Food and Chemical Toxicology 50 (2012) 3990–3995

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

The influence of fining agents on the removal of some pesticides from white wine of *Vitis vinifera* L. cv. Emir

K. Sen [a], T. Cabaroglu [b,*], H. Yilmaz [c]

[a] Nevsehir University, Engineering Architecture Faculty, Department of Food Engineering, Nevsehir 50300, Turkey
[b] University of Cukurova, Faculty of Agriculture, Department of Food Engineering, Balcali, Adana 01330, Turkey
[c] NetKim Laboratory Equipment Marketing Co. Ltd., Seyhan, Adana 01170, Turkey

ARTICLE INFO

Article history:
Received 1 December 2011
Accepted 3 August 2012
Available online 24 August 2012

Keywords:
Fining agents
Pesticide residues
White wine
Fining

ABSTRACT

The influences of fining agents (activated carbon, casein, kieselsol–gelatine, bentonite and polyvinylpolypyrrolidone) and their doses (low, middle, high) on the removal of six pesticides used in viticulture (vinclozolin, penconazole, α-endosulfan, imazalil, nuarimol and tetradifon) from white wine were investigated. The pesticides were added into white wine obtained from the Emir grape, and then the wine was clarified with the use of fining agents at low, middle and high doses. After the fining, extraction of pesticides from the wine was made by liquid–liquid extraction. Quantification and identification were performed by the multiresidual method using GC–MS and GC-ECD techniques. The effect of the fining agents on these pesticides ranged from little to large. Of the fining agents, activated carbon showed the largest effect on the removal of pesticides. The pesticide removal efficiencies of the fining agents were in the following order: activated carbon, casein, bentonite and kieselsol–gelatine. Polyvinylpolypyrrolidone (PVPP) had the least effect on the removal of pesticides. A linear relation was not found between fining agent doses and the amount of removed pesticides. α-Endosulfan, penconazole, imazalil and tetradifon were removed by the fining agents at the highest levels. Vinclozolin and nuarimol were the pesticides least affected, except activated carbon and casein.

1. Introduction

Although problems stemming from the use of pesticides have been known, recently the use of pesticides in viticulture has become an important practice all over the world (Farris et al., 1992). While their environmental effects are relatively safe at acceptable doses, some concerns about the toxicity of pesticide residues remain (Soleas and Goldberg, 2000). Pesticides on grapes depend largely on the concentration of pesticides used in the vineyard to protect the crop; the technique; the number of applications; and the length of time between the last application and harvest (Miller et al., 1985; Frank et al., 1990). Passage of these residues from the grapes to the wine depends on the physical properties of the active ingredients and, primarily, on their solubility in water and water: alcohol mixtures (Navarro et al., 1999). However many researchers have established that winemaking processes, such as maceration, pressing, racking, clarification and filtration, influence the concentration of pesticide residues (Farris et al., 1992; Soleas and Goldberg, 2000; Gennari et al., 1992; Cabras and Garau, 1995; Cabras et al., 1999; Navarro et al., 1999; Tsiroulos, 1999; Ruediger et al., 2004). Clarification of wines is an important process especially from the point of view of wine color and brilliancy (Cabras et al., 1999). Various substances have been used to clarify wines for many years, and many authors have reported that pesticide residues can be adsorbed and removed by fining agents (Farris et al., 1992; Soleas and Goldberg, 2000; Gennari et al., 1992; Cabras and Garau, 1995; Cabras et al., 1999; Navarro et al., 1999; Tsiroulos, 1999; Ruediger et al., 2004; Fernandez et al., 2005). Fining agents, which are all adsorptive compounds, commonly used in winemaking are grouped according to their general nature; earths (montmorillonite, bentonite, kaolin), animal proteins (gelatin, isinglass, caseins), wood charcoal (carbons) and synthetic polymers (polyvinyl polypyrrolidone – PVPP) (Marchal and Jeandet, 2009). Generally, adsorption contains the accumulation of molecules from a solvent onto the exterior and interior (i.e., pore) surfaces of an adsorbent. The surface phenomenon is a manifestation of complex interactions (vander Waals, resonance, and electrostatic forces and hydrogen bonding) between the adsorbent, adsorbate, and the solvent (Castellari et al., 2001).

The aim of this investigation was to determine the potential of fining agents (activated carbon, casein, kieselsol–gelatine, bentonite and polyvinylpolypyrrolidone) for removing six pesticides (vinclozolin, penconazole, α-endosuphane, imazalil, nuarimol and tetradifon) used in viticulture in Turkey from white wine. Although some of these pesticides have been studied, to the best of our

* Corresponding author. Tel.: +90 3223386173; fax: +90 3223386997.
E-mail address: tcabar@cu.edu.tr (T. Cabaroglu).

0278-6915/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2012.08.016

knowledge the current study is the first report on the removal of penconazole, imazalil, nuarimol, and tetradifon by use of fining agents.

2. Materials and methods

2.1. Wine

A non-clarified white wine produced from a Turkish variety of Emir was used. The compositional characteristics of the white wine are shown in Table 1.

2.2. Pesticide standard

The pesticides used in this investigation were of analytical grade. All of the pesticides were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Some properties of the pesticides such as octanol–water partition coefficient ($\log K_{ow}$), solubility and the doses used are shown in Table 2. $\log K_{ow}$ is an expression of adsorption degree of a substance to solid matter. High $\log K_{ow}$ values show that the compounds have a good degree of adsorption. Moreover low $\log K_{ow}$ values mean that the compounds generally remained in the water phase, in other words they do not have good adsorption abilities to solid matter (Roger, 1996; Jones et al., 2005). $\log K_{ow}$ values and solubility values in water of the pesticides preferred in this study are between 3.00–4.61 and 0.05–180 mg/l, respectively.

2.3. Fining agents

Commercially prepared fining agents were purchased from Erbslöh GmbH-Germany (betonite, gelatine, kieselsol), Merck KGaA-Germany (activated carbon), and Laffort-France (casein). Some physical properties and composition of the fining agents used are as follows:

Activated carbon: It is nonspecific adsorptive agents made from wood. The sponge like carbon binds with weakly polar molecules, especially those containing benzene rings. It has a BET surface area of 1100–1500 m^2/g, a particle diameter of 10–100 Å.

Casein: It has water-soluble potassium caseinate form with isoelectric points of 4.1. Casein is a positively charged protein that flocculates in acidic media such as wine.

Gelatine: It is a gelatin of animal origin in food quality for the treatment of wines. It is a product of high purity. The Bloom value is between 90–100 which is the ideal range for wine treatment. Gelatine is a positively charged protein with isoeletric points of 4.8.

Kieselsol: It has a strong and rapid action in white wines. It is used in combination with gelatine. Kieselsol is negatively charged and electrostatically bind to and adsorb positively charged proteins and initiate flocculation and settling. It has particle sizes ranging from approximately 30 to 100 nm in diameter and particle density in the range of 2.1–2.3 g/cm^3.

Bentonite: It is a natural, negatively charged, finest-grained sodium bentonite powder mined from a particularly pure bentonite layer. It has a surface area of 700–800 m^2/g and particle size of 1–2 mm.

PVPP: It is 100% polyvinylpolypyrrolidone of active ingredient, purity requirements comply with EU Regulation No. 3220/90. It has highest adsorptive capacity through best preparation, selection and finest grain size distribution. It is in a diameter exceeding 37 μm in over 90%.

2.4. Reagents

All of the reagents were of the highest analytical grade. Acetone, petroleum ether, dichloromethane, anhydrous sodium sulfate and sodium chloride were purchased from Merck KGaA-Germany.

Table 1
Composition of Emir white wine.

Compositional parameter	White wine
Density	0.9917
Alcohol (v/v)	11.75
pH	3.18
Total acidity (g/l)	6.32
Volatile acidity (g/l)	0.26
Total sugar (g/l)	1.12
Total SO_2 (mg/l)	103
Free SO_2 (mg/l)	28
Turbidity (NTU)	151
Optical density (420 nm)	0.312
Absorbance (280 nm)	3.913
Absorbance (520 nm)	0.201
Pesticide (μg/l)	Not detected

2.5. Pesticide treatments and fining procedure

The concentrations of vinclozolin, penconazole, α-endosulfan, imazalil, nuarimol and tetradifon used in this experiment are given in Table 2. All pesticides were added to the white wine. Then the treated wine was divided into 45 equal volumes and they were filled in glass cylinders with a capacity of 100 ml. Then, the wine was clarified by fining agents at low, middle and high doses. The procedure was carried out in the darkness by wrapping each glass cylinders with aluminium foil. A flowchart of the experiments are shown in Fig. 1. The doses of fining agents were adjusted according to values which were given in the literature (Boulton et al., 1996; Jackson, 2000). During the clarification, glass cylinders were held for 5 days in a room set to 18 °C, and all experiments were performed three times.

2.6. Extraction procedure

The extraction of wine samples was made using the method described by Makovi and McMahon (1994). 25 ml of wine sample was taken in a separatory funnel with 250 ml. 50 ml of acetone was added and the mixture was agitated for 1 min. At the end of this time, 25 ml of petroleum ether and 25 ml of dichloromethane were added, and the separatory funnel was shaken for 1 min again. The phases were allowed to separate, and all of the organic phase was taken and passed through anhydrous sodium sulfate for the purpose of removing the aqueous phase. The organic phase was evaporated to dryness, residue was dissolved in 1.8 ml of acetone and injected into the GC.

2.7. Quantification and identification of pesticides by GC-ECD and GC–MS

Quantification and identification of pesticides was performed according to the method described by Navarro et al. (2000)). Validation parameters of the method using GC-ECD are shown in Table 3.

Vinclozolin, penconazole, α-endosulfan, imazalil, nuarimol and tetradifon were determined by GC with an electron capture detector (ECD). In all cases, an HP Agilent 6890 gas chromatography was used. The capillary column was an HP-5MS (% 5 phenyl methylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Folsom, CA). The injector and detector were operated at 250 °C. The extract (1 μl) was injected in the split mode (30:1 split rate). The oven temperature was programmed as follows: initial 50 °C, held 2 min programming rate 25 °C/min (from 50 to 150 °C), 3 °C/min (from 150 to 200 °C), 8 °C/min (from 200 to 280 °C), and held 4 min at 280 °C. Helium was carried at 1.2 ml/min and nitrogen was carried at 60 ml/min for making up. A typical chromatographic separation of pesticide standards is shown in Fig. 2.

The pesticide standards were used to create a calibration curve (Table 3). The calibration range was from 2.5 to 250 μg/l. Calibration graphs for the pesticides were constructed with the external Standard method by measuring peak areas depending on concentrations. Calibration curves were linear over the specified range and coefficients (r^2) were between 0.9994 and 0.9999 for all pesticides.

Recovery experiments were carried out for the purpose of reliability and suitability of the multiresidue method. In this regard, an untreated sample of wine was fortified with 250 μg/l of each pesticides and processed according to the extraction procedure given above. At each fortification, six replicates were analyzed. The proposed procedures were validated by recovering pesticides from fortified samples. Average recovery of each pesticide for wine was utilized to calculate mean recovery and inter-replicate repeatability (expressed as the relative standard deviation RSD%). Recoveries ranged between 67.20–102.02%, and RSD between 1.65–9.70% were obtained by GC-ECD. LOD and LOQ were calculated according to SANCO Guide recommendations (SANCO, 2009). The injection concentration, which could be detected at the signal-to-noise ratio (S/N ratio) of 3, was considered to be the LOD for all pesticides. LOQ was set at a signal-to-noise ratio (S/N ratio) $\geqslant$10 by chromatography for individual pesticides in wine. As shown in Table 3, LOD and LOQ of pesticides in GC-ECD changed between 0.69–1.06 μg/l and 1.24–2.33 μg/l, respectively.

An Agilent 6890 equipped with Agilent 5973N mass selective detector (MSD) Hewlett–Packard 6890 gas chromatograph was employed to confirm the identity of all pesticides. An HP-5MS (% 5 phenyl methylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Folsom, CA) was used. The injector was operated at 250 °C. The operation conditions were: acquisition mode scan (mass range 50–450), voltage 1650 V, ionization foil temperature 230 °C, quadrupole temperature 150 °C. The sample (1 μl) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: initial 50 °C, held 2 min programming rate 25 °C/min (from 50 to 150 °C), 3 °C/min (from 150 to 200 °C), 8 °C/min (from 200 to 280 °C), and held 4 min at 280 °C. All pesticides were identified using their analytic standards and identification data are given in Table 4.

2.8. Statistical analysis

Analysis of variance (ANOVA) was performed by SPSS 15 software. Mean comparisons were performed by Duncan's multiple range test at $p = 0.05$, when appropriate.