Pesticide residue analysis in parsley, lettuce and spinach by LC-MS/MS

Okan Esturk · Yasin Yakar · Zehra Ayhan

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Abstract In this study, pesticide residues in parsley, lettuce and spinach (120 samples) were analyzed by the application of liquid chromatography-tandem mass spectrometry (LC-MS/MS). All samples of spinach, parsley or lettuce contained residues of three or more active substances. In parsley, carbendazim (100.0%), dichlorvos (100.0%), fenarimol (40.0%), pendimethalin (95.0%), in lettuce, diazinon (30.0%), dichlorvos (100.0%), pendimethalin (92.5%) phenthoate (12.5%), and in spinach, carbendazim (45.0%), cymoxanil (85.0%), dichlorvos (100.0%) and fenarimol (85.0%) were the significant active compounds. The maximum residue limits were exceeded in 28, 20 and 40 samples of parsley, lettuce and spinach, respectively. The results showed that there was a high occurrence of pesticide residues in parsley, lettuce and spinach samples from Hatay province, in which most of them were prohibited from use in Turkey for these vegetables. The contamination levels of these residues may be considered a serious public health problem according to the maximum residue limits (MRLs) of Turkey and the European Union (EU).

Keywords LC-MS/MS · Lettuce · Maximum residue limits · Pesticide · Parsley · Spinach

Introduction

Pesticides are widely used at various stages of cultivation and during post-harvest storage of fruits and vegetables. These chemicals help to control a wide range of pests and plant diseases that damage crops. Therefore, they play an important role in food production and quality preservation. However, in addition to destroying pests, these substances are often harmful to nontarget organisms. Consumers can be affected from the presence of pesticide residues in agricultural produces, especially when they consumed fresh. The toxic nature of these compounds makes the monitoring of pesticide residues obligatory in food products in order to assess the human exposure to pesticides through foods. Consumers are now much more conscious about the damage caused by pesticide residues. Therefore, there is a growing social desire to reduce the use of pesticides in food and food products and demand for better agricultural practices, transparency and traceability in the production and marketing of conventional food.

Hatay is an important region for cultivation and production of parsley and lettuce. In this region, 30.68% of the parsley and 18.02% of the lettuce of the whole country are produced. Total amount of parsley, lettuce and spinach production in Turkey is estimated at about 52.34, 439.64 and 225.75 thousand metric tons, respectively (FAO 2008).

Gas chromatography (GC) equipped with an electron-capture detector (ECD), nitrogen-phosphorous detector (NPD) or mass spectrometer (MS) has been the analytical technique more frequently applied to the analysis of pesticides in food for many years (Anastassiades

O. Esturk (✉) · Z. Ayhan
Department of Food Engineering, Faculty of Agriculture,
Mustafa Kemal University,
Tayfur Sokmen Campus 31034,
Antakya, Hatay, Turkey
e-mail: okanesturk@gmail.com

Y. Yakar
Department of Chemistry, Mustafa Kemal University,
Antakya, Hatay, Turkey

et al. 2003; Hernandez-Borges et al. 2009; Lehotay et al. 2005; Nguyen et al. 2008; Paya et al. 2007; Zawiyah et al. 2007). However, for highly polar, thermally liable and non-volatile compounds, application of alternative analytical techniques is necessary. Liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) is one of the most powerful techniques for pesticide residue analysis in fruits and vegetables. This technique provides excellent sensitivity/selectivity and discriminates analyte and matrix signal more efficiently than GC-MS (Alder et al. 2006) or LC-MS (Hernandez et al. 2006). In recent years, a number of reliable LC-MS/MS methods have been developed for pesticide residue analysis for the postharvest fungicide residues (thiabendazole, carbendazim, thiophanate-methyl, imazalil and prochloraz) in citrus juices (Dreassi et al. 2010), for guazatine in commercial citrus fruits (Scordino et al. 2008), for thiosultap sodium, thiocyclam, and nereistoxin in pepper (Ferrer et al. 2010), for multi-class pesticides residues in fresh grape samples (Venkateswarlu et al. 2007) and in olives (Ferrer et al. 2005).

The objective of this study was to determine the occurrence and concentrations of pesticide residues in parsley, lettuce and spinach from Hatay province of Turkey by LC-MS/MS.

Materials and methods

Reagents and materials

Parsley (*Petroselinum crispum* var. *neapolitanum*), lettuce (*Lactuca sativa* var. *longifolia*) and spinach (*Spinacia oleracea*) samples produced in Hatay were purchased from farmers market at various days between January–March 2010 and analyzed within 24 h, being stored at 4 °C until the analysis. Pesticide reference standards were purchased from Dr Ehrenstorfer (Augsburg, Germany) and Sigma-Aldrich (Seelze, Germany). Acetonitrile (MeCN) and methanol (MeOH) of HPLC quality were obtained from Merck (Darmstadt, Germany). Magnesium sulfate anhydrous (anhy. MgSO_4), sodium acetate (NaOAc) and glacial acetic acid (AcOH) were purchased from Merck. Bulk primary secondary amine (PSA) sorbent was obtained from Varian (Varian Incorporated, Harbor City, CA, USA), and graphitized carbon black sorbent was obtained from Supelco (Bellefonte, PA, USA).

The individual standard solutions were prepared in MeCN at the concentration of 1000 $\mu\text{g ml}^{-1}$. The composite working standard solution was prepared by mixing the appropriate amount of the individual standard solutions and diluting with MeCN to the final concentration of 10 $\mu\text{g ml}^{-1}$. All solutions were preserved at 4 °C.

Sample preparation

Samples were prepared according to the QuEChERS (quick, easy, cheap, effective, rugged and safe) method (Anastassiades et al. 2003) with some modifications. Sample preparation has the following steps: each vegetable was finely chopped and homogenized in a mixer (Robot Coupe R6-02VB, Jackson, MS, USA). Fifteen gram of thoroughly homogenized sample weighed into a 50 ml fluoroethylenepropylene (FEP) centrifugation tube (Nalgene, Rochester, NY, USA), and 15 ml of MeCN 1% AcOH (v v^{-1}) was added and shaken for 1 min by hand. Six gram anhydrous MgSO_4 and 1.5 g anhydrous NaOAc was added and shaken vigorously for 1 min by hand. The tubes were centrifuged at 3,450 relative centrifugal force (5,000 rpm on the centrifuge, Universal 320R, Hettich, Germany). Cleanup was performed according to Lehotay (2007). Four ml extract was transferred from the upper layer into a 50 ml FEP tube, and 0.6 g anhydrous MgSO_4 and 0.2 g PSA were added and shaken vigorously for 1 min by hand. The tubes were centrifuged at 5,000 rpm (Universal 320R, Hettich, Germany) for 1 min. The supernatant was transferred with a syringe and filtered through 0.45 μm filter (Millipore Millex-HN Nylon, Billerica, MA, USA). The permeate was transferred to a vial and stored at 4 °C until injection into the LC-MS/MS system.

LC-MS/MS analysis

LC analyses were performed using a prominence series liquid chromatography system equipped with DGU-20A3 degasser, LC-20 AD pump, SIL-20A autosampler, CTO-10ASvp oven and a CBM-20A controller (Shimadzu, Kyoto, Japan). The chromatographic separation was carried out on a Synergi 2.5 μm Fusion-RP 100A-C18 column (50×2.0 mm, Phenomenex, USA). The injection volume was 10 μl . A gradient elution program at 0.5 ml min^{-1} flow, in which both reservoirs contained 5 mM ammonium formate in (A) water and (B) MeOH, was used as follows: 5% solution B ramped to 90% linearly over 2 min then held for an additional 2 min. After 4 min, the mobile phase was returned to 5% solution B over the course of 2 min and allowed to equilibrate for 4 min.

MS/MS experiments were performed on a 3200 triple-quadrupole linear ion mass spectrometer equipped with a TurboIonSpray (Applied Biosystems, Foster City, CA, USA). The spectrometer was operated in positive ion mode with the multiple reaction monitoring (MRM) mode. Analyst software, version 1.5 was used for instrument control and data acquisition. Nitrogen was used as both nebulizing and drying gas with pressure settings of 275.79 and 413.69 kPa, respectively. The

capillary voltage was set at 5,000 V and the source temperature at 500 °C. The resolution was 0.6–0.8 (unit resolution) for both the first and third quadrupole. Mass spectra were recorded across the range 5 to 1,800 m/z .

Validation

The analysis method was tested to assess for sensitivity, recovery, precision and specificity. Linearity was evaluated both in solvent and matrix (lettuce), using matrix-matched calibration curves prepared as described above in the [sample preparation](#) section, in a concentration range of 0.0025–0.36 mg kg^{-1} . The matrix effect was studied by comparison of the responses of the standard solutions prepared in solvent with the responses of the standard solutions prepared in pesticides-free lettuce sample. Calculated matrix effects were less than $\pm 20\%$ for the most of pesticides. The matrix-matched calibration curves for the pesticides fell within a range of 0.003–0.36 mg kg^{-1} , and good linearity was detected for most pesticides with correlation coefficients (r^2) better than 0.99 (Table 1).

Recovery studies were carried out at two concentration levels (0.1 and 0.5 mg kg^{-1}) performing three replicates at each level. All of recoveries for the tested pesticides were in the range from 70% to 120% with relative standard deviations (RSD) lower than 20% (Table 1). The limit of determination (LOD) was established as the lowest concentration tested, which gave satisfactory recovery, precision, signal/noise ratio, and diagnostic ions relative abundances according to the validation and quality control criteria proposed by the European SANCO guidelines (European Commission 2009). Validation results for 80 pesticides showed that limits of detection ranged from 0.0025 to 0.160 mg kg^{-1} . Parameters are listed in Table 1.

Results and discussion

Eighty pesticides were chosen based on the information available on their use in the treatment of fruits and vegetables (Agriculture and Rural Affairs Ministry of the Republic of Turkey 2008). Table 1 shows the instrument acquisition parameters, LOD, correlation coefficient and individual pesticide recoveries at two different concentration levels.

A total of 120 parsley, lettuce and spinach samples were collected from farmers market at various days in January–March 2010 in Hatay, and analyzed according to the described procedure. Active substances detected in parsley, lettuce and spinach samples are shown in Table 2. Each parsley sample contained residues of seven or more active substances. Carbendazim (100.0%, in the concentration

range of 0.002–0.111 mg kg^{-1}), dichlorvos (100.0%, in the concentration range of 0.002–0.035 mg kg^{-1}), fenarimol (40.0%, in the concentration range of 0.004–0.032 mg kg^{-1}) and pendimethalin (95.0%, in the concentration range of <0.001–0.289 mg kg^{-1}) were the significant active compounds in parsley. Zawiyah et al. (2007) monitored the organochlorine and pyrethroid pesticide residues in fruit and vegetables from market in Malaysia and reported that cypermethrin was the only pesticide found in samples and six of Chinese parsley (30%) contained it with a mean value of 0.68 mg kg^{-1} .

Lettuce samples also contained seven or more active compounds. In lettuce, diazinon (30.0%, in the concentration range of 0.002–0.078 mg kg^{-1}), dichlorvos (100.0%, in the concentration range of 0.005–0.020 mg kg^{-1}), pendimethalin (92.5%, in the concentration range of 0.001–0.354 mg kg^{-1}) and phenthoate (12.5%, in the concentration range of 0.007–0.011 mg kg^{-1}) were the significant active compounds. Nguyen et al. (2008) studied the pesticide residues in 53 Korean cabbage and 46 Korean radish samples from central Korea by using GC-MS in selected ion monitoring mode (SIM). While procymidone was detected in 4 cabbage samples, alpha-endosulfan, bifenthrin, diazinon, fenprothrin, metalaxyl, pp'-DDE and sanmarton were detected in one cabbage sample each. Cesnik et al. (2006a) analyzed lettuce and spinach samples from Slovene producers for pesticide residues. While no residue was found in 7 spinach samples, dithiocarbamate was found in 1 out of 17 lettuce samples (5.9%) which was below the MRL.

Spinach samples contained three or more active compounds. Carbendazim (45.0%, in the concentration range of 0.003–1.840 mg kg^{-1}), cymoxanil (85.0%, in the concentration range of <0.001–0.161 mg kg^{-1}), dichlorvos (100.0%, in the concentration range of 0.002–0.071 mg kg^{-1}) and fenarimol (85.0%, in the concentration range of 0.001–0.052 mg kg^{-1}) were the significant active compounds. Gonzalez-Rodriguez et al. (2008) monitored pesticide residues in 75 samples of green and leafy vegetables (Swiss chards, spinaches and lettuces) using GC-MS in SIM mode. They have reported that pesticide residues were above the MRLs in 15 of the 75 analyzed samples, with a total of 18 violations of the MRL. The highest concentrations of fungicides were found in lettuce (procymidone, 12 mg kg^{-1}) and the highest concentrations of insecticides were found in Swiss chard (cypermethrin, 6 mg kg^{-1}). Fenoll et al. (2009a) investigated the dissipation rates of fenitrothion in lettuce samples grown in a greenhouse and during 4 weeks of cold storage using GC with NPD. The dissipation rate of fenitrothion in lettuce was much higher in the greenhouse experiment than under refrigeration, confirming the substantial influence of chemical degradation in pesticide dissipation caused by

Table 1 Instrument acquisition parameters used and LOD, correlation coefficient and recovery data obtained for the analysis of 80 pesticide residues by LC-MS/MS

No	Name	RT (min)	M	MS/MS (m/z)	DP (V)	CE (V)	LOD (mg kg ⁻¹)	r ²	Spiking level (mg kg ⁻¹)			
									0.1		0.5	
									Rec%	RSD%	Rec%	RSD%
1	Acetamiprid	2.38	222.7	223.3>126.1	41	29	0.003	1.000	87.2	5.2	85.3	6.9
2	Alachlor	3.00	269.8	270.3>238.3	26	13	0.010	0.977	94.4	6.5	97.9	5.4
3	Amitraz	3.29	293.4	294.4>163.2	26	23	0.010	0.997	77.3	2.8	76.9	10.3
4	Atrazine	2.82	215.7	216.4>174.1	51	25	0.010	0.994	100.2	8.6	112.3	3.1
5	Azoxystrobin	2.89	403.4	404.3>344.2	31	31	0.005	0.991	87.1	4.0	99.5	1.7
6	Bifenthrin	3.48	422.9	440.3>181.2	21	23	0.010	1.000	102.3	0.6	104.2	4.7
7	Bupirimate	3.02	316.4	317.4>166.2	51	35	0.003	0.980	97.1	6.8	99.8	12.5
8	Buprofezin	3.16	305.5	306.3>201.3	36	19	0.005	0.995	105.3	0.5	102.2	6.5
9	Carbaryl	2.74	201.2	202.3>145.2	21	15	0.005	0.997	87.0	9.3	84.5	8.4
10	Carbendazim	2.51	191.2	192.3>160.1	41	27	0.005	1.000	88.4	5.7	85.7	9.4
11	Carbofuran	2.69	221.3	222.3>165.2	41	19	0.005	0.999	86.9	7.5	86.3	7.2
12	Carbosulfan	3.42	380.5	381.2>118.1	36	29	0.010	1.000	78.3	4.5	76.6	5.3
13	Chlorflazuron	3.23	540.9	540.0>382.9	51	31	0.005	1.000	89.7	3.8	87.3	3.4
14	Chlorpropham	2.96	213.7	214.1>172.1	21	15	0.010	0.999	93.5	3.0	97.1	7.2
15	Chlorpyrifos	3.19	350.6	350.1>198.1	36	29	0.005	0.994	105.7	1.1	107.2	6.5
16	Clofentezine	3.08	303.2	303.2>138.2	31	23	0.005	0.990	86.1	12.9	80.6	5.3
17	Cycloate	3.11	215.4	216.3>134.2	31	19	0.003	0.990	90.4	5.0	96.3	7.5
18	Cymoxanil	2.46	160.2	199.3>128.0	26	17	0.005	1.000	92.5	11.7	89.5	12.6
19	Cypermethrin	3.25	416.3	433.2>190.9	21	23	0.010	0.999	98.4	2.0	98.7	2.1
20	Cyproconazole	2.96	291.8	292.3>70.0	36	33	0.003	0.991	95.1	3.5	93.2	12.7
21	Cyprodinil	3.05	225.3	226.3>93.1	61	49	0.005	0.988	99.9	5.2	103.6	9.2
22	Deltamethrin	3.25	505.2	523.2>281.0	26	25	0.010	0.999	96.1	4.5	94.4	3.4
23	Diazinon	3.06	304.4	305.3>169.1	41	31	0.005	0.985	96.1	7.4	99.0	11.6
24	Dichlofluanid	2.97	333.2	350.2>224.1	26	23	0.005	0.985	105.2	7.9	98.3	8.6
25	Dichlorvos	2.67	221.0	221.2>109.1	41	25	0.003	0.999	85.6	1.8	84.2	9.7
26	Difenoconazole	3.08	406.3	406.1>251.1	56	35	0.003	0.995	94.5	10.7	90.6	8.8
27	Dimethoate	2.38	229.3	230.2>199.1	31	15	0.005	0.999	94.9	9.4	85.2	6.9
28	Dimethomorph	2.92	387.9	388.3>301.2	56	29	0.010	0.996	94.4	4.0	105.3	3.8
29	Epoxiconazole	2.99	329.8	330.4>121.1	46	27	0.005	0.985	111.7	1.9	107.0	9.8
30	Ethiofencarb	2.76	225.3	226.3>107.2	16	25	0.003	0.995	86.1	5.8	81.7	10.3
31	Ethofumesate	2.89	286.3	304.3>121.1	21	29	0.005	0.987	82.7	7.6	105.7	2.0
32	Famoxadone	3.03	374.4	392.3>331.2	21	19	0.005	0.994	88.8	8.5	100.3	11.0
33	Fenarimol	2.99	331.2	331.3>268.1	31	31	0.005	0.989	97.1	8.3	89.8	8.9
34	Fenazaquin	3.36	306.4	307.4>161.2	46	23	0.005	0.999	96.2	7.3	95.9	8.8
35	Fenoxycarb	3.00	301.3	302.4>88.1	36	27	0.005	0.976	111.3	3.7	106.9	9.1
36	Fenpropathrin	3.21	349.4	350.4>125.3	26	21	0.010	0.998	108.3	1.1	106.2	5.6
37	Fenthion	3.04	278.3	279.3>169.2	36	27	0.003	0.993	94.6	10.1	99.6	5.2
38	Furathiocarb	3.14	382.5	383.4>195.3	41	25	0.010	0.997	110.3	2.3	107.3	9.1
39	Hexythiazox	3.18	352.9	353.4>228.1	56	23	0.005	0.997	97.2	3.7	97.5	8.1
40	Imidacloprid	2.28	255.7	256.3>175.1	36	29	0.003	0.999	86.9	11.2	86.4	6.1
41	Kresoxim-methyl	3.02	313.4	314.3>116.1	16	19	0.010	0.985	90.5	9.5	98.4	10.5
42	Lufenuron	3.17	511.2	511.1>158.2	51	27	0.005	0.998	95.1	4.1	86.8	7.5
43	Malathion	2.95	330.4	331.4>127.2	31	19	0.003	0.986	93.8	8.0	96.7	8.5
44	Metalaxyl	2.83	279.3	280.4>220.3	41	17	0.005	0.995	101.2	8.4	110.0	1.8
45	Metolachlor	3.00	283.8	284.2>252.2	31	19	0.003	0.980	104.0	3.5	98.1	12.3

Table 1 (continued)

No	Name	RT (min)	M	MS/MS (m/z)	DP (V)	CE (V)	LOD (mgkg ⁻¹)	r ²	Spiking level (mgkg ⁻¹)			
									0.1		0.5	
									Rec%	RSD%	Rec%	RSD%
46	Metribuzin	2.69	214.3	215.2>187.2	41	23	0.010	0.999	87.2	5.7	86.3	3.2
47	Monolinuron	2.77	214.6	215.3>126.0	31	25	0.010	0.987	90.3	5.9	90.2	7.6
48	Oxadixyl	2.60	278.3	279.3>219.2	31	17	0.005	0.999	96.8	3.1	103.1	10.9
49	Oxamyl	1.95	219.3	237.2>72.1	16	21	0.005	0.999	78.1	4.9	78.5	6.9
50	Oxyfluorfen	3.12	361.7	379.0>315.9	6	25	0.160	0.998	105.2	10.5	105.5	8.3
51	Penconazole	3.03	284.2	284.3>159.1	41	41	0.003	0.983	90.2	10.1	100.3	12.9
52	Pendimethalin	3.22	281.3	282.4>212.1	16	17	0.005	1.000	97.1	4.5	98.4	3.7
53	Permethrin	3.41	391.3	408.3>183.3	21	29	0.005	0.999	97.9	3.6	94.0	5.5
54	Phenthoate	3.02	320.4	321.1>163.2	21	17	0.003	0.976	94.6	6.7	97.7	8.9
55	Phosalone	3.06	367.8	368.2>182.0	31	27	0.003	0.980	100.0	5.0	105.0	7.5
56	Phosmet	2.89	317.3	318.2>160.1	26	23	0.005	0.984	84.2	6.5	103.0	2.6
57	Pirimicarb	2.79	238.3	239.4>71.9	36	29	0.003	0.994	90.2	2.6	96.8	7.2
58	Pirimiphos-methyl	3.08	305.3	306.3>108.2	46	43	0.003	0.985	94.0	7.4	94.2	5.6
59	Profenofos	3.12	373.6	373.1>302.9	41	25	0.005	0.986	96.0	3.0	93.2	10.8
60	Propargite	3.21	350.5	368.3>231.3	21	19	0.005	0.998	98.7	8.2	105.2	4.7
61	Propiconazole	3.04	342.2	342.3>159.0	56	43	0.010	0.983	97.4	5.5	109.3	5.9
62	Propyzamide	2.95	256.1	256.2>190.2	26	21	0.005	0.985	92.1	6.6	101.0	6.2
63	Prothiofos	3.33	345.3	345.2>241.0	36	23	0.010	0.999	89.9	9.8	93.6	11.6
64	Pyrazophos	3.09	373.4	374.3>222.1	51	33	0.010	0.990	102.0	10.4	111.7	4.4
65	Pyridaben	3.32	364.9	365.3>147.2	46	37	0.003	0.999	101.4	8.3	109.0	4.9
66	Pyridaphention	2.95	340.3	341.3>189.3	56	31	0.003	0.991	100.8	4.0	98.6	8.7
67	Pyriproxyfen	3.16	321.5	322.3>96.0	41	23	0.005	0.993	102.3	2.5	100.2	7.4
68	Simazine	2.70	201.7	202.3>132.1	46	27	0.003	0.999	89.3	5.6	82.9	9.7
69	Tau-Fluvalinate	3.31	502.9	520.0>208.2	16	25	0.005	0.997	92.0	9.4	99.3	8.2
70	Tebuconazole	3.03	307.8	308.4>70.0	46	39	0.003	0.985	99.3	9.4	102.9	9.4
71	Terbutryn	3.00	241.4	242.4>186.2	46	27	0.003	0.976	96.8	6.6	97.5	8.2
72	Thiamethoxam	2.09	291.7	292.2>211.2	31	21	0.010	1.000	77.7	3.3	75.4	4.9
73	Thiophanate-methyl	2.67	342.4	343.1>151.1	31	25	0.003	0.997	82.2	6.7	80.7	8.9
74	Tolyfluanid	3.02	347.3	347.1>137.2	31	37	0.010	0.975	80.5	6.9	86.0	7.6
75	Triadimefon	2.95	293.8	294.3>197.2	36	21	0.003	0.990	96.3	5.3	95.8	10.4
76	Triadimenol	2.96	295.8	296.2>69.9	21	27	0.003	0.994	94.9	6.1	97.0	9.4
77	Tri-allate	3.19	304.7	304.3>142.8	41	37	0.005	0.999	98.1	4.3	101.2	6.2
78	Trichlorfon	2.33	257.4	257.1>108.8	41	25	0.005	0.999	88.4	0.5	86.8	6.0
79	Trifloxystrobin	3.08	408.4	409.3>186.2	31	25	0.010	0.987	102.9	4.3	105.6	4.9
80	Triflumizole	3.10	345.7	347.0>278.7	21	17	0.005	0.994	85.7	5.2	90.2	5.1

M monoisotopic molecular mass; *RT* retention time; *DP* declustering potential; *CE* collision energy; *r*² regression coefficient

temperature and solar radiation. MRLs of detected pesticides in parsley, lettuce and spinach are shown in Table 3. The European Union (EU) has set the MRLs in or on food and feed of plant and animal origin to guarantee consumer safety and to regulate international trade under regulation (EC) No 396/2005 and amending Council Directive 91/414/EEC (European Commission 2005). Typically, MRLs range from 0.01 to 5.00 mg kg⁻¹ depending

on the commodity and the pesticide. Ethiofencarb and phenthoate are included in the Council Directive 91/414/EEC active substances list (European Commission 1991), however, no MRLs are established for them under the regulation, and so a default MRL of 0.01 mg kg⁻¹, according to Art 18(1)(b) Reg. 396/2005, is applied to these insecticides. In addition to these pesticides, assessment of cymoxanil, fenoxycarb, imidacloprid, metribuzin,

Table 2 Active substances found in parsley, lettuce and spinach samples (mg kg^{-1})

	Name	Parsley			Lettuce			Spinach		
		N ^a	min	max	N	min	max	N	min	max
1	Acetamiprid	–	–	–	12	<0.001	0.104	40	<0.001	0.001
2	Atrazine	38	<0.001	0.002	40	<0.001	0.001	–	–	–
3	Carbaryl	40	0.003	0.003	32	0.003	0.003	–	–	–
4	Carbendazim	40	0.002	0.111	40	0.002	0.007	18	0.003	1.840
5	Carbofuran	8	<0.001	<0.001	32	<0.001	0.001	0	–	–
6	Chlorpropham	28	<0.001	0.007	2	<0.001	<0.001	36	<0.001	0.019
7	Chlorpyrifos	18	<0.001	0.002	2	<0.001	<0.001	–	–	–
8	Cymoxanil	40	0.003	0.004	40	0.004	0.007	34	<0.001	0.161
9	Cypermethrin	40	0.003	0.020	35	0.004	0.016	–	–	–
10	Diazinon	–	–	–	12	0.002	0.078	–	–	–
11	Dichlorvos	40	0.002	0.035	40	0.005	0.020	40	0.002	0.071
12	Dimethoate	4	0.007	0.007	–	–	–	–	–	–
13	Ethiofencarb	40	0.002	0.003	40	0.002	0.002	–	–	–
14	Fenarimol	16	0.004	0.032	–	–	–	34	0.001	0.052
15	Fenoxycarb	2	0.003	0.003	12	<0.001	0.015	–	–	–
16	Furathiocarb	18	0.002	0.017	15	<0.001	0.018	6	<0.001	0.003
17	Imidacloprid	38	0.003	0.009	32	0.003	1.220	–	–	–
18	Kresoxim-methyl	–	–	–	–	–	–	6	<0.001	0.018
19	Metalaxyl	40	<0.001	<0.001	40	<0.001	0.015	–	–	–
20	Metribuzin	8	<0.001	0.005	23	<0.001	0.017	22	<0.001	0.004
21	Monolinuron	36	0.002	0.002	40	0.002	0.002	–	–	–
22	Oxadixyl	–	–	–	3	0.003	0.011	–	–	–
23	Oxyfluorfen	2	<0.001	<0.001	–	–	–	–	–	–
24	Pendimethalin	38	<0.001	0.289	37	0.001	0.354	–	–	–
25	Phenthoate	–	–	–	5	0.007	0.011	–	–	–
26	Pirimicarb	10	0.006	0.006	7	0.006	0.006	–	–	–
27	Pirimiphos-methyl	40	0.001	0.020	40	0.001	0.024	40	0.001	0.020
28	Simazine	40	0.002	0.015	40	<0.001	0.005	30	<0.001	0.007
29	Thiamethoxam	8	0.001	0.001	22	0.001	0.069	2	0.001	0.001
30	Trichlorfon	40	<0.001	0.002	40	<0.001	0.001	–	–	–

^aNo. of samples with residue. Forty samples were analyzed for each vegetable

oxadixyl, oxyfluorfen, pirimicarb, simazine, thiamethoxam and trichlorfon is ongoing and they are on the provisional list of Turkey. According to Turkey's communiqué of the maximum residue limits of pesticides, most of the pesticides detected in the samples were not permitted for use in parsley, lettuce or spinach (Table 3). In addition to that, all parsley and lettuce samples contained ethiofencarb and 6 lettuce samples contained phenthoate, which are non-authorized pesticides in Turkey.

According to the EU regulations, there were many MRLs violations exceeding the allowed limits. In parsley, two samples (5.0%) exceeded the MRLs for carbendazim (0.100 mg kg^{-1}) in the concentration range of $0.102\text{--}0.111 \text{ mg kg}^{-1}$, twenty eight samples (70.0%) exceeded the MRLs for dichlorvos (0.010 mg kg^{-1}) in the concentration range of $0.011\text{--}0.035 \text{ mg kg}^{-1}$, six

samples (15.0%) exceeded the MRLs for fenarimol (0.020 mg kg^{-1}) in the concentration range of $0.023\text{--}0.032 \text{ mg kg}^{-1}$ and four samples (15.0%) exceeded the MRLs for pendimethalin (0.050 mg kg^{-1}) in the concentration range of $0.269\text{--}0.289 \text{ mg kg}^{-1}$.

In lettuce, eight samples (20.0%) exceeded the MRLs for diazinon (0.010 mg kg^{-1}) in the concentration range of $0.012\text{--}0.078 \text{ mg kg}^{-1}$, sixteen samples (40.0%) exceeded the MRLs for dichlorvos (0.010 mg kg^{-1}) in the concentration range of $0.010\text{--}0.020 \text{ mg kg}^{-1}$, eight samples (20.0%) exceeded the MRLs for pendimethalin (0.050 mg kg^{-1}) in the concentration range of $0.149\text{--}0.354 \text{ mg kg}^{-1}$ and two samples (5.0%) exceeded the MRLs for phenthoate (non-authorized pesticide in the EU, 0.010 mg kg^{-1}) in the concentration range of $0.010\text{--}0.012 \text{ mg kg}^{-1}$. Cesnik et al. (2006b) analyzed the pesticide

Table 3 Maximum residue limits of pesticides (mg kg⁻¹) in parsley, lettuce and spinach in EU and Turkey

	Name	Category ^a	Parsley		Lettuce		Spinach	
			EU ^b	Turkey ^c	EU	Turkey	EU	Turkey
1	Acetamiprid	in	5.00	—	5.00	5.00	3.00	—
2	Atrazine	hb	0.05	—	0.05	—	0.05	—
3	Carbaryl	in, pg	0.05	0.05	0.05	0.05	0.05	0.05
4	Carbendazim	fu	0.10	—	0.10	—	0.10	—
5	Carbofuran	in, ne, ac	0.02	—	0.02	—	0.02	—
6	Chlorpropham	pg, hb	0.05	—	0.05	—	0.05	—
7	Chlorpyrifos	in, ac	0.05	—	0.05	—	0.05	—
8	Cymoxanil ^e	fu	0.05	—	0.20	—	0.05	—
9	Cypermethrin	in, ac	2.00	—	2.00	—	0.70	—
10	Diazinon	in, ac	0.01	—	0.01	—	0.01	—
11	Dichlorvos	in, ac	0.01	—	0.01	—	0.01	—
12	Dimethoate	in, ac	0.02	—	0.02	0.50	0.02	—
13	Ethiofencarb ^d	in	0.01	—	0.01	—	0.01	—
14	Fenarimol	fu	0.02	—	0.02	—	0.02	—
15	Fenoxycarb ^e	in	0.05	—	0.05	—	0.05	—
16	Furathiocarb	in	0.05	—	0.05	—	0.05	—
17	Imidacloprid ^e	in	2.00	—	2.00	—	0.05	—
18	Kresoxim-methyl	fu	0.05	—	0.05	—	0.05	—
19	Metalaxyl	fu	2.00	—	2.00	2.00	0.05	0.05
20	Metribuzin ^e	hb	0.10	—	0.10	0.10	0.10	—
21	Monolinuron	hb	0.05	—	0.05	—	0.05	—
22	Oxadixyl ^e	fu	0.01	—	0.10	—	0.01	—
23	Oxyfluorfen ^e	hb	0.05	—	0.05	—	0.05	—
24	Pendimethalin	hb	0.05	—	0.05	—	0.05	—
25	Phenthoate ^d	in	0.01	—	0.01	—	0.01	—
26	Pirimicarb ^e	in	5.00	5.00	5.00	2.00	2.00	—
27	Pirimiphos-methyl	in	0.05	—	0.05	—	0.05	—
28	Simazine ^e	hb	0.10	—	0.10	—	0.10	—
29	Thiamethoxam ^e	in	0.05	—	5.00	5.00	0.05	—
30	Trichlorfon ^e	in	0.50	—	0.50	—	0.50	—

^aac, acaricide; fu, fungicide; hb, herbicide; in, insecticide; ne, nematocide; pg, plant growth regulator

^bEU MRLs sorted by pesticide. http://ec.europa.eu/sanco_pesticides/public/index.cfm. Accessed November 2010.

^cAgriculture and Rural Affairs Ministry of the Republic of Turkey. Communiqué of the maximum residue limits of pesticides allowed in foodstuffs. 2008. <http://www.kkgm.gov.tr/TGK/Teblig/2008-26.html>. Accessed November 2010.

^dNon-authorized pesticides in the EU.

^ePesticides on the provisional list of Turkey.

“—” means not permitted to use for the corresponding product in Turkey

residues in lettuce samples of the Slovene origin and MRLs was exceeded by three samples (3.1%) with values from 0.14 to 6.36 mg kg⁻¹.

In spinach, twelve samples (30.0%) exceeded the MRLs for carbendazim (0.100 mg kg⁻¹) in the concentration range of 0.109–1.840 mg kg⁻¹, eight samples (20.0%) exceeded the MRLs for cymoxanil (0.050 mg kg⁻¹) in the concentration range of 0.051–0.161 mg kg⁻¹, thirty four samples (85.0%) exceeded the MRLs for dichlorvos (0.010 mg kg⁻¹) in the concentration range of 0.011–0.071 mg kg⁻¹ and fourteen samples (35.0%) exceeded the MRLs for fenarimol (0.020 mg kg⁻¹) in the concentration range of 0.021–0.052 mg kg⁻¹. These findings indicate that pesticides were extensively used in parsley, lettuce and spinach cultivation in Hatay. Wang et al. (2008) analyzed eight organophosphorus pesticide residues

(OPP) in fresh vegetables retailed in agricultural product markets of Nanjing (China). While dimethoate (0.118–0.124 mg kg⁻¹) was detected in 4 samples (26.7%), methyl-parathion (0.0924 mg kg⁻¹) in 3 samples (20.0%), chlorpyrifos (0.106–0.204 mg kg⁻¹) in 4 samples (26.7%), fonofos and fenthion were not detected in any of the samples. Dimethoate, chlorpyrifos, and methyl-parathion were the most common OPPs applied in vegetable production. Despite a high occurrence of pesticide residues in the fresh vegetables tested, no sample exceeded their respective MRLs, thus the contamination levels of these residues may not be considered a serious public health problem according to Chinese regulations.

Food composition and processing have a significant effect on the dissipation of pesticide residues. Pico and Kozmutza (2007) studied the effect of natural antioxidants

on the degradation rate of four pesticides (aldicarb, demeton-S-methyl, fenamiphos, methiocarb) in grape juices and their results indicated that natural antioxidant compounds present in fruit juices can reduce the pesticide degradation rate through an oxidative mechanism, increasing the persistence of the compounds. Ticha et al. (2008) and Fenoll et al. (2009b) investigated the dissipation of various insecticides and fungicides in apples and in peppers, respectively. They have reported that pesticide residue contents were decreased during storage. Randhawa et al. (2007) investigated the effect of washing, peeling and cooking on residue levels of chlorpyrifos in spinach, cauliflower, potato, eggplant, tomato, okra by capillary GC and reported that washing, peeling and cooking have significant effect on the declining of chlorpyrifos residue in those vegetables. Uygun et al. (2008) investigated the carryover of malathion, fenitrothion, chlorpyrifos-methyl, and pirimiphos-methyl residues in durum wheat, semolina and pasta using GC. While the storage period was generally not effective enough to reduce the residues in wheat or semolina to levels below the MRLs, spaghetti processing significantly reduced residue concentrations. Fernandez-Cruz et al. (2006) analyzed the dissipation of residue levels of captan and trichlorfon in field-treated kaki fruits. While levels of captan decreased by more than 90% after peeling and completely after cooking, cooking resulted in a decrease of 27% of residue levels of trichlorfon. All these studies showed that while cold storage has limited effect on pesticide dissipation, washing, peeling, extrusion and cooking have significantly affected pesticide dissipation. Therefore, we may expect to see a decrease in pesticide residue content of spinach since it is washed and boiled or cooked before consumption. However, parsley and lettuce are usually consumed fresh and washing process will have a limited effect on pesticide residue content of these vegetables.

Conclusions

A total of 120 spinach, parsley and lettuce samples harvested and commercialized in the farmers market of Hatay province (Turkey) were analyzed in order to determine the presence of a group of 80 pesticides. All of the spinach, parsley and lettuce samples tested contained detectable residues. According to Turkey's communiqué of the maximum residue limits of pesticides, most of the pesticides were not permitted for use in the corresponding vegetables. The MRLs was exceeded in 28 parsley (70%), 18 lettuce (45%) and 40 spinach (100%) samples, according to EU regulation. The repeated use of the pesticide in the field, particularly

closer to harvest significantly contributes to a higher residue level. The results suggested that the farmers in Hatay are either unconscious about the safe and judicious use of pesticides or have a tendency to overdose the pesticides to ensure a high external quality of the produce. The findings of this study pointed to the recommendation that there is a need for revising the MRLs of pesticides in Turkey in or on food and feed of plant and animal origin parallel to EU Regulation (EC) No 396/2005 and implement a monitoring program for residues in or on food and food products at the national level.

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