Determination of the Level of Organophosphorous Pesticides in Tomato Using GC-MS/MS and the Concentration of Some Heavy Metals Using FAAS

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***Abstract*:** *This study was carried out to develop and apply a quick, yet efficient, analytical method for simultaneous determination of the residues of the 86 pesticides repeatedly detected in food commodity in Al-Rass province of Al-Qassim Region, Saudi Arabia. The suggested method is based on QuEChERS extraction procedures based on acetonitrile followed by a dispersive solid-phase extraction (d-SPE) with primary–secondary amine (PSA) and bulk of carbograph for clean-up, was applied prior to GC-MS/MS analysis, focusing in particular on tomato crops. The second aim of the study was to measure the levels of some heavy metals using FAAS technique, particularly, Cu, Cd, Zn, Fe and Pb. The third aim was to evaluate the effectiveness of some common household-type preparations and handling procedures for tomato on reducing the levels of the detected pesticide residues as well as the heavy metals. The current findings could provide that the proper home preparation of tomato samples, in particular, soaking in 2% sodium chloride solution and soaking in commercial 5% acetic acid has led to effective elimination of the pesticide ethion from those samples. However, results have shown that there was no significant effect on the levels of heavy metals in the investigated tomato samples after applying the common types of household treatments.*

Keywords: *FAAS; GC-MS/MS; Heavy Metals, Pesticides; Tomato.*

1. Introduction

Pesticides are commonly used in vegetation activities. Great attentions are paid by governments, health organizations, as well as many other institutions to the issue of food contamination caused by pesticides due to their dangerous impacts on public health [1]. Finding an efficient, economical and reliable method for studying these chemicals can help minimize the risks on our health [2]. To minimize the risks of these chemicals on health and environment, a maximum residual limit (MRL) for such chemicals is established by law and recommended by codex in food and feed to ensure the appropriate concentration of pesticides in the food commodity [3].A big attention has been given to organo-phosphorous pesticides as they are globally used in agricultural activities for various crops, in addition to their believed neurological impacts on humans as a result of excess exposure [4]. The "Quick, Easy, Cheap, Effective, Rugged, and Safe" (QuEChERS) method for extraction and cleanup of pesticides is chosen for rapid sample preparation [5, 6]. Ethion [(O,O,O′,O′-Tetraethyl S,S′-methylene bis(phosphorodithioate)] is a congener of the organo-phosphorous pesticides family, and it is registered to be used for many crops. Ethion is a non-systemic insecticide which is utilized to control leaf-feeding insects, scales and mites. Ethion is exerted by inhibiting an enzyme of the nervous system of the insect (Acetylcholinesterase). The boiling point of ethion is 165°C and it is insoluble in water but soluble in most organic solvents [7]. Exposure to ethion residues by ingestion of contaminated foods can cause many

harmful health issues [8]. Tomatoes (*Lycopersicon lycopersicum*) are among the most important vegetables in the world. In 2014, world production of tomatoes was about 171 million tonnes [9]. Tomato has high nutritive valuesand it can be consumed in several formseitheras fresh or processed products such as juices, ketchup, sauce, past and tomato powder [10]. Pesticides can be chemically degraded in various ways such as hydrolysis, redox reactions, and suitable temperature and pH values. These factors are greatly dependent on the chemical nature of the pesticide, type of commodity, as well as the characteristics of location. In food commodities, the degradation of pesticides can occur by cooking, boiling, soaking in salts and chemicals or steaming [11].Analyzing of the extracts of pesticides from plants can be done using liquid or gas chromatography coupled with various detectors [12]. On the other hand, plants have several essential elements for normal and healthy growth. Some elements may become of a toxic concern when ingested in high doses. Humans can be exposed to metallic elements via water or food consumption. Cadmium and lead are the highest toxic among other elements in food [13, 14] while cupper, iron and zinc are naturally present in human diet but may cause health issue under specific circumstances [15].The reasons that might cause higher levels of such elements in human diets include emission of industrial processes, the land disposal of waste and the contamination resulting from soil or untreated irrigation water [16]. Researchers are paying a lot of attentions to poisoning caused by metals in foods using the wet ashing approach (wet digestion or wet oxidation) for the determination of dissolved heavy metals in food. The concentrated nitric acid is used for decomposing the organic materials in the tissues of the plant, which is favorable for Fe, Cu and Zn due to the possibility of some other metals, such as Pb, to partially volatilize during the process of dry ashing [17]. Combination of different acids and oxidants is mostly implemented in the process of preparation prior to the elemental analysis [18]. Flame atomic absorption spectroscopy (FAAS) is an effective solution for the determination of metals in foods because of its simplicity, economic and easiness of operation [19]. Tomato samples for this study were collected from Al-Rass province in Al-Qassim region, which lies approximately at the center of the Arabian Peninsula [20].There is a group of 86 repeatedly detected pesticides in the agricultural food commodities in Al-Qassim region, Saudi Arabia. Currently, there are no published reports on the contamination of tomatoes in Al-Qassim region with those pesticides or heavy metals as the well as ways to reduce the pesticide residues level in tomato using household-type handling and processing procedures. Therefore, the present study was carried out to create, test and run a simple method for simultaneous determination of those 86 pesticide residues in agricultural products, particularly in tomato. The method is based on liquid- liquid extraction combined with QuEChERS sample preparation procedures prior to analysis using GC-MS/MS technique following. The second aim was to determine the concentration of copper, cadmium, lead, iron and zinc contents in the common agricultural products grown in Al-Rass province. The third aim was to figure out an efficient, easy and cheap method that can be adopted at home for the removal of pesticide residues, particularly ethion, in addition to other heavy metals from tomato.

2. Materials and Methods

2.1. Pesticide Residues Analysis

All the this section is fully explained in our previous research [21] starting from the preparation of the standard solutions of the 86 pesticides then, the determination of pesticides residues using GC-MS/MS following SANCO guidelines [22] and the fitness for purpose of analytical methods [23], it is worth mentioning that the preparation of the sample was including collecting of tomato from the local vegetables market of Al-Rass province, Al-Qassim region, Saudi Arabia, during the summer season in 2017, samples were cutting mixing, and homogenization, then, sample Extraction/partitioning and dispersive solid-phase extraction (SPE) cleanup was processed using an Agilent Bond Elut QuEChERS dispersive SPE, finally the extracts were capped and shaken by vortex mixer thoroughly and transferred to the GC auto-sampler for GC– MS/MS analysis using Thermo Scientific™ TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS pesticide analyzer system and the same thermal program and the TSQ 8000 system which automatically optimized acquisition windows and set instrument duty cycle using timed-selected reaction monitoring (t-SRM) for maximum sensitivity. After that, Identification of

compounds was based on comparison of their mass spectra with those recorded in the (NIST/EPA/NIH Mass Spectral Library - NIST MS Search Program v 2.0g, 2011). Xcalibur™ 2.2 SP1.48 software was used for data acquisition.

Fig. 1: Schematic diagram of the determination of pesticide residues using GC-MS/MS

2.2. Drying and Ashing of the Samples

A mass of 150-300 g of each tomato sample were homogenized thoroughly in an electrical grinder (CH550, Kenwood, China). A mass of 65-70 g of the homogenized sample were transferred into a porcelain crucible and dried-shed at 105 °C overnight by using a drying and heating oven (ED 53, BINDER, GmbH, Germany) to remove the moisture content from the samples. The dried samples were re-weighed to determine the moisture content [24].then, the dried samples were ground and homogenized into a fine powder. A mass of 5-7 g of the powder were then weighed in a porcelain crucible, then samples were thermally treated by a muffle furnace (P330, Nabertherm, GmbH ,Germany) at 550°C for 12 h with a heating/cooling ramp of 5°C/min to remove most of organic content from samples [24, 25].

2.3. Elemental Analysis of the Heavy Metals

By using the method of Wet Ashing for the the dried samples were carefully ground and 1 gm was weighed in a glass tube using an analytical balance (AE5-4C, Kern, Germany). The sample was then dissolved in 5 ml of 69% nitric acid which was transferred by single channel micropipette (CAPP, Denmark) and the sample was immersed in a sand path and heated up to 120°C overnight on a digital hotplate (SD 300, Stuart, Bibby Scientific Ltd, UK). Then, additional 5 ml of 69% nitric acid were added to the digested solution to indicate that the brownish fumes are ceased till the observation of dense white fumes. Later, A few drops of hydrogen peroxide (30% w/v) were added to the solution until it becomes clear. After that, the interior wall of the glass tube was washed and swirled with deionized water to avoid any loss of the contents. The solution was then allowed to cool down to room temperature. The solution was filtrated using a qualitative filter paper (601:2.5µm - medium speed, AHLSTROM, USA) which is commonly used in the food industry to separate foodstuff in order to avoid the interfering of particles into the solution. The filtrated solution was then transferred quantitatively to 50 mL in a glass volumetric flask (class A with PE stopper, Duran) and diluted by adding deionized water using (Purelab Flex, ELGA, Veoila Water Solution & Technologies, UK) with specific resistivity of 18 MΩ. The solution was then mixed well prior to the elemental analysis [24, 26]. After that, Samples were analyzed using flame atomic

absorption spectroscopy FAAS (iCE 3500 AA system, Thermo SCIENTIFIC, UK) with impact beads nebulizer and using hollow cathode lamb for each element: Cu, Cd, Zn, Fe and Pb. The flame type is Air/acetylene. It can be depicted in fig. 2.

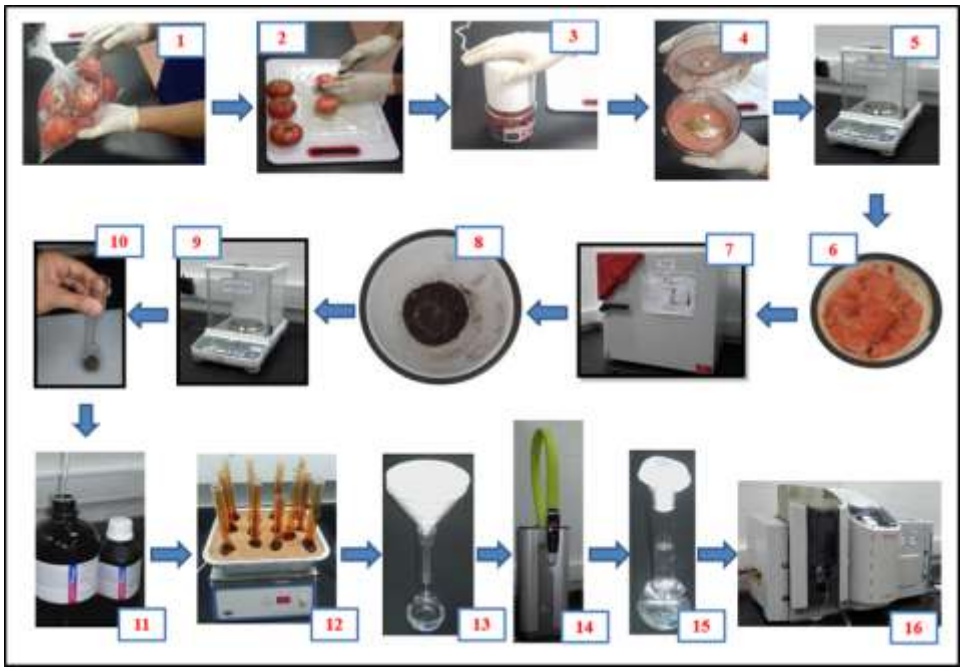

Fig. 2: Schematic diagram of the determination of heavy metals in tomato using FASS

The results are means of three replicates; nebulizer uptake time of 4 seconds and normal segmented curve method was used. SOLAAR software v11.03 was used for data acquisition. The working parameters are shown in Table I.

TABLE I: The working parameters for FAAS

Parameters	Cu	Cd	Zn	Fe	Pb
Fuel Flow	1.1	1.1	1.2	0.9	1.1
Wavelength (nm)	324.8	228.8	213.9	248.3	217
Calibration Levels	0.25, 0.50, 1.00, 2.00	0.05, 0.10, 0.20, 0.40	0.125, 0.250, 0.500, 1.0	0.50, 1.00, 2.00, 4.00	0.05, 0.10, 0.20, 0.40
Lamp Current (mA)	5	8	10	15	10
Signal (mg/A)	3.5 /0.4	1.5 /0.4	1 /0.4	5 /0.4	7 /0.4
Bandpass (width) (nm)	0.5	0.5	0.2	0.2	0.5

3. Results and Discussion

3.1. Pesticide Residues Analysis and Effect of Processing on the Level of Ethion

The parameters for the 86 pesticides analyzed by GC-MS/MS are fully illustrated in our previous research [21].The maximum residual limit (MRL) of these pesticide residues is recommended by the European Union standards [22, 24]. The samples of tomatoes have followed different methods of home preparation 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 sec, thirdly, samples were boiled in tap water and finally samples were soakedin5%solution of commercial acetic acid as well as 2% sodium chloride aqueous solution. The concentration of ethion after each process can be seen in table II. As previously mentioned in literature, the level of pesticide residues is rather higher in the exposed parts of the plant than its inner parts [27]. It is found that washing of tomato with running tap water for 30-45 sec has resulted in a significant reduction in ethion levels by about 8.7%. Reducing the levels of pesticides can also be done by kitchen-type processing procedures, such as

boiling [28], where the loss of pesticides can be in the form of degradation, evaporation or co-distillation, etc [29, 30]. In our study, a reduction by88% in the level of ethion has been detected in tomato after boiling in tap water.

TABLE II: The Levels of Ethion in Fresh Tomato and Processed Tomato Samples

No.	Description	Ethion conc. (ppb)
1	Sample from the market	195
2	washed sample	169
3	washed sample after soaking in2% sodium chloride solution for 15 min	1.73
4	washed sample after soaking in2%sodium chloride solution for 30 min	1.79
5	washed sample after soaking in5%commercial acetic acid for 15 min	10.64
6	washed sample after soaking in5%commercial acetic acid for 30 min	11.24
7	boiled sample	22

Other methods are used to remove or reduce the level of pesticide residues in food commodities such as washing with dilute chemical solutions of tomato samples under investigation were soaked in an acidified solution of tap water, 5% solution of an aqueous acetic acid. There was only a slight difference in the levels of ethion in case of soaking for 30 min instead of 15 min. The results have also shown that soaking in 2% sodium chloride solution is highly effective in reducing ethion in the samples. There was no much difference in case of soaking for 30 min instead of 15 min.

3.2 Moisture and Ash Content in Tomato

The moisture percentage in the samples was calculated according to the following equation: % moisture content $= \frac{W_1 - W_2}{W_1} \times 100$ eq. (1) where, W_1 is the initial weight of the sample before drying, and W_2 is the weight of the sample after drying. The ash content can be calculated as follow: % ash content $= \frac{W_2 - W_3}{W_1} \times 100$ eq. (2) where, W_3 is the final weight of the dried sample after ashing. Results show that, the moisture content and ash contents of original tomato and tomato samples after handling and processing is almost the same for all the samples (about 95%) and ranging from 94.83 to 95.61% (about 0.5%) ranging from 0.49-0.57% respectively.

3.3 Heavy Metals Analysis and the Effect of Processing and Handling on them

To check the quality and usefulness of the optimized method for determination of copper, cadmium, lead, iron and zinc contents in food, the accuracy of the method was evaluated by means of percentage recovery as can be seen in table III

TABLE III: FAAS Method Parameters for Determination of Heavy Metals in Tomato Samples

NO.	Laboratory Control Sample (LCS)	Recovery parameters				
		Fe	Cu	Zn	Pb	Cd
1	0.25 (ppm)	0.27	0.246	0.27	0.053/0.05	0.040 /0.05
2	0.5(ppm)	0.53	0.506	0.52	<LOD	<LOD
3	REC%	106-108%	98.4–104%	104-108%	106%	80 %
4	LOD	0.0043	0.0045	0.0033	0.013	0.0028
5	LOQ	0.013	0.014	0.0103	0.04	0.0088
6	Corr. Co-eff.	0.999	0.9999	0.996	0.999	0.996

The obtained results have shown that the levels of Cd, Cu and Pb are considered to be negligible and not detectable in all investigated samples. The low levels of Pb might be attributed to the low traffic density in the area in which tomato has grown and marketed as the main cause of Pb contamination in crops is the heavy traffic [31]. As can be seen in table IV, there was no difference in the results between the original and other treated samples which means that these specific treatment procedures have no significant effect on the levels of the heavy metals in tomato.

TABLE IV: The Levels of Heavy Metals in the Treated Samples of Tomato

No.	Samples	Cd	Pb	Cu	Zn	Fe
1	Fresh sample from the market	<LOQ	<LOQ	<LOQ	0.02	0.05
2	Washed sample	<LOQ	<LOQ	<LOQ	0.03	0.04
3	Washed sample after soaking in 2%sodium chloride solution for 15 min	<LOQ	<LOQ	<LOQ	0.04	0.03
4	Washed sample after soaking in 2% sodium chloride solution for 30 min	<LOQ	<LOQ	<LOQ	0.04	0.05
5	Washed sample after soaking in 5%commercial acetic acid for 15 min	<LOQ	<LOQ	<LOQ	0.04	0.05
6	Washed sample after soaking in 5%commercial acetic acid for 30 min	<LOQ	<LOQ	<LOQ	0.04	0.04
7	Boiled sample	<LOQ	<LOQ	<LOQ	0.03	0.04
Range		<LOQ	<LOQ	<LOQ	0.02-0.04	0.03-0.05

4. Conclusion

A quick, yet efficient, analytical method for simultaneous determination of the residues of 86 pesticides found in food commodities of Al-Rass province, Saudi Arabia has been developed. The suggested method is based on QuECherS extraction procedures based on acetonitrile and utilizing GC-MS/MS instrumental technique with a case study focusing particularly on tomato. The contents of the heavy metals copper, cadmium, lead, iron and zinc in tomato samples has also been analyzed using FAAS. The aim of this study was to evaluate the effectiveness of some household preparations and handling procedures on fresh tomato, on reducing the levels of pesticide residues. It has been found that the proper home preparation of tomato samples, in particular, soaking in2% sodium chloride solution and soaking in commercial 5% acetic acid leads to effectively reduce ethion in the investigated tomato samples. However, the results show that there is no change in the levels of heavy metals in the samples after the treatments. Further studies may be required to investigate the effect of such household-like type of treatments on different pesticide residues that might be observed in other tomato samples and samples of other crops marketed in the same area.

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