



COMPARISON OF THE EFFECTS OF WATER AND THERMAL PROCESSING ON PESTICIDE REMOVAL IN SELECTED FRUIT AND VEGETABLES*

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Abstract

Fruit and vegetables are a valuable and essential component of a human diet. Unfortunately, the widespread and increasingly popular use of pesticides has largely magnified quantities of pesticide residues in these plant products. Among the best methods for removal of these contaminants from food of plant origin are food processing technologies, which affect the levels of pesticide residues to various degrees. The aim of this study was to compare the effects of different water and thermal processing treatments on pesticide residue concentrations in selected fruit and vegetables obtained from controlled field and tunnel trials. Black currants, broccoli, strawberries and tomatoes sprayed with plant protection products were analyzed. Washing by immersion in chlorine and in ozonated water as well as boiling were used to assess the removal of eleven pesticides in blackcurrants, broccoli, strawberries and tomatoes. Processing factors, which were determined for each combination of a pesticide, commodity and processing method, ranged between 0.03 and 1.66. Washing in ozonated water was more effective than washing in chlorinated water. However, high temperature at boiling caused a significant decrease in the concentration of most compounds (up to 97%), although there were some exceptions. The thermal treatment proved to be the most effective technological process removing pesticide residues from different commodities. The water and thermal processing technologies tested in this experiment are promising methods for fast and simple removal of pesticide residues from broccoli, black currants, strawberries, tomatoes and possibly other commodities.

Keywords: processing treatments, fruits, vegetables, pesticide.

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INTRODUCTION

Fruit and vegetables are an important component of a human diet because they provide essential nutrients and vitamins required for the proper bodily functions (OGUNTIBEJU et al. 2013). Unfortunately, fruit trees and vegetable plants are often infested by insects and fungal diseases during their growth. In order to control losses of crops and to maintain their high quality, pesticides are used widely. The food crops treated with pesticides invariably contain unpredictable amounts of these chemicals, therefore solutions for decontamination of agricultural products must be searched for, especially as consumers worry about the harmful effect of chemicals on human health.

Many processing methods used in households and at food plants have been reported to affect, albeit differently, the pesticide level in foods. Most food processing techniques and methods usually reduce residual amounts of pesticides, but some may cause an increase in the residue content due to the concentration effect. Water processing like washing with water or soaking in different solutions, chemicals and detergents is reported to be highly effective in reducing the level of pesticides (ANGIONI et al. 2004, LING et al. 2011, CHANDRA et al. 2015). Thermal processing treatments like pasteurization, blanching, boiling, cooking, steaming, canning etc., have been found to reduce effectively various pesticides (BALINOVA 2006, KUMARI 2008, KAUSHIK 2009).

The key elements to the effective removal of residues are: the nature of pesticide molecules (physicochemical properties of the pesticide), location of residue (the pesticide may be absorbed by the plant surface, waxy cuticle and root surfaces; it may enter the plant's transport system, i.e. systemic presence, or stay on the surface of the plant, i.e. contact presence), type, size and texture of a given plant product as well as the processing method – temperature and type of treatment (HOLLAND et al. 1994). Knowledge of the effect of food processing on the level of pesticide residues in fruit and vegetables is used to calculate dietary exposure (KEITKOTLHAILE et al. 2010).

The aim of this study has been to compare the influence of water and thermal processing treatments on residual concentrations of pesticides (seven fungicides and four insecticides most frequently detected in fruits and vegetables) in plant material (ŁOZOWICKA et al. 2009, 2012, 2013). The study compared effects of washing in chlorinated and ozonated water and boiling of berry fruits (black currants and strawberries), brassica and fruiting vegetables (broccoli and tomatoes) according to the processing factors (PFs) for each pesticide/commodity/processing method combination.

MATERIAL AND METHODS

Chemicals

Acetone, acetonitrile and hexane for analyses of pesticide residues were provided by J.T. Baker (Deventer, The Nederland), Florisil (60-100 mesh) was supplied by POCH (Gliwice, Poland), anhydrous sodium sulphate, Celite and octadecyl silica gel C₁₈ (200-400 mesh) were purchased from Fluka (Seelze-Hannover, Germany). Silica gel (230-400 mesh) was obtained from Merck (Darmstadt, Germany). QuEChERS sorbents kits and pouches of salts were purchased from Agilent Technologies (Santa Clara, CA, USA). The following sorbents were used in this study: PSA and pouches of salts: Magnesium Sulphate, Sodium Chloride, Sodium Citrate, Citric Acid Disodium Salt.

The origin of the plant material

Fruit and vegetables were collected from controlled field and tunnel trials set up in north-eastern Poland (Podlasie) and conducted for three years with no previous pesticide applications. When crops grew in open field, pesticides were allowed to undergo natural weathering before harvest. The total area of each experimental plot was approximately 100 m². Blackcurrants and broccoli were grown in Sokółka, while the strawberry plantation was located in Nowy Dwór and tomatoes were cultivated in a greenhouse at the Plant Protection Institute, the National Research Institute in Białystok.

Pesticide spraying

Chemical treatments based on the application of plant protection products (PPP) were carried out during the cultivation of crops on isolated plots. The purpose was to produce crops exposed to the selected pesticides (each plant protection product was applied separately) – Figure 1.

Spraying treatments were carried out by a specialized operator using a double dose of the recommended application amount (Polish Ministry of Agriculture) to ensure sufficient pesticide primary deposit for the following processing. Spray boom equipment at normal settings and timing was used.

Sample preparation

Fruit and vegetables were randomly collected 14 days after the application of PPPs to obtain approximately 10 kg samples. In the case of broccoli, the heads were cut into florets. Samples were packed in polyethylene bags and transported under refrigerated conditions to a laboratory. Each sample was divided into four representative analytical subsamples and stored in a refrigerator at 4°C prior to analysis.

Each analytical sample was divided into two parts. One part was not subjected to any processing (unprocessed sample) and was used to evaluate

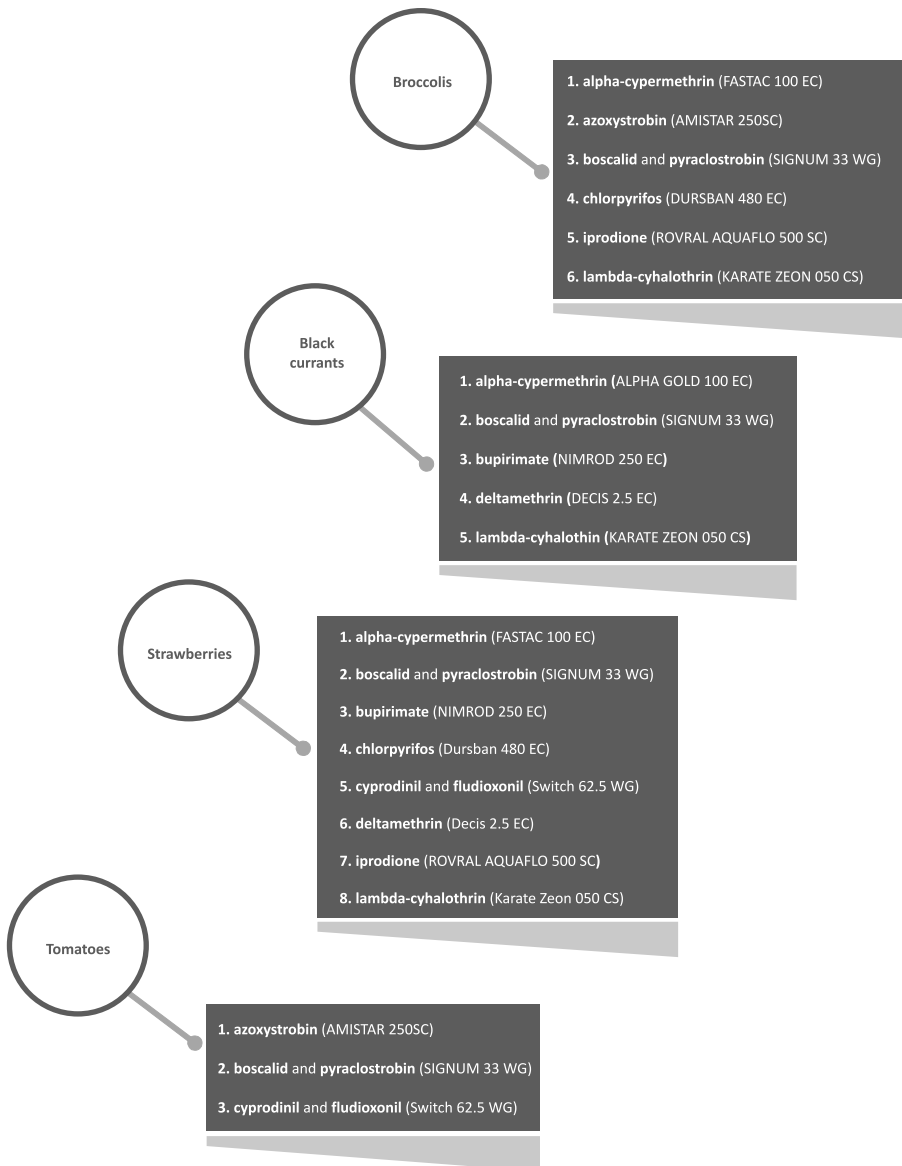


Fig. 1. List of pesticide applications on particular crop

the initial concentration of pesticides. This part of a subsample was homogenized in a Waring blender (Waring Laboratory Science, Stamford CT, USA) and frozen until analysis. The other part was divided into 300 g samples and processed. Immediately after processing, all of these samples were blended and deep-frozen (-20°C) until analysis.

Processing treatments

The processing conditions reproduced as closely as possible the actual conditions that are normally in place in households and at food processing plants. Samples (300 g) were processed by soaking at different water parameters for 5 min. Chlorinated (tap) water washing was done by immersing a sample in 1 l chlorinated water (20°C; 0.1 mg Cl₂ l⁻¹). In the ozonated water washing technology, a sample was immersed in 1 l ozone solution (20°C; 1 mg O₃ l⁻¹). Thermal processing was performed by placing samples into a stainless steel basket, which was then immersed into 1 l of boiling tap water (100°C) for 5 min. The processing variants are shown in Figure 2.

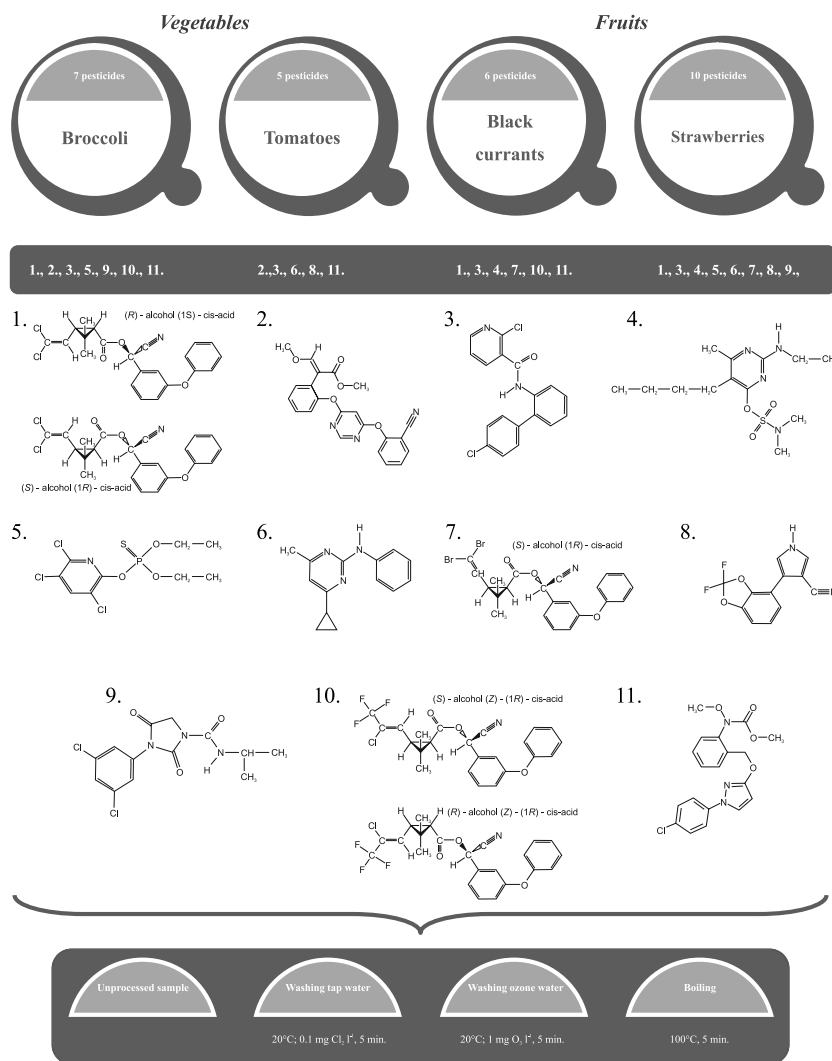


Fig. 2. Pesticides analyzed in each commodity with sample preparation scheme

Processing factors (PFs)

Processing factors for all the combinations of pesticide/commodity/processing method were calculated as a ratio of pesticide residue concentration in processed product and pesticide residue concentration in raw fruit or vegetables. When the PF is lower than 1, it indicates a reduction of the pesticide concentration (reduction factor), while a PF value greater than 1 means a higher pesticide concentration in the processed product (concentration factor).

Determination of pesticide residues

Extraction procedures

Pesticides were extracted using the validated multi-residue method (MRM) based on the matrix solid phase dispersion (MSPD) method and a modified QuEChERS (Quick Easy Cheap Effective Rugged and Safe) method.

Fruit samples – MSPD method: 2 g of a homogenized sample was put in a mortar with 4 g of solid support (5% silica gel, prepared by adding 5 mL of distilled water to 95 g of activated silica gel). The solid support and sample were manually blended together using a pestle to produce homogeneous mixture. The mixed materials were transferred to a glass column with 5 g anhydrous sodium sulphate and 2.5 g silica gel. Adsorbed analytes were eluted using 15 ml of a mixture of hexane/acetone (8:2, v/v) and 15 ml of a mixture of hexane/diethyl ether/acetone (1:2:2, v/v/v). The extract was evaporated to dryness in a rotary vacuum evaporator at the temperature of about 40°C. The residue was dissolved in 2 ml volume of a mixture of hexane/acetone (9:1, v/v). The final solution was put into a GC vessel and placed onto a rack in an autosampler.

Vegetable samples – QuEChERS method: 10 g of a homogenized vegetable sample was weighed in a 50 ml polypropylene centrifuge tube. The sample was extracted with 10 ml of acetonitrile and vortexed for 5 min using a digital Vortex-Mixer (Velp Scientifica, Usmate, Italy). After vortexing, salts containing 4 g MgSO_4 , 1 g NaCl, 1 g trisodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) and 0.5 g disodium hydrogen citrate sesquehydrate ($\text{Na}_2\text{HC}_6\text{H}_5\text{O}_7 \cdot 1,5\text{H}_2\text{O}$) were added. The tubes were immediately shaken for 1 min, vortexed in a Vortex-Mixer for 5 min at 4500 rpm and then centrifuged at 10 000 rpm (Hettich, Tuttlingen, Germany). The upper layer (acetonitrile extract) was transferred into the dSPE tubes containing 150 mg anhydrous MgSO_4 and 25 mg PSA. The tubes were vortexed for 30 s and centrifuged at 5000 rpm for 5 min. One ml of the final extract was filtered through a 0.2 m hydrophilic PTFE filter, transferred into the appropriately labeled autosampler vial and subsequently analyzed via GC.

Instrumental analysis

The qualitative and quantitative analyses of the final fruit and vegetable extracts were performed by gas chromatography. GC was conducted using an Agilent 7890 A series gas chromatograph (Waldbronn, Germany) equipped

with two selective detectors: ^{63}Ni electron capture (ECD) and nitrogen-phosphors (NPD) (ŁOZOWICKA et al. 2009) and HP 6890 autosampler and split/splitless injector. A capillary column HP-5 (5%-phenylmethylpolysiloxane) (30 m x 0.32 mm, 0.5 μm film thickness) was used. The temperature of the injector and detectors was set at 210°C and 300°C, respectively. The oven temperature was programmed as follows: 120°C to 190°C at a rate of 16°C min^{-1} , increased to 230°C at 8°C min^{-1} and then to 285°C at 18°C min^{-1} , and remained there for 18 min. Helium was used as carrier gas at a flow rate of 3.0 ml min^{-1} . Nitrogen was used as make-up gas; the EC detector and NP detector were set at 57 and 8 ml min^{-1} , respectively. The air and hydrogen (for NPD) gas flows were set at 60 and 3 ml min^{-1} , respectively. The injection volume was 2 μl . The total time of analysis was 20 minutes. The GC was controlled by a personal computer system using Chemstation software (Hewlett-Packard).

RESULTS AND DISCUSSION

Validation of the methods

During this study, a number of quality control recovery tests were conducted on fruit and vegetable samples. In the present investigations, recovery experiments were carried out at different levels to establish the reliability and validity of the analytical methods and to recognise the efficiency of extraction procedures (European Commission, 2014). The blank blackcurrant, strawberry, tomato and broccoli samples previously determined not to contain any residues (unprocessed, juice and jam) were spiked at three fortification levels: 0.01, 0.5 and 2.50 mg kg^{-1} . Percentages of the mean recovery and relative standard deviation (RSD) were acceptable and ranged from 71% to 109%, with the relative standard deviation below 15% for all the tested matrices. The linearity of the methods, which was evaluated with matrix-matched calibration curves, was good, with an excellent coefficient of determination ($r^2 \geq 0.99$). The LODs of the analytes were determined by considering a signal-to-noise (S/N) ratio of 3, whereas the LOQs were determined via the S/N ratio = 10. The limit of quantification (LOQ) ranged 0.005-0.010 mg kg^{-1} and the limit of detection (LOD) was between 0.001-0.005 mg kg^{-1} , respectively. These values were determined following the guidelines established by the European Commission for method validation and quality control procedures for pesticide residue analysis in food and feed (European Commission 2014).

Properties of studied 11 pesticides

The active substances studied belong to various pesticide classes and have different modes of action. Pesticides with systemic action are absorbed through the leaves, stems or roots and then transported within the treated

plant by the plant's vascular system. Contact pesticides are applied to surfaces of plants and must come into direct contact with the pest to be effective. The main physical and chemical properties of the pesticides sorted by their mode of action are presented in Table 1. The pesticides studied in each commodity and their structures are shown in Figure 2.

Table 1

Properties of the pesticides according to their mode of action

Mode of action	Pesticide	Group	Polarity log P	Solubility in water (mg l ⁻¹)	Boiling point (°C)	Molecular mass
Systemic	azoxystrobin	strobilurin	2.7	6.7	581.0	403.40
	boscalid	carboxamide	2.96	4.6	447.7	343.21
	bupirimate	pyrimidinol	3.68	13.06	463.2	316.42
	pyraclostrobin	strobilurin	3.99	1.9	501.1	412.87
	cyprodinil	anilinopyrimidine	4.0	13	405.985	225.29
Non-systemic	iprodione	dicarboximide	3.1	12.2	not available	330.17
	fludioxonil	phenylpyrrole	4.12	1.8	420.4	248.19
	deltamethrin	pyrethroid	4.6	0.0002	535.8	505.2
	chlorpyrifos	organophosphate	4.7	1.05	395.8	350.89
	alpha-cypermethrin	pyrethroid	5.5	0.004	826.0	416.30
	lambda-cyhalothrin	pyrethroid	6.9	0.005	498.9	449.85

Unprocessed samples

The unprocessed, raw samples of fruit and vegetables were used to calculate the PFs and these values describe the efficiency of reducing the pesticide residue level in food processing. Initial concentrations of the pesticides in unprocessed samples are summarized in Table 2.

Comparison of water and thermal processing treatments

To achieve the aim of this study, water and thermal processing technologies were carried out: washing with chlorinated water, washing with ozonated water and boiling. Removal effectiveness for each pesticide/commodity/processing method combination was determined and expressed as % of reduction (Table 2). Processing factors of 7 fungicides and 4 insecticides, which were also calculated, were generally below 1, except for the boiling process, where three insecticides (alpha-cypermethrin, deltamethrin and lambda-cyhalothrin) demonstrated PFs above 1. The comparison of processing effectiveness of each pesticide/commodity/processing method combination is presented in Figure 3 and discussed below.

Washing is a preliminary step in the preparation of fruit and vegetables, and in this study it was done using chlorinated and ozonated water. In the

Table 2

Processing removal effectiveness (28 combinations of pesticide/commodity/processing method)

No.	Pesticide (type of pesticide)	Commodity	Initial concentration (mg kg ⁻¹)	Washing with chlorinated (tap) water	Washing with ozonated water	Boiling
				reduction (%)		
1.	alpha-cypermethrin (I)	black currants	0.12	22	17	-
		broccoli	0.06	38	49	34
		strawberry	0.17	45	50	-
2.	azoxystrobin (F)	broccoli	0.34	41	49	81
		tomatoes	0.14	40	27	82
3.	boscalid (F)	black currants	4.41	49	38	56
		broccoli	1.99	24	54	69
		strawberry	0.35	36	59	41
		tomatoes	0.19	47	22	97
4.	bupirimate (F)	black currants	0.77	11	45	58
		strawberry	0.12	14	38	49
5.	chlorpyrifos (I)	broccoli	1.04	24	55	43
		strawberry	0.10	54	65	41
6.	cyprodinil (F)	strawberry	0.27	34	36	43
		tomatoes	0.23	17	37	86
7.	deltamethrin (I)	black currants	0.11	8	12	-
		strawberry	0.15	20	54	-
8.	fludioxonil (F)	strawberry	0.10	34	43	34
		tomatoes	0.10	66	48	69
9.	iprodione (F)	broccoli	4.34	46	49	87
		strawberry	1.34	27	35	42
10.	lambda-cyhalothrin <i>n</i> (I)	black currants	0.08	12	37	-
		broccoli	0.03	6	26	34
		strawberry	0.28	18	31	-
11.	pyraclostrobin (F)	black currants	1.23	18	33	72
		broccoli	0.42	23	44	52
		strawberry	0.91	26	50	91
		tomatoes	0.11	30	37	75

F – fungicide, I – insecticide, bolded – PF > 1

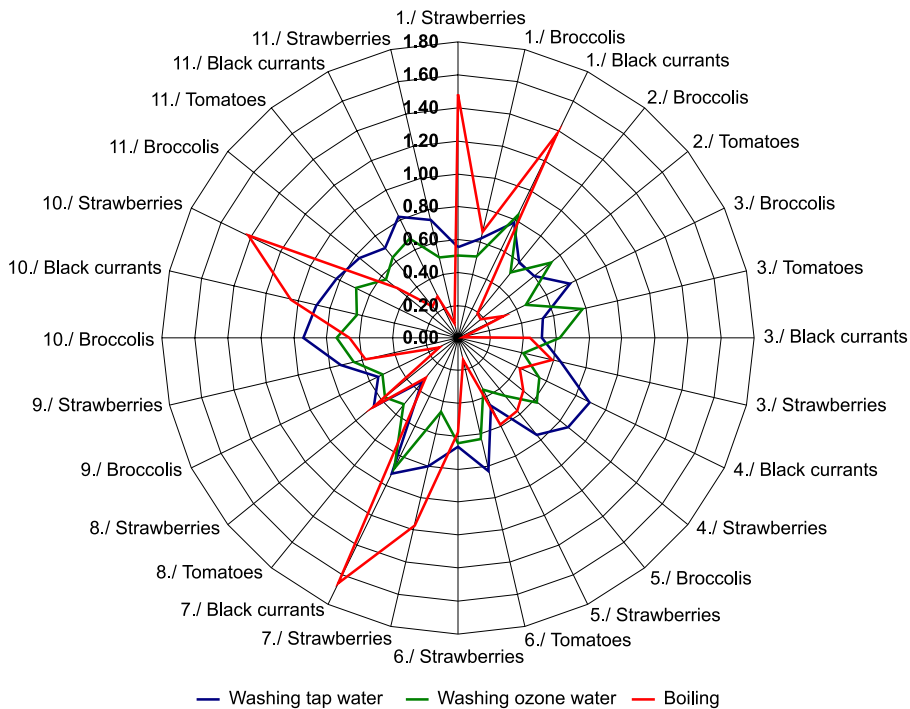


Fig. 3. Comparison of the effect of water and thermal processing on pesticide removal in different commodities:

(1. – alpha-cypermethrin, 2. – azoxystrobin, 3. – boscalid, 4. – bupirimate, 5. – chlorpyrifos, 6. – cyprodinil, 7. – deltamethrin, 8. – fludioxonil, 9. – iprodione, 10. – lambda-cyhalothrin, 11. – pyraclostrobin)

former treatment, the pesticide removal efficiency resulted in a 4% reduction for lambda-cyhalothrin in broccoli and 66% for fludioxonil in tomatoes, with $PF = 0.96$ and $PF = 0.34$ respectively (Table 2, Figure 3). After the latter treatment, pesticide residues were reduced by between 12% ($PF = 0.88$) for deltamethrin in black currants and 65% ($PF = 0.25$) for chlorpyrifos in strawberries (Table 2, Figure 3). Washing with chlorinated water significantly reduced (by over 50%) the concentrations pesticide residues in just two cases: chlorpyrifos in strawberries and fludioxonil in tomatoes (Table 3). While soaking in ozone solution, reduction was achieved in seven cases: alpha-cypermethrin, boscalid, chlorpyrifos, deltamethrin, pyraclostrobin in strawberries and boscalid, chlorpyrifos in broccoli using ozonated water (Table 3).

Comparing the above results, washing with ozonated water was more effective as a pesticide removal technology than washing with chlorinated water. Ozone is considered to be the best at removing pesticide residues from fruit and vegetables (GABLER et al. 2010), and dissolved ozone generates hydroxyl radicals that are highly effective in decomposing organic molecules such as pesticide residues (SUMIKURA et al. 2007). CHEN et al. (2013) con-

Table 3

Comparison of the effects of processing treatments

Treatment/ removal effectiveness		Water processing		Thermal processing
		washing with chlorinated water	washing with ozonated water	boiling
PF range	the lowest PF	0.34 fludioxonil/tomatoes	0.35 chlorpyrifos/ strawberries	0.03 boscalid/tomatoes
	the highest PF	0.96 lambda-cyhalothrin/ broccoli	0.88 deltamethrin/black currants	1.66 deltamethrin/ blackcurrants
Reduction factor PF < 1	PF < 0.2 (strong removal)	-	-	6 combinations
	PF 0.21+0.50	2 combinations	7 combinations	6 combinations
	PF 0.51+1.00	26 combinations	21 combinations	9 combinations
Concentration factor PF > 1		-	-	6 combinations

cluded that removal efficiency increased when samples were treated with ozone, and our results confirmed this finding.

The third processing treatment consisted of the boiling of fruit and vegetables. In this method, heat can increase volatilization, hydrolysis or other forms of degradation and therefore reduce most residue levels (HOLLAND et al. 1994). In our study, most of the pesticide residues were highly reduced after the thermal processing of broccoli, blackcurrants, strawberries and tomatoes. The reduction ranged between 44% for lambda-cyhalothrin in broccoli (PF = 0.66) and 97% for boscalid in tomatoes (PF = 0.03) which was almost completely eliminated after boiling (Table 2, Figure 3). High temperature caused higher reduction of pesticides in the commodities processed by heating in aqueous solution than either of the two types of washing. Decontamination above 50% was observed in twelve cases (Table 3), including azoxystrobin, iprodione in broccoli and azoxystrobin, boscalid, cyprodinil in tomatoes and pyraclostrobin in strawberries, which showed a removal degree of over 80% after boiling. We can presume that polar, water-soluble pesticides are more readily removed than low-polarity materials (HOLLAND et al. 1994). These pesticides have low octanol-water partition coefficient ($\log P \leq 4.00$), hence they were more easily removed using hot water.

Other pesticides such as alpha-cypermethrin, deltamethrin and lambda-cyhalothrin (insecticides from the pyrethroid class) were not reduced after

boiling of berry fruits. Their residues were concentrated as the water evaporated in the final product by a factor of $PF > 1$ (AMVRAZI 2011). These three pesticides also present very low solubility in water of 0.004 mg l^{-1} , 0.0002 mg l^{-1} and 0.005 mg l^{-1} , respectively (Table 1). In contrast to water-soluble compounds, the lipid cell membrane of a plant is a weak barrier to lipid-soluble compounds, which can freely penetrate it. Potentially damaging lipid-soluble toxins can therefore gain free access to cellular interiors of strawberries or black currants, and are much more difficult to remove.

Similar findings were obtained by RASMUSSEN et al. (2003), who found that boiling did not reduce chlorpyrifos, cypermethrin, deltamethrin, diazinon, endosulfan (alpha, beta and endosulfan sulphate), fenpropathrin, iprodione, kresoxim methyl, lambda-cyhalothrin, quinalphos and vinclozoline residues in apples. Interestingly, no increases of residue concentration were noted in broccolis. This fact, can be explained by the different size and texture of the analyzed fruits and vegetables. Penetration of those pesticides was easier in the case of small soft fruits than broccoli florets.

CONCLUSIONS

Summarizing all the results, thermal processing proved to be more effective than water treatments as a technological process in terms of pesticide removal from different commodities, although there were some exceptions. Decreasing amounts pesticide residues during thermal processing could be due to their decomposition by heat, stronger adsorption of pesticides to plant tissues and/or the solubility of pesticides in water (HOLLAND et al. 1994).

The water and thermal processing treatments used in this experiment are promising methods for fast and simple removal of pesticide residues from broccoli, black currants, strawberries, tomatoes and possibly other fruit and vegetables. The current study has demonstrated that processing procedures like washing and boiling are a good and effective solution which prevents chemical contamination in foodstuffs from chemically treated crops.

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