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Investigations on fungicide removal from broccoli by various processing methods

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ABSTRACT

The removal of five fungicide residues from the broccoli matrices were achieved by various processing methods. The influence of washing with chlorinated and ozonated water, blanching and cooking on the concentration of azoxystrobin, boscalid, iprodione, metalaxyl, and pyraclostrobin has been studied on the broccoli samples collected from experimental plots over two weeks after the application. The analysis of five pesticides was carried out by gas chromatography with nitrogen-phosphorous and electron capture detection (GC-NPD/ECD) and matrix solid-phase dispersion as the sample preparation method. Fungicides levels in the unprocessed broccoli samples were 0.16–4.34 mg/kg. The washing of the matrices with chlorinated and ozonated water was not as effective as cooking in the removal of residual pesticides. Washing with chlorinated and ozonated water reduced concentration up to 45.9 and 49.0%, respectively. Blanching and cooking decreased residues up to 50 and 87%, respectively. Our results show that the cooking process has a great potential in the process of removing residual pesticides from broccoli.

Keywords: Processing methods; Fungicide residue; Broccoli

1. Introduction

In the era of intensive chemization of agriculture, pesticides are widely used to increase the yield of vegetables, maintain their quality and destroy pests, and prevent diseases during cropping. However, the use of pesticides often leads to the presence of pesticide residues after harvest below permitted limits (maximum residue limits) [1,2].

Broccolis are susceptible to disease attacks, therefore, in cultivation during one growing season plant protection products (PPP) are applied. Thus, pesticides can be often detected in those vegetables [3,4]. Occurrence of various pesticide residues in vegetables has been widely studied and published by many researchers [5–7]. However, the data of fungicide residue occurrence in broccoli are limited.

Broccolis, among *Brassica*, are important vegetables in the human diet because they are rich in glucosinolates. These compounds (sinigrin, glucoraphanin, glucobrassicin and gluconasturtin) are essential phytonutrients for health and play a cancer-protective role. Today consumers eat broccoli both cooked and

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raw, and it is important to consider the effect of removed pesticide residues on the quality of both raw and cooked broccoli.

In Poland, cultivation of broccoli is very common. Many PPP to control agrophages are registered, and label instructions can be found at the Polish Ministry of Agriculture website [8]. The most popular PPP used against fungi diseases include azoxystrobin, boscalid, iprodione, metalaxyl, and pyraclostrobin. They are a new broad spectrum fungicides from different chemical classes (Table 1), and high residual levels have been detected in crops [9,10]. Azoxystrobin (methyl(*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) is a fungicide with protectant, curative, eradicant, and systemic properties. Boscalid (2-chloro-*N*-(4′-chlorobiphenyl-2-yl)nicotinamide) is a protectant fungicide, foliar absorption, translocates, inhibits spore germination and germ tude elongation. Iprodione (3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide) is used to prevent the germination of fungal spores and it has a contact action with that protectant and some eradicant activity. Metalaxyl (methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-*DL*-alaninate) is a systemic fungicide with a curative and protective function. It works by suppressing sporangial formation, mycelial growth, and prevents new infections. Pyraclostrobin (methyl (2*E*)-2-(2-{[3-(4-chlorophenyl)-1-methylpyrazol-5-yl]oxymethyl}phenyl)-3-methoxyacrylate) plays a protective and curative role. Physicochemical properties of fungicides used in field experiment, their chemical structures, water solubility, log K_{ow} data, the type of activity and maximum residue levels are included in Table 1 [11].

It is well known that food processing can reduce the level of pesticide residues. Over the last decade, various studies have been performed to measure the concentration of pesticide residues after home or industrial processing [12,13]. For many years, it has been assumed that processing methods like washing, boiling, and blanching lead to a significant decrease in pesticide residues [14,15]. The ratio between the residue level in the processed commodity and the residue level in the commodity to be processed is called processing factor (PF). However, many PFs remain unknown and are necessary to estimate the total concentration in processed products [16].

Therefore, the aim of this study was to estimate the influence of four processing methods: washing chlorinated and ozonated water, blanching, and cooking on pesticide residues levels in broccoli. Additionally, PFs for five fungicides on broccoli were evaluated.

2. Experimental

2.1. Reagents and materials

Acetone, *n*-hexane, diethyl ether and toluene (pesticide residue grade) were obtained from J.T. Baker (Deventer, Holland). Florisil (60–100 mesh) was supplied by J.T. Baker (Deventer, Holland) and anhydrous sodium sulfate from Fluka (Seelze-Hannover, Germany). Silica gel was obtained from Merck (Darmstadt, Germany). All sorbents were activated at 600˚C in an oven for eight hours.

Pesticides were purchased from the Dr Ehrenstorfer GmbH (Augsburg, Germany) with certified purity above 95%. Individual stock standard solutions were prepared in acetone and stored at 4˚C. Working standard solutions for gas-chromatographic analysis were prepared by suitable dilution of the stock solution with a mixture hexane/acetone (9:1, v/v).

2.2. Field experiments

2.2.1. Cultivation

The open field trials were conducted in a plantation of broccoli located in Sokółka, the northeastern Poland (Podlasie). The total area of the field was 100 m^2 and five plots (20 m^2 each) were separated. A double dose of the recommended dose of PPP was applied by a specialized operator using spray boom equipment at normal settings and timing, providing 50–70 drop/cm^2 with a drop size between 150 and 250 μm (Table 2). The total rainfall was 40.1 L/m^2 and the daily maximum/minimum/medium temperatures ranged between 21/9/18˚C from the day of spraying until harvest.

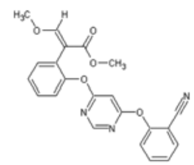
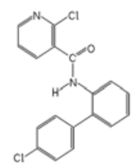
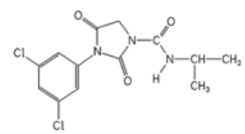
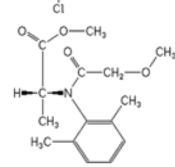
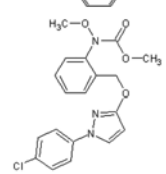
2.2.2. Harvest

Broccoli samples were collected 14 d after the application of PPP. Vegetables were cut using knives at 1–2 cm above the ground level. The knives had been cleaned with water prior to use and sterile disposable gloves were worn. Diseased and oversized plants were excluded from sampling. Forty heads of broccoli were randomly collected to obtain 12 kg of samples, packed in polyethylene bags, and transported in refrigerated condition to the laboratory. The samples were stored in a refrigerator at 4˚C before analysis.

2.3. Sample preparation

As for 12 kg of samples, the broccoli were cut into small part (florets) and divided into four

Table 1
Characteristics of the analyzed fungicides

Acive substance	Substance group	Solubility[a]	log K_{ow}[b]	Chemical structure	Chemical formula	Molecular mass (g/mol)	Mode of action	MRL[c] (mg/kg)
Azoxystrobin	Strobilurin	6.7	2.5		$C_{22}H_{17}N_3O_5$	403.4	Systemic	5.0
Boscalid	Carboxamide	4.6	2.96		$C_{18}H_{12}Cl_2N_2O$	343.21	Contact	5.0
Iprodione	Dicarboximide	12.2	3.1		$C_{13}H_{13}Cl_2N_3O_3$	330.17	Contact	0.1
Metalaxyl	Phenylamide	7,100	1.65		$C_{15}H_{21}NO_4$	279.33	Systemic	0.2
Pyraclostrobin	Strobilurin	1.9	3.99		$C_{22}H_{21}ClN_2O_4$	412.87	Systemic	0.1

[a]In water at 20°C (mg/L).
[b]log K_{ow} octanol-water partition coefficient at pH 7, 20°C.
[c]EU Pesticide Database [17].

Table 2
Characteristic of applied PPP

PPP	Active substance (a.s.)	The recommended dose of PPP[a]	The double dose of PPP used in experiment	Fungi causal agent
Amistar 250 SC	Azoxystrobin	0.3 L a.s./ha	0.6 L a.s./ha	*Alternaria brassicae* *Alternaria brassicola* *Alternaria alternata*
Signum 33 WG	Boscalid pyraclostrobin	1.0 kg a.s./ha	2.0 kg a.s./ha	*Alternaria brassicae* *Alternaria brassicola* *Alternaria alternata*
Rovral Aquaflo 500 SC	Iprodione	1.0 L a.s./ha	2.0 L a.s./ha	*Botrytis squamosa* *Botrytis aclada* *Botrytis cinerea Pers* *Alternaria brassicae* *Sclerotinia sclerotiorum*
Ridomil Gold MZ 67.8 WG	Metalaxyl	2.0 kg a.s./ha	4.0 kg a.s./ha	*Albugo candida* *Puccinia horiana*

[a]The recommended dose of PPP according to Polish Ministry of Agriculture web site [8].

representative analytical subsamples (about 3 kg). In order to minimize the variability factor, broccoli florets were taken randomly. Fig. 1 shows the sampling scheme and processing procedure.

As shown in Fig. 1, each analytical subsample was divided into two parts. One part (1.5 kg taken randomly) was not the object of any processing (unprocessed sample) and was used to evaluate the initial concentration of five fungicides. This part of subsample was homogenized in Waring Blender (USA) and frozen until further analysis. The other part of broccoli florets (the remaining 1.5 kg taken randomly) were divided into 500 g samples and washed with chlorinated, ozonated water, blanched and cooked to obtain processed samples. Immediately after processing, all these samples were blended and kept deep-frozen (−20˚C) before being analyzed.

2.4. Processing factor

The unprocessed broccoli samples were used to calculate some PFs. The PF is a ratio between residues concentration in the processed commodity and the same in the raw commodity [Eq. (1)]:

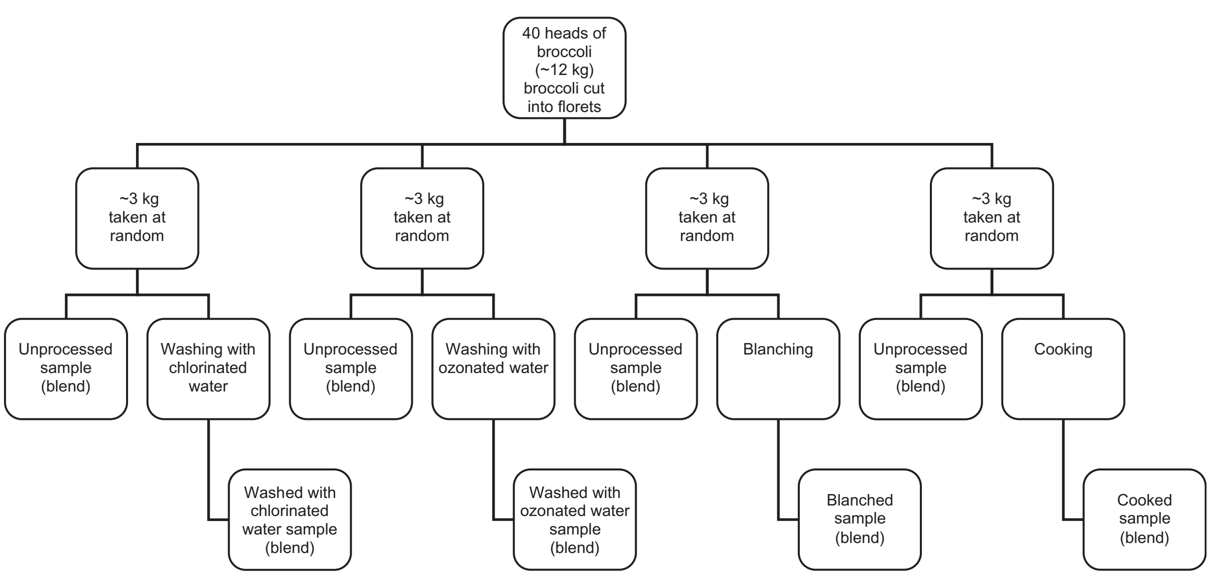


Fig. 1. Processing scheme.

$$\mathrm{PF} = \frac{c_{\mathrm{processed}}}{c_{\mathrm{raw}}} \times 100 \quad (1)$$

where PF—processing factor; $c_{\mathrm{processed}}$—residue concentration in the processed commodity; c_{raw}—residue concentration in the raw commodity.

2.5. Processing methods

2.5.1. Washing with chlorinated water

Five hundred grams of broccoli florets was washed using 1 L chlorinated (tap) water (10˚C, 0.1 mg Cl_2/L) by immersion for 5 min. After that period of time, the florets were taken out and dried by absorbent paper ("washed with chlorinated water" sample).

2.5.2. Washing with ozonated water

Ozone was generated with an GL-2186 ozone generator (WRC Multiozon, Poland) at a flow rate of 1 L/min. The maximum amount of ozone that could be dissolved under these conditions was 1 mg O_3/L. Then 500 g of broccoli florets were immersed in this solution (10˚C, 1 mg O_3/L) for 5 min. After that time, the florets were taken out and dried by absorbent paper ("washed with ozonated water" sample).

2.5.3. Blanching

500 g of broccoli florets was put into a stainless steel basket to 1 L of boiling water (100˚C) for 1 min. After the thermal process, the broccoli was cooled in water (10˚C, 1 L per 500 g) and dried by the absorbent paper ("blanched" sample).

2.5.4. Cooking

Five hundred grams of broccoli florets was put into a stainless steel basket to 1 L of boiling water (100˚C) for 20 min. After that time, the basket with florets was taken out and dried by the absorbent paper ("cooked" sample).

2.6. Sample preparation and chromatographic analysis

Broccoli samples were prepared according to the original matrix solid-phase dispersion method described in our previous publication Łozowicka et al. [4]. The scheme of the extraction procedure is presented in Fig. 2.

The qualitative and quantitative analysis of the samples was performed using Agilent 7890 A gas chromatograph (Waldbronn, Germany) equipped with a nitrogen-phosphorous (NP) and an electron-capture (EC) detector. The chromatographic separation was carried out on a HP-5 column containing 5%-phenyl-methylpolysiloxane (30 m × 0.32 mm and film thickness 0.50 μm). The operating conditions were as follows: for detectors—injector temperature: 210˚C; carrier gas: helium at a flow-rate of 3.0 mL/min; detector temperature: 300˚C (EC and NP); make up gas: nitrogen at a flow-rate of 57 mL/min (EC) and 8 mL/min NP, hydrogen 3.0 mL/min, air 60 mL/min; for oven—the initial temperature: 120˚C increase to 190˚C at 16˚C/min, then to 230˚C at 8˚C/min and finally to 285˚C at 18˚C/min and hold 10 min (EC and NP). The volume of the final sample extract injected at 210˚C in splitless mode (purge off time 2 min) was 2 μL. The total time of the analysis was 20 min.

3. Results and discussion

3.1. Recovery studies

During the study, a number of quality control recovery tests were conducted on blank broccoli samples. Three concentration levels of five pesticide standards ranging between the limit of quantification (LOQ) and 200× the LOQ had been used for calibration curve, and the correlation coefficients (R^2) were >0.998 (Table 3). The LOQ had been determined with a signal-to-noise ratio $(S/N) = 10$ and the limit of detection (LOD) $(S/N) = 3$. These values had been determined following the guidelines established by the European Commission [18], in which LOQ is defined as the lowest validated spike level meeting the method performance criteria.

The blank broccoli samples had been spiked at three fortification levels and analyzed by the methodology described in point *2.6*. Mean recovery values and the corresponding relative standard deviations (RSD) obtained for five pesticides are indicated in Table 3. In all those cases, the results of the recovery tests are in accordance with the validation and quality control criteria for pesticide residue analysis (mean recovery in the range 70–120% with an RSD < 20%) [18].

3.2. Unprocessed broccoli

The purpose of the field experiment was to produce samples containing residues of five selected pesticides in order to determine the initial concentrations of raw, unprocessed broccoli. It was necessary to calculate PFs, which described the efficiency of food processing on pesticide residue level. The concentrations

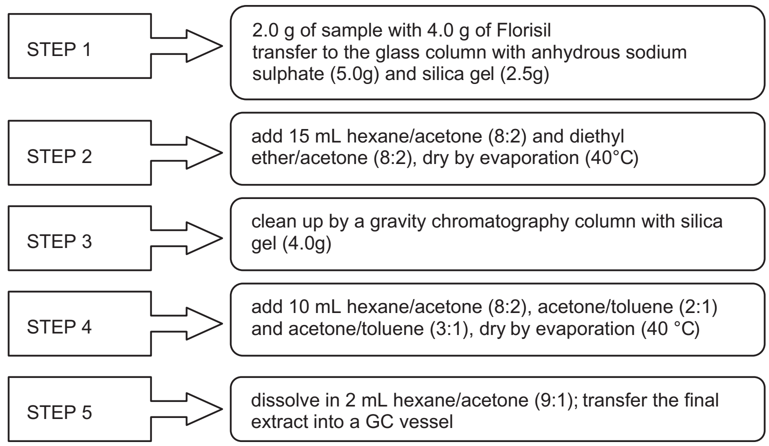


Fig. 2. Sample preparation scheme.

Table 3
Method validation parameters

		Level 1, 0.01 mg/kg	Level 2, 0.10 mg/kg	Level 3, 2.00 mg/kg		
Pesticide	R^2	Mean recovery ± RSD (%)	Mean recovery ± RSD (%)	Mean recovery ± RSD (%)	LOQ (mg/kg)	LOD (mg/kg)
Azoxystrobin	0.99970	95.10 ± 3.31	88.83 ± 3.45	92.30 ± 3.10	0.010	0.002
Boscalid	0.99795	102.57 ± 2.15	105.10 ± 2.86	107.57 ± 1.86	0.010	0.002
Iprodione	1.00000	104.93 ± 4.62	106.07 ± 3.50	93.43 ± 0.64	0.010	0.005
Metalaxyl	0.99839	104.77 ± 1.72	96.23 ± 1.78	85.43 ± 7.02	0.010	0.005
Pyraclostrobin	0.99989	102.57 ± 1.90	99.87 ± 4.86	101.15 ± 1.26	0.010	0.005

of the analyzed pesticides in unprocessed samples are summarized in Table 4.

3.3. Effect of processsing

To achieve the aim of this study, several processing methods were carried out: washing chlorinated and ozonated water, blanching, and cooking. To evaluate the effects of processing on the pesticide residues in broccoli, PFs related to each process were determined and expected to be below 1. The processing conditions were established in accordance with the industrial practice corresponding as closely as possible to the actual conditions that are normally used in practice. The changes of fungicide residues during processing are presented in Table 5.

3.4. Effects of washing with chlorinated water on residue levels

Washing is the most common form of processing and it is often the first step in various types of treatment, especially in household conditions. In this study, washing was done in cold chlorinated (tap) water. As shown in Fig. 3, this process removed a portion of the pesticide residues between 19.4% (PF = 0.81) for metalaxyl and 45.9% (PF = 0.54) for iprodione. That decrease could be explained by physicochemical

Table 4
Fungicide residues in unprocessed broccoli

Pesticide	Azoxystrobin	Boscalid	Iprodione	Metalaxyl	Pyraclostrobin
Concentration (mg/kg) ± RSD[a] ($n = 4$)	0.343 ± 0.1310	1.998 ± 0.7551	4.342 ± 1.5982	0.165 ± 0.0471	0.418 ± 0.1716

[a]RSD—relative standard deviation.

Table 5
Effect of processing on fungicide residues in broccoli

Pesticide	Washing with chlorinated water: Concentration (mg/kg) ± RSD[a] ($n=2$)	Reduction (%)	PF[b]	Washing with ozonated water: Concentration (mg/kg) ± RSD[a] ($n=2$)	Reduction (%)	PF[b]
Azoxystrobin	0.204 ± 0.0619	40.5	0.59	0.175 ± 0.0822	48.9	0.51
Boscalid	1.516 ± 0.3015	24.2	0.76	1.117 ± 0.1185	44.1	0.56
Iprodione	2.351 ± 0.7143	45.9	0.54	2.216 ± 0.9107	49.0	0.51
Metalaxyl	0.133 ± 0.0255	19.4	0.81	0.106 ± 0.0299	35.5	0.64
Pyraclostrobin	0.289 ± 0.0486	30.8	0.69	0.232 ± 0.1030	44.4	0.56
Pesticide	**Blanching: Concentration (mg/kg) ± RSD[a] ($n=2$)**	**Reduction (%)**	**PF[b]**	**Cooking: Concentration (mg/kg) ± RSD[a] ($n=2$)**	**Reduction (%)**	**PF[b]**
Azoxystrobin	0.167 ± 0.0115	51.2	0.49	0.064 ± 0.0186	81.2	0.19
Boscalid	0.923 ± 0.4362	53.8	0.46	0.612 ± 0.3554	69.4	0.31
Iprodione	1.410 ± 0.3832	67.5	0.32	0.563 ± 0.2994	87.0	0.13
Metalaxyl	0.072 ± 0.0108	56.4	0.44	0.055 ± 0.0121	66.7	0.33
Pyraclostrobin	0.203 ± 0.0043	51.5	0.49	0.123 ± 0.1055	70.6	0.29

[a]RSD—relative standard deviation.
[b]PF—processing factor.

properties like water solubility of the pesticide (Table 1). For example, iprodione is a relatively more polar (log $K_{ow} = 3.1$) pesticide than metalaxyl (log $K_{ow} = 1.65$).Thus, some significant decrease was expected.

3.5. Effects of washing with ozonated water on residue levels

Ozone is a strong oxidizing agent and has a wide application in water treatment, food processing, and other environmental areas. In this study, ozonated water was more effective in pesticide removal than tap water. The pesticide residues reduced between 35.5% (PF = 0.64) for metalaxyl and 49.0% (PF = 0.51) for iprodione (Fig. 3). As in the case of washing with chlorinated water, the lowest reduction for metalaxyl was also observed (Table 5). Such a large reduction may be possible since the dissolved ozone generates hydroxyl radicals that are highly effective at decomposing organic molecules like fungicide residual [19,20].

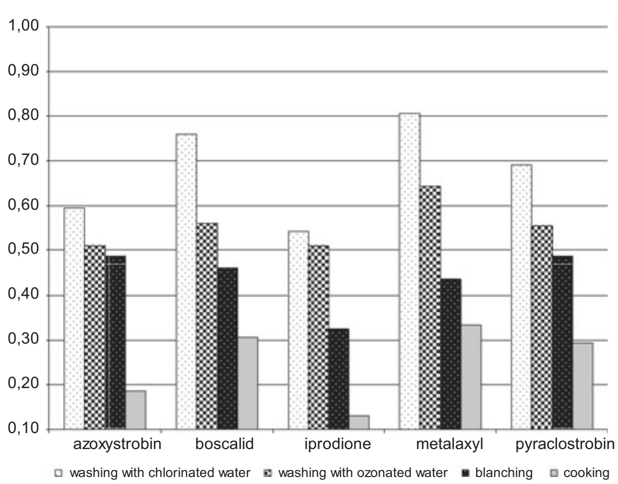


Fig. 3. PFs after various processing methods for five fungicides in broccoli.

3.6. Effects of blanching on residue levels

Blanching is a thermal process used mostly for vegetable tissues prior to freezing, drying, or canning. Vegetables are plunged into hot water at the temperature of 100˚C or below and then removed after a short time to be finally plunged into cool water to halt the cooking process. Blanching was more effective to eliminate pesticide residues than both types of washing. As shown in Fig. 3, the PFs for blanching are less than 0.5. After the blanching step, all studied fungicide residues: azoxystrobin, boscalid, iprodione, metalaxyl, and pyraclostrobin were reduced by over 51%. The highest removal efficacy for iprodione (67.5%, PF = 0.32) was detected (Table 5).

3.7. Effects of cooking on residue levels

Cooking is the act of preparing food conducted in high temperatures which is often used prior to consuming vegetables. The concentration level of fungicide residues was strongly reduced in this process and PFs was below 0.33 for all active substances. Iprodione was highly reduced up to 87.0% (PF = 0.13) and the lowest efficacy for metalaxyl (66.7% with PF = 0.33) removal was noted (Table 5). These results can be explained by the fact that the main effect of cooking is a process of pesticide degradation, because volatilization, hydrolysis, and thermal breakdown occur *during* the boiling process.

4. Summary

In this study, four processing methods: washing with chlorinated and ozonated water, blanching and cooking were investigated to evaluate the removal efficiency of the most common fungicide residue in broccoli. PFs for azoxystrobin, boscalid, iprodione, metalaxyl, and pyraclostrobin were determined. The obtained PF values ranged between 0.13 and 0.81. The washing of the matrices with chlorinated and ozonated water was not as effective to remove pesticide residues as thermal processing. It can be explained by physicochemical properties of pesticides, and on the other hand, by conditions of the process. Water solubility and polarity described as the log K_{ow} had an impact on removal efficiency of pesticides. Iprodione is a relatively more polar pesticide than metalaxyl with lower log K_{ow}, therefore, the obtained PF was lower. The most effective processing method regarding the removal of pesticide residues was cooking due to the high temperature used in the process.

Additionally, the different mode of action of pesticides in broccoli may influence changes in the residues level. Three active substances revealed systemic properties and two substances revealed the contact activity. Iprodione is a contact pesticide, so it remains on the leaf surface, acting as a shield providing a barrier to protect against infection. In contrast, systemic metalaxyl penetrate the broccoli to the deeper layers of tissue, and it is more difficult to remove. In the non-systemic action mode, residues were amenable to simple processing operations.

The present results demonstrated that processing technologies seem promising and could be useful to remove residual fungicides from a wide range of vegetables under domestic and industrial conditions.

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