

Studying the Effect of Household-Type Treatment and Processing on the Residues of Ethion and Profenofos Pesticides and on the Contents of Capsaicinoids in Green Chili Pepper Using GC-MS/MS and HPLC

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Abstract The first aim of this study was to create, test, and apply a quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, based on acetonitrile extraction, for simultaneous determination of pesticide residues in green chili pepper (fresh and dried forms). There is a group of 86 repeatedly detected pesticides in the agricultural food commodity in Al-Qassim region, Saudi Arabia. The suggested method is utilizing GC-MS/MS for simultaneous determination of those 86 pesticides. The limit of quantitation (LOQ) for each pesticide was set as the lowest fortification level that achieved acceptable recoveries in the range 70–120% with precision (RSD \leq 20%). The results indicated that two organophosphorus pesticides, ethion and profenofos, in the analyzed chili pepper samples have significantly exceeded the European maximum residual limit (MRL) for pesticides. The second purpose of the study was to evaluate the effect of some different household-type treatment and processing procedures on the detected pesticide residues and on capsaicinoids (particularly, capsaicin and dihydrocapsaicin) which are responsible for the pungency of chili pepper, using GC-MS/MS and

HPLC. Some recommendations were concluded for the best practices.

Keywords Pesticides · GC-MS/MS · HPLC · QuEChERS · Chili pepper

Introduction

Pesticide residues on agricultural products are among the most common reasons for transferring chemical contaminants to humans. Pesticides are widely used during the production of fruits and vegetables in order to control the growth and reproduction of agricultural pests and to prolong the duration of storage and the quality of crops post-harvest. Research on detecting and identifying of these residues can help minimize the risks on human health. Pesticide residues in foods have a maximum residual limit (MRL). MRL is established by law and recommended by codex in food and feed to minimize health and environmental issues by ensuring that the concentration of pesticides in the food commodity is at levels that should not be harmful upon consumption (FDA 1993; Koesukwiwat et al. 2009; Masahiro et al. 2005). Great attentions are paid to the organophosphorus pesticides because of their health impacts on humans as a result of excess exposure and the spread of use in agricultural activities for various crops (Rawn et al. 2008).

Peppers are the fruits of plants from the genus *Capsicum* belong to the family *Solanaceae*. They are widely consumed as a food additive throughout the world, due to their unique pungency, color and aroma characteristics. Pungency, a commercially important attribute of peppers, is related to the presence of capsaicinoids (Perucka and Materska 2001). The two most abundant capsaicinoids congeners in peppers are capsaicin (8-methyl-*N*-vanillyl-*trans*-6-nonenamide) and

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dihydrocapsaicin. Both chemicals constitute about 90%, where capsaicin accounts for about 71% of the total capsaicinoids in most of the pungent varieties (Kosuge and Furuta 1970). Capsaicin—the most pungent of the group capsaicinoids—content of peppers is one of the major parameters that determine its commercial quality (Hachiya et al. 2007; Kawabata et al. 2006; Ohnuki et al. 2001; Zhang et al. 2007). Capsaicin is sparingly soluble in water, but it is very soluble in fats.

Hydrophobic compounds like capsaicin, analogs of capsaicin dihydrocapsaicin, and the saturated analogue of capsaicin (Laohavechvanich et al. 2006) are also considered as an active principle which accounts for the pharmaceutical properties of peppers. It has been used as an analgesic against arthritis pain and inflammation (Deal et al. 1991). However, high levels of capsaicin can lead to negative health impacts (López-Carillo et al. 1994). The amount of capsaicin in peppers is highly dependent on the light intensity and temperature at which the plant is grown, the age of the fruit, and the position of the fruit on the plant (Scoville 1912).

“Quick, Easy, Cheap, Effective, Rugged, and Safe” (QuEChERS) sample preparation method for the determination of pesticide residues was developed and applied for the extraction and cleanup of pesticides. The proposed method replaces the other conventional preparations techniques (Anastassiades et al. 2003; Lehotay 2006).

Ethion is a member of the organophosphorus family, and it is a non-systemic insecticide which is used to control leaf-feeding insects, scales and mites and registered to be used for many crops. It is insoluble in water and soluble in most organic solvents and its boiling point is 165 °C and it is exerted by inhibiting an enzyme of the nervous system of the insect (Acetylcholinesterase) (EPA 1989).

Profenofos, *O*-(4-Bromo-2-chlorophenyl)-*O*-ethyl-*S*-propyl phosphorothioate, is an organophosphate insecticide/miticide and is used to control some pests like cotton bollworm, armyworm, whiteflies, spider mites, and plant bugs. Its boiling point is 110 °C, and it is soluble in water (20 mg/L at 20 °C; 25 mg/L at 25 °C) and readily miscible with most organic solvents, and it has some risks on human health (Rao et al. 2003).

The degradation of a pesticide depends on several factors such as the chemical nature of pesticide, the type of commodity, and the characteristics of location. The degradation can take place by hydrolysis, redox reactions, and suitable temperature and pH values. Additionally, some preparation steps are well known to reduce the levels of pesticides such as peeling which removes the pesticide residues from the exposed part of the plant; this is not applicable in the case of many vegetable plants. Other processes can be useful too, such as cooking, boiling, soaking with salts and chemicals, or steaming (Bajwa and Sandhu 2014). The extracts of pesticides from plants can be analyzed using liquid or gas chromatography

coupled with various detectors (Fenoll et al. 2008; Tuzimski 2011).

This study was conducted in Al-Rass province, Al-Qassim region, which lies approximately at the center of the Arabian Peninsula. Al-Qassim region is divided into 11 provinces (Mohieldein et al. 2011). Chili pepper samples for the current study were collected from the local market of Al-Rass province. According to our lab data, the residues of 86 pesticide compounds are repeatedly detected in different food commodity in Al-Qassim region, Saudi Arabia.

Currently, there are no published reports on the contamination pepper caused by those pesticides in Al-Qassim region. Therefore, the first aim of the present study was to create, test, and apply a simple method for the determination of those 86 pesticide residues in the commonly consumed vegetables. It was based on solid-phase extraction and GC-MS/MS technique following the QuEChERS sample preparation procedures.

The second goal of this study was to investigate the extent to which the household-type handling and processing of pepper would affect the concentration of pesticides, particularly ethion and profenofos. The last aim was to qualitatively estimate the presence of capsaicin and dihydrocapsaicin that may be present in some pepper samples after some household-type processing and handling of the pepper samples from the local markets of Al-Rass province, AL-Qassim region.

Materials and Methods

Standard Solutions

Pesticide reference standards (86 standards) were purchased from Riedel-de-Häen (Seelze, Germany) and Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA). Ultrapure water was produced using (Purelab Flex, ELGA, Veolia Water Solution & Technologies, UK) with specific resistivity of 18 MΩ. A stock standard solution for each compound (1000 mg/L) was prepared in acetonitrile (HPLC grade, Fisher Scientific, UK). All standard solutions with a concentration of 10 g/mL were prepared for the 86 pesticides and then were stored in glass-stoppered flasks at 4 °C to be used for the preparation of a calibration curve and for the spiking of the contaminated samples of pepper.

Method of Pesticide Determination Using GC-MS/MS

The analytical method was tested using the evaluation criteria based on SANCO guidelines (SANCO 2015) and the fitness for purpose of analytical methods (Magnusson and Örnemark 2014). Matrix-matched calibration curves were carried out by fortification of blank pepper extracts with working standard solutions to check the linearity. The limit of detection (LOD)

of all tested analytes were taken where $S/N \geq 3$. The limit of quantitation (LOQ) for each pesticide was set as the lowest fortification level that achieved acceptable recoveries in the range 70–120% with precision (relative standard deviation, $RSD \leq 20\%$). Precision in case of repeatability, represented by the relative standard deviation for repeatability (RSD_r), was determined by fortifying six blank pepper samples with the analyzed pesticides at three different concentration levels of 10, 50, and 100 $\mu\text{g}/\text{kg}$ on the same day, operator, and same instrumental configuration. Precision in case of reproducibility, represented by the relative standard deviation for reproducibility (RSD_R), was determined by fortifying two sets of blank samples at concentration level of 10 $\mu\text{g}/\text{kg}$ and analyzed on two different days with different operators and same instruments' configuration. The accuracy of the method was evaluated by means of percentage recovery from spiked samples set in repeatability study.

Sample Preparation

Sample Collection

The samples of green chili pepper were collected from the local market in Al-Rass province, Saudi Arabia, during the autumn season of 2017, and all samples were of *Capsicum annuum* L. which were imported from India. The weight of each representative samples ranged from 150 to 300 g. The samples were kept in sterile polyethylene bags and transported to the laboratory in an ice chest box. In the laboratory, samples either analyzed immediately or stored at 4 °C until the process of analysis.

Sample Extraction

It consists of three steps which includes sample mixing and homogenization, extraction/partitioning, and dispersive SPE cleanup. The process is reported in details in a previous application notes (Zhao et al. 2007). All reagents were HPLC grade and were purchased from (Fisher Chemical Scientific, UK) except for the commercial natural vinegar which was purchased from (Haley, Saudi Arabia), and it is made from sugar cane diluted with water to 5% acetic acid strength for table use.

We have studied two forms of chili pepper samples: fresh (untreated), and sun-dried samples. The fresh pepper samples were homogenized thoroughly in an electrical grinder (Kenwood CH550, China). A 15-g (± 0.1 g) amount of homogenized sample was placed into a 50-mL centrifuge tube (blue cap, sterile polypropylene, Elkay lab, UK). Samples were fortified with appropriate QC spiking solutions (100 μL) when necessary if the concentration of the pesticide residues is higher than the expected values or to confirm the exact amount of the pesticide residues in the sample itself.

Samples' tubes were shaken by vortex (Select Bio Products, SBS100-2, USA), 15 mL of acidified acetonitrile (0.01% acetic acid glacial) was added to each tube using the analog adjustable bottle-top dispenser (Dispensette, Brand, Germany), and placing a ceramic homogenizer (50 mL, Agilent Technology, USA) to ensure the homogeneity of the sample as acetonitrile is an effective solvent for the simultaneous extraction of pesticide multi-residues. To each tube, 6 g of anhydrous magnesium sulfate (ACROS Organics, Belgium) and 1.5 g of anhydrous sodium acetate (Panreac, Spain) were added to make a buffer solution with acidified acetonitrile in order to control pH at 6–7. The buffer system is used to protect the target compounds, and is suitable for pesticides. The neutral condition was created to enhance the stability of the organophosphorus pesticides which are easily hydrolyzed, so the pesticides prefer acetonitrile to water (Qu et al. 2010). Sample tubes were capped tightly and hand-shaken vigorously for 1 min and then shaken by vortex mixer for another 1 min. After that, tubes were transferred to a refrigerated centrifuge (Pro-Research K241R, Centurion Scientific, UK) for 5 min at 5000 rpm at 6 °C.

For the dried samples, 150 g of pepper were sun-dried for 7–10 days without any heat treatment. Sun drying is a traditional method which is used to reduce the moisture content in pepper to 10–15% and found to be in a good agreement with our sun-dried samples (Condori et al. 2001). The dried samples were then homogenized thoroughly using an electrical grinder to obtain fine powder of pepper. Then, 2 g of the dried powder was added to 10 mL deionized water into 50 mL centrifuge tube and was shaken for 10 s and then kept for 30 min to equilibrate. 10 mL of acetonitrile were added to the mixture and shaken by vortex for 1 min. Six grams of anhydrous magnesium sulfate and 1.5 g of anhydrous sodium acetate were added to the tubes. The sample tubes were then capped tightly and hand-shaken vigorously for 1 min and then shaken by vortex for another 1 min. After that, tubes were centrifuged at 5000 rpm for 5 min at 6 °C (Young et al. 2015).

Sample Cleanup

For both fresh and dried samples, the same procedures were followed: A 4-mL aliquot (the top-layer) was transferred by single channel micropipette (CAPP, Denmark) into an Agilent Bond Elut QuEChERS-dispersive SPE 15 mL (Pigment sample, AOAC, USA) in 15 mL centrifuge tube containing the sorbent consisting of 400 mg of primary-secondary amine (PSA) used to remove fats, 400 mg of bulk of carbograph which is used to remove the pigments from the extract, and 1200 mg of anhydrous magnesium sulfate which is used to remove pigments in the extract. Then, the 15-mL tubes were centrifuged at 5000 rpm for 5 min at 6 °C.

A volume of 1.5 mL of the extract was transferred into a glass syringe (Jena-Glass, with hollow handle, Metal record

Table 1 Parameters for the common 86 pesticides in Al-Rass province, Al-Qassim region, Saudi Arabia, determined using GC-MS/MS

No.	Pesticide	R.T	Rec. % (ppb)	LOD (ppb)	LOQ (ppb)	Chemical formula	M. wt (ppm)	MRL (ppm)	M/Z1		M/Z2		M/Z3				
									Precursor ion	Product ion	CE	Product ion	CE	Precursor ion	Product ion	CE	Precursor ion
1	Dichlorvos	5.22	105	2.22	4.44	C ₄ H ₂ Cl ₂ O ₄ P	220	0.01	109	79	6	185	93	12	186.9	93	12
2	Allidochlor	5.46	71.36	4.26	8.52	C ₈ H ₁₂ ClNO	173	0.01 ^a	132	49	24	132	56.1	8	138.1	95.9	6
3	Dichloroamine-3,5	6.06	116.4	4.26	8.53	C ₆ H ₅ Cl ₂ N	161	0.01 ^a	161	90	20	161	98.9	22	161	125.5	14
4	Mevinphos	6.11	119.07	2.5	5.01	C ₇ H ₁₃ O ₆ P	224	0.01	127	95	14	127	109	10	192	127	10
5	Vernolate	6.21	105.18	4.66	9.32	C ₁₀ H ₂₁ NOS	203	0.01 ^a	128.1	41.1	16	128.1	43.1	8	160.9	160.1	8
6	Chloroneb	6.52	114.6	1.07	2.13	C ₈ H ₈ Cl ₂ O ₂	206	0.01 ^a	190.9	113	14	190.9	141	10	206	190.9	12
7	Chlorfenprop-methyl	6.91	110.25	1.89	3.77	C ₁₀ H ₁₀ Cl ₂ O ₂	232	0.01 ^a	165	102.1	18	165	137	10	195.9	165	10
8	Propachlor	7.02	109.2	1.87	3.73	C ₁₁ H ₁₄ ClNO	211	0.05	120	50.9	35	120	77	15	176.1	57.1	10
9	Ethalfuralin	7.14	111.55	1.27	2.53	C ₁₃ H ₁₄ F ₃ N ₃ O ₄	333	0.01	276	202	14	276	248.1	8	315.9	276.1	8
10	Benfluraline	7.21	90.4	4.09	8.18	C ₁₃ H ₁₆ F ₃ N ₃ O ₄	335	0.05	292	159.7	20	292	206.1	10	292	264	8
11	Cadusafos	7.33	105.35	3.09	6.17	C ₁₀ H ₂₃ O ₂ PS ₂	270	0.01	159	96.9	16	159	130.9	8	213	89.1	12
12	Atrazine	7.58	104.25	1.98	3.95	C ₈ H ₁₄ ClN ₅	215	0.05	200	122.1	8	200	132	8	215.1	58.1	12
13	Dichloran	7.59	109.27	4.54	9.08	C ₆ H ₄ Cl ₂ N ₂ O ₂	206	0.01 ^a	175.9	148	10	205.9	147.9	20	205.9	176	10
14	Propyzamide	7.76	113.55	2.64	5.28	C ₁₂ H ₁₁ Cl ₂ NO	255	0.2	172.9	74	38	172.9	109	26	172.9	145	14
15	Diazinon	7.78	102.6	1.73	3.46	C ₁₂ H ₂₁ N ₂ O ₃ PS ₂	304	0.02	137.1	54.1	20	137.1	84.1	12	179.1	121.5	26
16	Pyrimethanil	7.82	109.47	4.17	8.33	C ₁₂ H ₁₃ N ₃	199	0.01 ^a	198.1	117.9	30	198.1	157.6	18	198.1	182.9	14
17	Fonofos	7.83	77.15	4.15	8.3	C ₁₀ H ₁₅ O ₂ PS ₂	246	0.01 ^a	137	109	6	246	109	14	246	137	6
18	Mexacarbate	7.92	113.7	2.22	4.44	C ₁₂ H ₁₈ N ₂ O ₂	222	0.01 ^a	150.2	76.9	25	164.1	148.1	15	165.1	134.1	10
19	Primicarbe	8.03	103.28	3.81	7.62	C ₁₁ H ₁₈ N ₄ O ₂	238	0.01 ^a	166.1	55	18	166.1	96	12	238.1	166.1	10
20	Iprobenphos	8.03	104.75	4.57	9.14	C ₁₃ H ₂₁ O ₃ PS	288	0.01 ^a	91.1	65	16	203.9	91.1	8	203.9	121	28
21	Desmetyrn	8.14	111.65	5.83	11.66	C ₈ H ₁₅ N ₅ S	213	0.01 ^a	213.1	58.1	10	213.1	170.9	8	213.1	198.1	8
22	Formothion	8.18	85.4	4.32	8.63	C ₆ H ₁₂ NO ₄ PS ₂	257	0.02	93	63.1	10	125	47	15	125	79	10
23	Dichlorfenthion	8.18	103.2	3.66	7.31	C ₁₀ H ₁₃ Cl ₂ O ₃ PS	314	0.01 ^a	222.9	205	12	250.9	223	8	279	223	12
24	Metribuzin	8.21	72.19	4.59	9.18	C ₈ H ₁₄ N ₄ OS	214	0.1	198	55	26	198	82.1	16	198	110	10
25	Vinclozolin	8.26	118.56	6.68	13.35	C ₁₂ H ₉ Cl ₂ NO ₃	285	0.02	241.1	58.1	12	241.1	184.1	10	284.9	269.9	12
26	Chlorpyrifos-methyl	8.29	118.43	4.87	9.73	C ₇ H ₇ Cl ₃ NO ₃ PS	321	0.05	125	47	12	125	79	6	285.9	93	20
27	Tolclofos-methyl	8.34	104	3.77	7.54	C ₉ H ₁₁ Cl ₂ O ₃ PS	300	0.02	265	219.9	20	265	250	12	266.8	252	12
28	Heptachlor	8.45	112.15	1.53	3.06	C ₁₀ H ₅ Cl ₇	370	0.01	99.8	39	26	99.8	65	12	271.8	236.9	12
29	Pirimiphos-methyl	8.49	114.25	2.46	4.93	C ₁₁ H ₂₀ N ₃ O ₃ PS	305	0.01 ^a	290.1	125	20	290.1	233	8	305.1	180.1	8
30	Ethofumesate	8.51	118.77	3.36	6.72	C ₁₃ H ₁₈ O ₅ S	286	1.5	161.1	77.1	28	161.1	105.1	10	207.1	137.1	10
31	Fenitrothion	8.51	116.8	4.56	9.12	C ₉ H ₂₁ NO ₃ PS	277	0.02	125	79	8	277	109	16	277	260	6
32	Malathion	8.55	117.55	3.26	6.52	C ₁₀ H ₁₉ O ₆ PS ₂	330	0.02	92.8	63	8	125	79	8	173.1	99	12
33	Phorate-sulfone	8.68	116.6	3.02	6.04	C ₁₂ H ₂₁ N ₂ O ₃ PS ₂	292	0.02	125	97	5	153	96.9	10	199	143	10
34	Isomethiozin	8.68	113.77	4.87	9.74	C ₁₂ H ₂₀ N ₄ OS	268	0.01 ^a	198.1	82.1	15	198.1	110.1	10	225	57.09	15

Table 1 (continued)

No.	Pesticide	R.T	Rec. %	LOD (ppb)	LOQ (ppb)	Chemical formula	M. wt (ppm)	M/Z1	M/Z2		M/Z3						
									Precursor ion	Product ion	CE	Product ion	CE	Product ion	CE		
35	Fenthion	8.68	115	3.43	6.85	C ₁₀ H ₁₅ O ₃ PS ₂	278	0.01	245.3	125	12	278	109	18	278	169	14
36	Chlorpyrifos	8.69	118.7	2.22	4.44	C ₉ H ₁₁ Cl ₃ NO ₃ PS	283	0.01 ^a	196.7	107	36	196.7	168.9	12	313.9	257.9	12
37	Metolachlor	8.69	117.7	2.44	4.88	C ₁₅ H ₂₂ ClNO ₂	348	0.01 ^a	162.1	132.9	14	238.1	132.8	26	238.1	162.2	10
38	Triadimefon	8.72	105.73	4.84	9.68	C ₁₄ H ₁₆ Cl ₃ N ₃ O ₂	293	0.1	208	111	20	208	126.7	12	208	180.8	8
39	Dicofol	8.77	115.73	1.92	3.84	C ₁₄ H ₆ Cl ₅ O	367	0.02	111	74.9	12	139	111	12	250.9	139	12
40	Parathion(oxon)	8.77	104.8	2.22	4.44	C ₁₀ H ₁₄ NO ₃ PS	291	0.05	139	109	6	109	81	8	149	91.1	10
41	Primiphos-ethyl	8.82	119.25	4.2	8.39	C ₁₃ H ₂₄ N ₃ O ₃ PS	333	0.01 ^a	304	168.1	12	318.1	166.1	12	318.1	182.1	10
42	Bromophos-methyl	8.89	117.4	3.37	6.75	C ₈ H ₈ BrCl ₂ O ₃ PS	363	0.01 ^a	125	79	6	328.9	313.8	14	330.8	315.8	14
43	Pendimethalin	8.99	114.25	2.46	4.93	C ₁₃ H ₁₉ N ₃ O ₄	281	2	252.1	161	14	252.1	162	8	252.1	191.3	8
44	Fipronil	9.15	104.05	3.07	6.14	C ₁₂ H ₄ Cl ₂ F ₆ N ₄ O ₅	435	0.005	366.9	212.9	28	366.9	244.9	20	368.8	214.9	30
45	Procyimidone	9.16	109.88	4.8	9.59	C ₁₃ H ₁₁ Cl ₂ NO ₂	283	0.02	95.9	53	16	95.9	67.1	8	283	96.1	8
46	Bromophos-ethyl	9.25	108.7	4.44	8.88	C ₁₀ H ₁₂ BrCl ₂ NO ₃ PS	391	0.02	96.9	65	16	96.9	78.9	12	302.7	284.8	14
47	Tetraclor-vinphos	9.32	113.7	2.22	4.44	C ₁₀ H ₆ Cl ₄ O ₄ P	363	0.01 ^a	109	79	6	328.9	109	18	330.8	109	18
48	Paclbutrazol	9.32	111.6	3.15	6.31	C ₁₅ H ₂₀ ClN ₃ O	293	0.02	125	89	18	236	125	12	236	167	10
49	Butachlor	9.33	119.07	2.97	5.94	C ₁₇ H ₂₆ ClNO ₂	311	0.01 ^a	160	131.7	12	176.1	146.9	12	188.1	160.1	10
50	Fluorodifen	9.4	83.7	2.22	4.44	C ₁₃ H ₇ F ₃ N ₂ O ₅	328	0.01 ^a	126	75	12	190	75	20	190	126	8
51	Fenamiphos-sulfoxide	9.41	111.6	4.77	9.55	C ₁₃ H ₂₂ NO ₄ PS	319	0.02	80.1	79.3	10	122.1	80	10	122.1	121.1	10
52	Chlorfenvinphos	9.42	93.7	2.71	5.42	C ₁₂ H ₁₄ Cl ₃ O ₄ P	357	0.02	266.9	159	16	266.9	203	10	323	266.9	14
53	Bromofenvinphos	9.5	108.93	3.1	6.2	C ₁₂ H ₁₄ BrCl ₂ O ₄ P	401	0.01 ^a	266.9	159	14	266.9	203	10	323.1	266.9	10
54	Prothiophos	9.5	110.5	3.15	6.31	C ₁₁ H ₁₅ Cl ₂ O ₂ PS ₂	343	0.01 ^a	266.7	220.9	18	266.7	238.9	8	308.9	239	14
55	Profenofos ^b	9.51	78.05	4.24	8.48	C ₁₁ H ₁₅ BrClO ₃ PS	371	0.05	296.7	268.9	10	336.9	266.9	12	336.9	308.9	8
56	Iodofenphos	9.51	114.25	3.09	6.17	C ₈ H ₈ Cl ₃ O ₃ PS	411	0.01 ^a	125	47	12	125	79	6	376.8	361.8	16
57	Oxadiazon	9.52	101.3	1.82	3.64	C ₁₅ H ₁₈ Cl ₂ N ₂ O ₃	344	0.05	174.9	76	28	174.9	112	12	174.9	147.2	6
58	Kresoxim-methyl	9.61	107.8	1.95	3.91	C ₁₈ H ₁₉ NO ₄	313	0.02	116	62.9	24	116	89	14	130.9	130.1	10
59	Buprofezin	9.63	104.25	2.82	5.65	C ₁₆ H ₂₃ N ₃ O ₅	305	4	105.1	50.9	32	105.1	77	18	175	132.1	12
60	Fluazifop- <i>p</i> -butyl	9.67	95.03	1.37	2.74	C ₁₉ H ₂₀ F ₃ NO ₄	383	0.02	282	91.1	18	282	238.1	16	383.1	282.1	14
61	Chlorfenpyr	9.74	111.45	2.55	5.11	C ₁₅ H ₁₁ BrClF ₃ N ₂ O	405	0.02	136.9	102	12	248.9	112	24	248.9	137.1	18
62	Nitrofen	9.78	104.7	3.33	6.66	C ₁₂ H ₇ Cl ₂ NO ₃	282	0.01	202	139	24	283	202	10	283	253	10
63	Chlorobenzilate	9.84	108.75	4.2	8.39	C ₁₆ H ₁₄ Cl ₂ O ₃	324	0.02	111	75.1	14	139	74.9	26	139	111	12
64	Fenthion-sulfoxide	9.94	93.15	2.95	5.91	C ₁₀ H ₁₅ O ₄ PS ₂	294	0.01	109	79	8	125	47	14	125	79	8
65	Ethion ^b	9.94	102.4	2.71	5.42	C ₉ H ₂₂ O ₄ P ₂ S ₄	383	0.01	153	97	10	230.9	128.9	22	230.9	174.9	12
66	Chlorthiophos	10.05	114.73	2.86	5.71	C ₁₁ H ₁₅ Cl ₂ O ₃ PS ₂	359	0.01 ^a	268.9	205	14	296.9	268.9	8	324.9	269	12
67	Carbophenothion	10.18	114.3	1.51	3.01	C ₁₁ H ₂₆ Cl ₂ O ₂ PS ₃	341	0.01 ^a	157	45	12	199	142.9	10	342	157	10
68	Cyanofenphos	10.21	107.95	4.11	8.22	C ₁₅ H ₁₄ NO ₂ PS	303	0.01 ^a	157	77.1	22	169	77.1	22	169	141	8

Table 1 (continued)

No.	Pesticide	R.T	Rec. %	LOD (ppb)	LOQ (ppb)	Chemical formula	M. wt	MRL (ppm)	M/Z1	M/Z2		M/Z3					
										Precursor ion	Product ion	CE	Product ion	Precursor ion	CE		
69	Dichlorofop-methyl	10.36	113.4	2.8	5.6	C ₁₆ H ₁₄ Cl ₂ O ₄	340	0.01 ^a	253	161.1	15	253	162	15	253	190.1	10
70	Diflufenican	10.37	118.13	3.31	6.63	C ₁₉ H ₁₁ F ₅ N ₂ O ₂	394	0.02	266	238.1	12	266	246.1	10	394	266.1	12
71	Spiromesifen	10.61	101.78	3	5.99	C ₂₃ H ₃₀ O ₄	370	0.02	99	57.1	6	254.1	209.1	10	272.1	254.2	8
72	Bifenthrin	10.66	119.9	1.03	2.06	C ₂₃ H ₂₂ ClF ₃ O ₂	422	0.05	165.1	163.6	24	181	165.9	10	181	179	12
73	Bifenazate	10.72	118.75	2.86	5.73	C ₁₇ H ₂₀ N ₂ O ₃	300	0.02	198.9	184.1	12	258	196.1	12	258	199.1	12
74	Phosmet	10.76	102.55	3.35	6.71	C ₁₁ H ₁₂ NO ₄ PS ₂	316	0.05	160	50.9	38	160	76.9	22	160	133	10
75	Fenpropathrin	10.76	90.3	2.09	4.17	C ₂₂ H ₂₃ NO ₃	349	0.01	97.1	55.1	6	181	126.8	28	181	151.9	22
76	Lambda-cyhalothrin	11.05	98.15	1.84	3.69	C ₂₃ H ₁₉ ClF ₃ NO ₃	449	1	181	152.1	25	197.1	141	10	208.1	192	15
77	Tetradifon	11.05	113.25	1.75	3.51	C ₁₂ H ₆ Cl ₄ O ₂ S	353	0.01	159	74.8	32	159	111	20	159	131	10
78	Amitraz	11.2	115.3	3.11	6.22	C ₁₉ H ₂₃ N ₃	293	0.05	121	106.1	10	131.9	117.1	16	161.9	132	8
79	Leptophos	11.2	105.35	3.93	7.86	C ₁₃ H ₁₀ BrCl ₂ O ₂ PS	409	0.01 ^a	171	51	38	171	77.1	18	171	124.3	10
80	Pyrazophos	11.41	119.5	2.66	5.33	C ₁₄ H ₂₀ N ₃ O ₅ PS	373	0.02	221	148.7	14	221	193.1	8	231.9	204.1	10
81	Fenarimol	11.5	119.1	1.33	2.66	C ₁₇ H ₁₂ Cl ₂ N ₂ O	330	0.02	139	74.9	26	139	111	14	219	107	10
82	Azinphos-methyl	11.53	102.3	4.93	9.86	C ₁₀ H ₁₂ N ₃ O ₃ PS ₂	317	0.05	132	77	12	160	50.9	34	160	77	16
83	Azinphos-ethyl	11.54	117.2	2.18	4.35	C ₁₂ H ₁₆ N ₃ O ₃ PS ₂	345	0.02	132	51	26	132	77	12	160	77	16
84	Permethrin	11.82	111.6	3.89	7.78	C ₂₁ H ₂₀ Cl ₂ O ₃	390	0.05	163	91.1	15	183.1	165.1	10	183.1	168.1	10
85	Fenvalerate	13.35	88.83	2.07	4.14	C ₂₃ H ₂₂ ClNO ₃	419	0.01 ^a	125	89	18	167	89	32	167	125	10
86	Esfenvalerate	13.57	98.3	1.73	3.46	C ₂₅ H ₂₂ ClNO ₃	419	0.05	125	89.3	20	167	125	10	225.1	119.1	18

CE collision energy

^a Pesticides that have no MRL are considered to be 0.01 according to EU

^b Pesticides under investigation in green chili pepper samples in this research

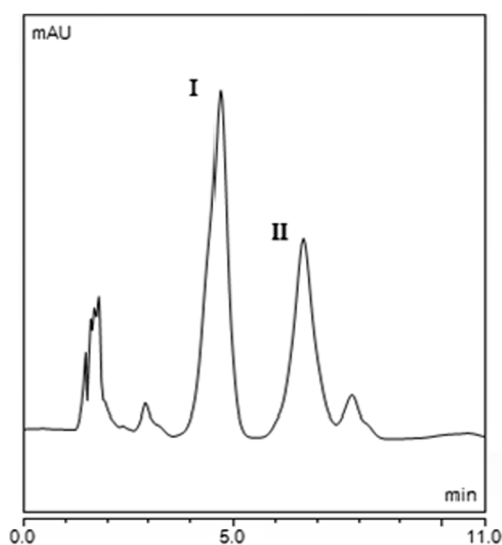
Table 2 HPLC quantified percentage and ratios for identified components (capsaicin and dihydrocapsaicin)

Samples	Component		
	Capsaicin (%)	Dihydrocapsaicin (%)	(Capsaicin/dihydrocapsaicin)
Fresh pepper sample	382	264	1.45
Dried pepper sample	276	198	1.39
Soaked in acetic acid for 15 min	245	155	1.58
Soaked in acetic acid for 30 min	215	135	1.59
Washed with running tap water for 40 s	194	125	1.55
Boiled in water for 30 min	201	127	1.58
Soaked in NaCl for 15 min	236	158	1.49
Soaked in NaCl for 30 min	334	268	1.25

cone no.4522, ELN no. 15336620, Germany), fitted with a nylon syringe filter (diameter 25 mm with pore size 0.22 μm , ChromTech, UK) fitted into an auto-sampler vial (1.5 mL wide open short screw-thread vial with write on spot, 9 mm thread, clear 11.6 \times 32 mm, ChromTech, UK) and the cap of the vial is red (PTFE/white silicone septa, 9 mm blue short screw thread, polypropylene cap, 6 mm center hole, ChromTech, UK). The extracts were capped and shaken by vortex mixer thoroughly and transferred to the GC auto-sampler for GC-MS/MS and HPLC analysis.

The Effect of Household Handling and Processing on the Levels of Pesticide Residues and on Capsaicinoids in Chili Pepper

The samples of chili pepper follow different methods of household handling and processing: firstly, samples were collected from the market with no special processing; secondly, samples were rinsed with running tap water for 30–45 s;

**Fig. 1** HPLC separation chromatogram as *I* is capsaicin, and *II* is dihydrocapsaicin

thirdly, samples were boiled at 100 °C for 30 min in tap water; and finally, samples were soaked in 5% solution of

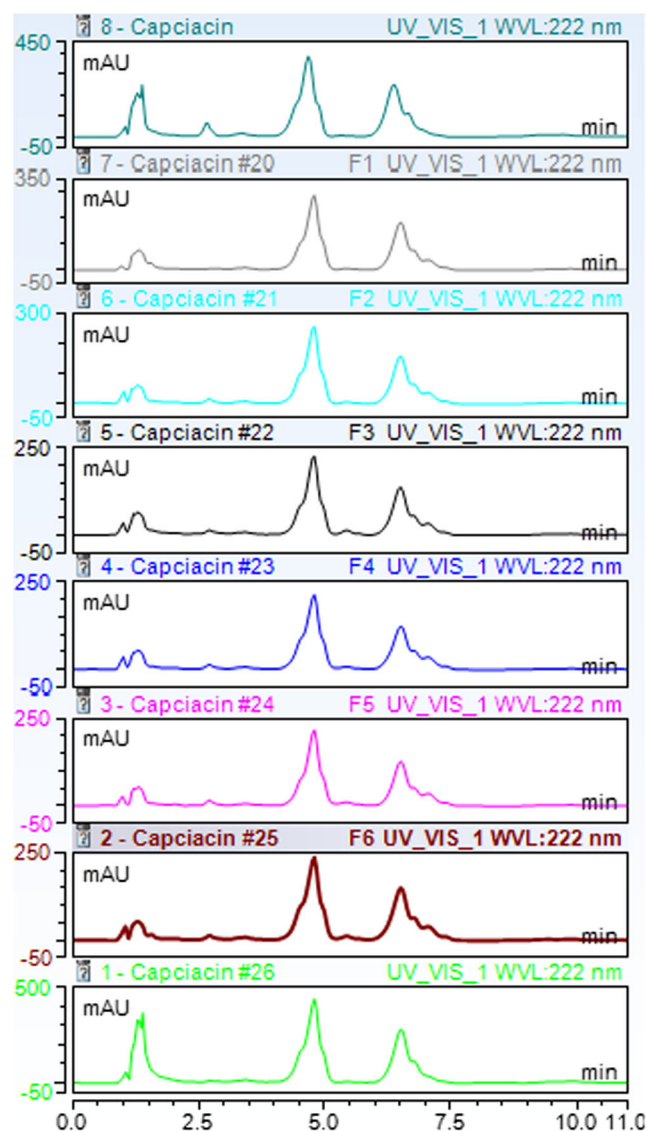
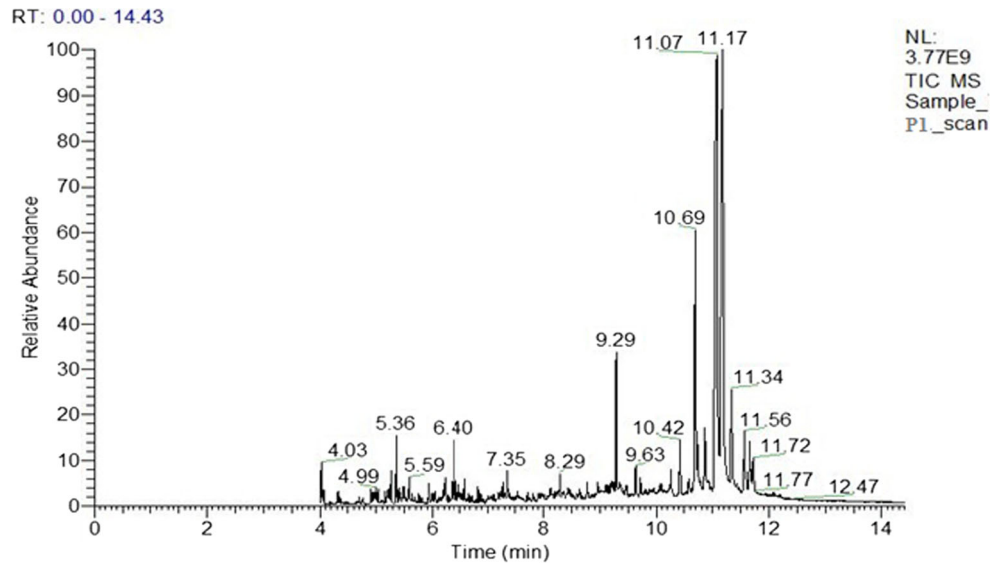
**Fig. 2** HPLC separation chromatogram overlay of the fresh and treated samples

Fig. 3 GC-MS/MS full scan chromatogram of the fresh sample

commercial acetic acid where 750 mL of tap water and 25 mL of commercial acetic acid (5% solution of an aqueous acetic acid) were used for 150 g of pepper and 2% sodium chloride aqueous solution (where 15 g of sodium chloride were dissolved in 750 mL of tap water for 150 g of pepper).

GC-MS/MS

The Thermo Scientific™ TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS pesticide analyzer system was utilized for all chemical quantitative and qualitative analysis in this study. The system consisted of a Thermo Scientific TRACE GC 1300 gas chromatograph (SN no.: 713001292) equipped with an auto sampler (AL 1310, Thermo Scientific, USA), temperature programmable injection system, and the TSQ™ 8000 Triple Quadrupole GC-

MS/MS detection system equipped with a TG-5MS capillary column (30 m length, 0.25 mm internal diameter, thickness 0.25 μm , Thermo Scientific, USA) (part no.: 26098–1420, SN: 1215669). The oven temperature was programmed from 80 $^{\circ}\text{C}$ and held for 1.5 min then raised to 210 $^{\circ}\text{C}$ at the ramp of 30 $^{\circ}\text{C}/\text{min}$, and then afterwards, raised to 300 $^{\circ}\text{C}$ and held for 4 min. The injector and ion source temperatures were both set to 250 $^{\circ}\text{C}$. The detector voltage was 70 eV, and the MS spectra were scanned in the mass range of m/z 45–600 as recommended by the manufacturer (Thermo Scientific, USA). It is worth mentioning that organophosphorus pesticides are always of low molecular weight. Helium was used as the carrier gas (99.9999% purity), and the column flow rate was 1.0 mL/min. One microliter of the sample was injected into the capillary column with a splitless Surge mood. Surge pressure was 200.0 kPa. The TSQ 8000

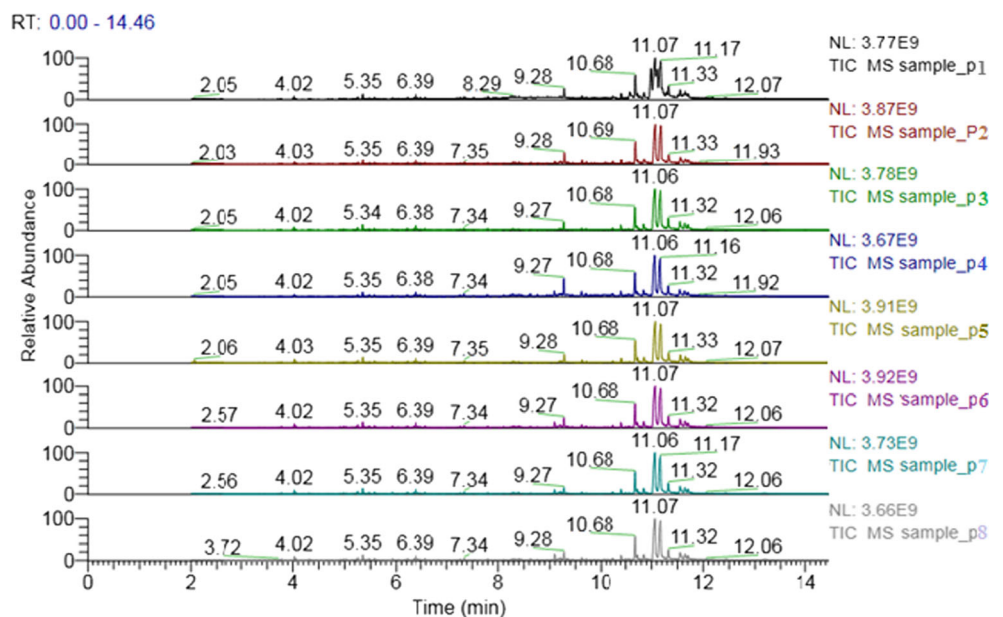
Fig. 4 GC-MS/MS separation chromatograms overlay of the fresh and treated samples

Table 3 Data from the full scan of the ingredients of the fresh samples

No.	Component	Retention time	Molecular formula	Molecular weight
1	Capsaicin	11.07	C ₁₈ H ₂₇ NO ₃	305
2	Dihydrocapsaicin	11.17	C ₁₈ H ₂₉ NO ₃	307
3	Dihydrocapsaicin	11.34	C ₁₈ H ₂₉ NO ₃	307
4	Capsaicin	11.56	C ₁₈ H ₂₇ NO ₃	305
5	Dihydrocapsaicin	11.66	C ₁₈ H ₂₉ NO ₃	307

system automatically optimized acquisition windows and set instrument duty cycle using timed-selected reaction monitoring (t-SRM) for maximum sensitivity and another time for full scan mode. Identification of compounds was based on comparison of their mass spectra with those indexed by The National Institute of Standards and Technology, USA (NIST/EDA/NIH Mass Spectral Library - NIST MS Search Program v2.0g 2011). Xcalibur™ v2.2 SP1.48 software was used for data acquisition. The results are means of three replicates. Measurements were done against pesticides standard solutions.

HPLC

HPLC analysis was carried out using Thermo Ultimax 3000 HPLC system with a MICROLITER™ syringe for manual injection (100 µL, HAMMILITON™ #710, USA) plus DIONEX Ultimate 3000 quaternary pump and a DIONEX Ultimate 3000 Diode array detector. The chromatographic conditions were as follows:

- Betasil C18 column (length 250 mm, internal diameter 4.6 mm, particle size 5 µm, pore size (A) 150, part number: 7150-254630, S.N: 12173941V1)
- Column temperature was kept at 60 °C, sample temperature 20 °C, and sample volume 20 µL.
- UV detection wavelength was adjusted at 222 nm.
- Mobile phase: binary mixture water/acetonitrile at a 50:50 ratio and flow rate of 2 mL/min as the run time for the complete separation is 7.30 min.
- Chromeleon 7.0 software was used for data acquisition.

Table 4 GC-MS/MS quantified percentage and ratios for identified components (capsaicin and dihydrocapsaicin)

Samples	Component		
	Capsaicin (%)	Dihydrocapsaicin (%)	(Capsaicin/dihydrocapsaicin)
Fresh pepper sample	26.93	17.50	1.54
Dried pepper sample	18.90	14.64	1.29
Soaked in acetic acid for 15 min	18.60	13.70	1.36
Soaked in acetic acid for 30 min	17.70	12.77	1.39
Washed with running tap water for 40 s	16.45	12.00	1.37
Boiled in water for 30 min	16.80	11.40	1.47
Soaked in NaCl for 15 min	17.90	12.97	1.38
Soaked in NaCl for 30 min	23.20	17.83	1.30

Results and Discussion

Method of Pesticide Determination Using GC-MS/MS

Table 1 shows the parameters for the 86 selected pesticides analyzed by GC-MS/MS. Ions in *italics* are quantification ions, the others are identification ions. Selection of the precursor ion was carried out trying to choose the ion with higher *m/z* ratio (increase in selectivity) and abundance (increase in sensitivity) (Bolaños et al. 2007). Both ethion and profenofos have shown noticeably higher levels in pepper samples than those recommended by the European maximum residual limit (MRL) standard (SANCO 2015). Both ethion and profenofos were selected as representatives for pesticides to investigate the effect of household-like handling and kitchen-type processing of green chili pepper samples.

The Effect of Processing and Handling on the Levels of Ethion and Profenofos

Samples of green chili pepper have passed through the following routes during processing and handling as they were collected from the local market with no further processing, other samples were boiled in tap water, other samples were rinsed with running tap water for 40 s, and other samples were soaked in 5% solution of commercial acetic acid and 2% sodium chloride solution. It is well known that leaching can be useful to remove the pesticides if they are soluble in water (Jaggi et al. 2000) and the loss of pesticides can be in the form of degradation, evaporation or co-distillation, etc. (Nagayama

Table 5 Comparison between (capsaicin and dihydrocapsaicin) percentages for both quantification techniques

Analytical technique	GC-MS/MS	HPLC
Samples	(Capsaicin/ dihydrocapsaicin)	(Capsaicin/ dihydrocapsaicin)
Fresh pepper sample	1.54	1.45
Dried pepper sample	1.29	1.39
Soaked in acetic acid for 15 min	1.36	1.58
Soaked in acetic acid for 30 min	1.39	1.59
Washed with running tap water for 40 s	1.37	1.55
Boiled in water for 30 min	1.47	1.58
Soaked in NaCl for 15 min	1.38	1.49
Soaked in NaCl for 30 min	1.30	1.25

1996; Sharma et al. 2005). Additionally, pesticide residues can be removed by washing or soaking with chemical or salt solutions (Gupta 2006).

In our study, we found that soaking with 2% sodium chloride solution for 15 min exhibited the best results in aspects of reducing the amount of profenofos by 60% to the initial concentration which is in a good agreement with literature (Lalitha et al. 1998). Other treatments had no significant effect on the levels of pesticides in the samples of green chili pepper, but sun drying of the samples leads to an increase in the level of profenofos by three times when compared to its level in the fresh samples.

As for ethion residues in the samples, it is found that soaking of samples with 5% solution of an aqueous acetic acid for 15 min and 2% sodium chloride solution for 15 min showed the highest reduction of levels of ethion by 50 and 60%, respectively, which is in a good agreement with literatures which assure the fact that washing with dilute chemical solutions could be useful to lower the load of contaminants from the surface of food commodities (Lalitha et al. 1998; Soliman 2001). Other treatments had no significant effect on the levels of pesticides in the samples of green chili pepper, but sun drying of the samples leads to an increase in the level of ethion by two times when compared to its level in the fresh sample.

The Effect of Processing and Handling on the Levels of Capsaicin and Dihydrocapsaicin

HPLC Separation

HPLC was performed with water-acetonitrile eluents, and the working solutions were prepared daily by an appropriate procedure for the deionized water. It is obvious that the total capsaicinoid content can be considered as the index of pungency degree so the main aim was to achieve efficient separation of composition of pepper including their main constituents such as (capsaicin and dihydrocapsaicin) before and

after the different handling and processing procedures as shown in Table 2. It is worth mentioning that the retention time of separation is 4.79 and 6.52 min for capsaicin and dihydrocapsaicin, respectively, which is in a good agreement with literature (Othman et al. 2011) as it can be seen in HPLC separation chromatograms in Figs. 1 and 2.

GC-MS/MS Separation

A full scan mood was performed on the investigated samples as can be shown in Figs. 3 and 4, respectively. Table 3 shows details on capsaicin and dihydrocapsaicin. Additionally, Table 4 shows the ratio of the concentration of capsaicin to dihydrocapsaicin.

As it can be seen in Table 5 for GC-MS/MS, the mean values for concentrations of capsaicin to dihydrocapsaicin have been with the ratio ranged from 1.29:1 to 1.54:1, respectively. On the other hand, for HPLC, the mean values for concentrations of capsaicin to dihydrocapsaicin have been with the ratio ranging from 1.25:1 to 1.59:1 respectively.

Conclusion

In an endeavor to help research in the area of minimizing the detrimental effects of pesticides residues on human health, this study has set out to apply a quick, yet efficient, analytical method for simultaneous determination of the residues of the 86 pesticides found in food commodity of Al-Qassim region, Saudi Arabia. Since these 86 pesticides are common in this area, a method as such is much needed to be able to determine whether or not their residue levels are within the internationally acceptable levels. Green chili pepper samples were collected from the local market of Al-Rass province, Al-Qassim region, and then samples were analyzed for pesticide residues using this method. The suggested method is based on QuEChERS extraction procedures based on acetonitrile and utilizing GC-MS/MS instrumental technique. The results have shown that the levels of those pesticides were within the European maximum residual limit (MRL)

(standard no. 836/2011, European Commission, 2011) except for ethion and profenofos, which exceeded the globally tolerated levels. The criteria of the method including the accuracy (average recoveries), precision (RSD_r), linear range, correlation coefficient, LOD, and LOQ have provided evidence that the method is valid for the analysis of the targeted groups of pesticides. Another goal of this study was to evaluate some household methods of handling green chili pepper and check their effectiveness in reducing the levels of the pesticide residues. It has been found that the proper handling and processing of green chili pepper samples could be highly effective to reduce the levels of ethion and profenofos in those samples, which exceeded the European MRL.

Evaluation of the effect of treatment of processing and handling on the ingredient of the samples of green chili pepper was obtained by comparing their percentage compared to the whole matrix where results show that there is a slight effect on concentration of the capsaicinoids in the whole matrix of the green chili pepper.

Further studies may be required to investigate the validity of the method with other crops grown and/or marketed in Al-Qassim region, Saudi Arabia.

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Compliance with Ethical Standards

Funding Not applicable.

Conflict of Interest Adel A. Abdalla declares that he has no conflict of interest. Ahmed S. Afify declares that he has no conflict of interest. Islam E. Hasaan declares that he has no conflict of interest. Amr Mohamed declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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