high rate
April 24, 1997	Euparen WG, 50% dichlofluanid	2.0	12.0
May 5, 1997	Rovral, 50% iprodione	1.0	6.0
May 16, 1997	Sumisclex WG, 50% procymidone	0.75	4.5

sepals were removed manually. Equal amounts of fruits and sucrose were mixed, and 80 mL/kg 5% pectin solution (Classic AF-401, Herbstreith&Fox, Neuenbuerg, Germany) was added. The mixture was cooked to 60 °Brix and adjusted to pH 3.0 by the addition of a 50% citric acid solution to initiate gelation. The jam was filled hot into preheated glasses with twist-off caps (250 mL), cooled, and stored at ambient temperature.

Chemicals. Extrelut and pesticide grade solvents were obtained from Merck, Darmstadt, Germany. Standard material for fungicides and the internal standard carbophenothion were purchased from Riedel-de Haën, Seelze, and Dr. Ehrenstorfer, Augsburg, Germany.

Sample Processing. *Strawberry Mash, Pomace, and Jam.* Sample preparation was done according to the German multiresidue method DFG S-8 as modified after Becker and Schug (1990). Briefly, 50 g of the thawed sample is mixed with 1 mL of internal standard solution (freshly prepared working solution of carbophenothion with 10 mg/L in toluene) and extracted with 100 mL of acetone. The extract is filtered and made up to 200 mL with 70% acetone. To 20 mL of this solution are added 50 mL of water and 5 mL of saturated NaCl solution. The mixture is extracted twice with 10 mL of dichloromethane; after phase separations, the dichloromethane phases are pooled and dried over 5 g of Na_2SO_4. The dried extract is filtered and evaporated to dryness. The residue is dissolved in 1 mL of a mixture of dichloromethane/acetone/toluene (10:2:2, v/v/v) and loaded onto a small glass column filled with active carbon and silica gel (2 mL). The column is eluted with 15 mL of the above mixture. After evaporation of the column eluate, the residue is dissolved in 1 mL of toluene and analyzed by GC/MS. All evaporation steps were carried out with a rotary evaporator.

Juices, Wines. Fungicides were extracted by liquid−liquid extraction using Extrelut cartridges. The cartridges are filled with a mixture of 20 g of Extrelut and 10 g of NaCl. A 20 mL sample is mixed with 1 mL of internal standard solution (freshly prepared working solution of carbophenothion with 5 mg/L in ethanol) and loaded onto the cartridge. After a 10 min distribution time of the sample, fungicides are eluted with 60 mL of hexane/dichloromethane (9:1, v/v). The eluate is evaporated in a rotary evaporator to 1−2 mL; 0.2 mL of toluene is added. Residual hexane/dichloromethane is carefully removed under a nitrogen stream, the resulting toluene extract (∼200 μL) is transferred into autosampler microvials and analyzed by GC/MS.

GC/MS Analysis. A Hewlett-Packard gas chromatograph 5890 coupled to an MS engine 5989B mass spectrometer was used. The capillary column was an HP-5 MS (25 m × 0.25 mm i.d., 0.25 μm film thickness). Injector and transfer line were heated to 250 and 280 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 80 °C for 1 min, raised to 175 °C (40 °C/min), held for 1 min, raised to 275 °C (6 °C/min), and held for 4 min. The carrier was helium with a flow rate of 1.1 mL/min kept constant by electronic pressure programming. MS detection was done with characteristic ion fragments of the substances in the EI-SIM mode (ions 224, 167, and 123 for dichlofluanid; ions 285, 283, and 96 for procymidone; ions 315, 245, 189, and 187 for iprodione; and ions 341, 157, 153, and 121 for the internal standard carbophenothion). Quantitation was carried out with known concentrations of the internal standard using experimentally determined response factors. Residue analysis was done in duplicate.

Recovery Assays. Recoveries were checked with four replications at two fortification levels of each fungicide. For the determination we took mash and juice from the untreated control sample. The samples were spiked with 0.5 and 1.0 mg/kg and 0.025 and 0.05 mg/L, respectively, of the active ingredients.

RESULTS AND DISCUSSION

Recovery. In the nonspiked control samples no residues of the corresponding substances could be detected. Table 2 shows the average recoveries in mash and juice carried out with the fortified control sample. The recovery values of both methods were in an acceptable range (Hill, 1998) between 87 and 110%. The relative standard deviations (RSD) were between 2.1 and 7.8%. In routine work the determination limits were 0.01 mg/kg with the DFG S 8 method and 0.005 mg/L with the Extrelut method for dichlofluanid and procymidone. For iprodione, we obtained determination limits of 0.02 and 0.01 mg/kg, respectively, because of the lower MS response. The determination limits were estimated using the calibration curve method after Frehse and Thier (1991).

Determination of Residues in Strawberry Juice. As expected, we found no residues of the monitored fungicides in the control samples. Table 3 summarizes the residues during the processing steps from the mash stage to clear juice. Due to the 6-fold higher application dose there are distinct higher residue values in the juices from this sample. In all processing stages, the highest residue levels in the readily processed juices were found for procymidone and iprodione. Dichlofluanid was applied at the beginning of the flowering stage; procymidone was applied 10 days later and iprodione 21 days later. Probably, the lower concentrations for dichlofluanid residues may result not only from the earlier application date but also from a higher degradation rate. The low concentration of dichlofluanid in the processed products could also be due to its physicochemical properties. The high K_{ow} of dichlofluanid relative to those of the other two fungicides indicates that it has a very low water solubility and is more likely to be associated with the solid organic matrix. This is supported by the high levels found in the pomace relative to the juice.

The harvested strawberries were not analyzed separately, but with respect to the residues they may be compared with the freshly prepared mash. Generally, the residue concentrations of the standard treated strawberries are in a low range of 0.01−0.15 mg/kg. After pressing, there is a distinct enrichment of the fungicides in the pomace. Regarding the sample with the high application dose of the fungicides, even in the pomace, which is a waste product of fruit processing, maximum residue limits (MRLs) were not exceeded. Because of the strong adsorption power of the pomace, low residue levels were observed in the raw juices. A further decrease occurs during the clarification process.

Determination of Residues in Strawberry Wine. In the mash and in the wines of the control sample no fungicide residues could be detected. The strawberry juices and the corresponding wines were produced from the same mash, so the residue values for the mash can be taken from Table 3. As Table 4 shows, traditional mash fermentation of the high-rate-treated strawberries did not lead to excessive residue values in the resulting wines. The particular values for both series are com-

Table 2. Mean Recoveries (Percent) and Relative Standard Deviations (RSD) of the Monitored Fungicides from Untreated Strawberry Mash and Juice at Various Fortification Levels

	fortification levels							
	strawberry mash method DFG S 8				strawberry juice Extrelut method			
fungicide	0.5 mg/kg	RSD	1.0 mg/kg	RSD	0.025 mg/L	RSD	0.050 mg/L	RSD
dichlofluanid	87	3.7	89	2.1	101	5.8	104	3.7
procymidone	98	3.6	98	2.9	93	3.3	100	7.8
iprodione	108	5.9	89	5.8	107	4.5	110	6.8

Table 3. Fungicide Residues in Strawberry Juice Processing

	mash		pomace		juice after enzymatic treatment		juice after clarification and filtration	
ai	standard	high rate	standard	high rate	standard	high rate	standard	high rate
dichlofluanid	0.01[a]	0.17	0.08	0.88	0.01	0.01	nd[b]	nd
procymidone	0.15	1.40	1.88	3.30	0.11	0.71	0.05	0.31
iprodione	0.08	1.27	1.34	3.62	0.04	0.56	0.02	0.39

[a] Mash and pomace, mg/kg; juices, mg/L; mean values from duplicate analyses. [b] nd, not determined (L_{DM} < 0.005 mg/L).

Table 4. Fungicide Residues in Strawberry Wine

	pomace after fermentation		wine after racking		wine after clarification and filtration	
fungicide	standard	high rate	standard	high rate	standard	high rate
dichlofluanid	0.06[a]	0.23	nd[b]	nd	nd	nd
procymidone	0.59	4.01	0.05	0.35	0.05	0.30
iprodione	0.35	3.84	0.02	0.29	0.02	0.27

[a] Pomace, mg/kg; wines, mg/L; mean values from duplicate analyses. [b] nd, not determined (L_{DM} < 0.005 mg/L).

parable to those from juice production except for the pomace, for which slightly higher amounts could be found. Probably this is a result from adsorption of fungicides by the yeast. Neither in the bottled wine from standard samples nor from the high-rate treatment could dichlofluanid be detected.

Determination of Residues in Strawberry Jam. All jam samples could not be analyzed for iprodione because of a large matrix peak appearing at the same retention time already in the untreated control sample. Regarding the correspondent mass spectra, it was a question of phthalates migrated from the compound mass of the cap gasket into the jam. Dichlofluanid and procymidone were not found in jam from the control sample. In the standard treated sample we measured 0.03 and 0.08 mg/kg, respectively. In case of the sample treated with the high application dose, we measured 0.04 mg/kg dichlofluanid and 0.44 mg/kg procymidone. The whole fruit is processed, and cooking is the only processing step here. There are no diminishing steps such as pressing or clarification as there are in juice or wine production.

Treatments with fungicides within the horticultural practice can lead to residues on strawberries. However, if application follows good agricultural practice and if preharvest intervals are adhered to, generally no residues above MRLs occur. For berry fruits and corresponding processed products, the German MRL law tolerates 5 mg/kg in the case of iprodione and procymidone and 10 mg/kg in the case of dichlofluanid. As could be shown here, residue-free fruits can be harvested only if synthetic fungicides are not applied. It is important for manufacturers and consumers that processing of strawberries into juice or wine decreased residues of the fungicides applied in this study. This is not true, for example, for fruit preparations or jams, for which the whole fruit is processed and only sugar and some minor ingredients are added. Even in the juices, the fruit wines, and the jams from the high-rate-treated sample we found no values exceeding the legally fixed German MRLs.

During processing of strawberry juice and wine the main portion of the monitored fungicides remained in the pomace. This may be compared to wine-making from grapes. In a residue study from vine to wine we found an up to 100-fold reduction of dichlofluanid from grapes to the clarified wine (Will et al., 1996). In conclusion, there is a strong enrichment of the substances in the pomace. The main part of the residues is adsorbed by the waste material of the juice or wine production. Farris et al. (1992) obtained similar results. They observed a distinct decrease of insecticide residues in must and wine after pressing the grapes. As could be shown here, clarification steps decrease the fungicide residues additionally. Cloud particles, which are able to adsorb active ingredients, are precipitated by fining and mechanically removed by filtration.

Today, industrial fruit wine production starts mainly from concentrates. Strawberry concentrates are available independent from the harvest season, and they are easier to handle. Traditionally, fruit wines from red berries are fermented for several days on the mash. During mash fermentation the alcohol extracts the anthocyans from the solid into the liquid phase and provides an attractive color. Obviously the fungicides monitored here are not extractable with this low ethanol concentration. Despite an alcohol concentration of ~4−5% vol after 3 days of mash fermentation and pressing, the main part of the fungicides remained adsorbed to the pomace or to the yeast.

We expected low residue levels at our determination levels for strawberry juice and wine using fruits treated with recommended doses. Thus, samples treated with application rates 6-fold higher than recommended were examined in parallel to achieve a better residue monitoring of the single processing steps above the analytical limits. The freshly prepared mash is the starting point

for juice and wine processing. Six-fold higher application doses led to residue values from 0.17 to 1.40 mg/kg in the mash. In no case were German MRLs exceeded. In manufacturing of clear strawberry juice or wine, we found reduction rates from nearly 100% for dichlofluanid and to 70−80% in the case of procymidone and iprodione.

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