Original Article

Residue Levels and Risk Assessment of Pesticides in Pistachio Nuts in Iran

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ABSTRACT

Background: Pistachio is one of the main nutrients, not only as a strategic crop but also as a main type of nut, in Iranians' food cycle. The aim of this study was to measure the relative safety of Iranian pistachio based on the standard pesticide's residue limits, which should be monitored and assessed in the cultivation of pistachio in order to confirm its public health.

Methods: Fifty samples of pistachios of different brands were collected from Tehran markets in 2015. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) sampling method was used in order to determine the pesticide's residue in the pistachio nuts by Gas chromatography/Mass spectrometry (GC/MS).The method was validated with related parameters. Recovery took place at five concentration rates (n=3) ranging from 81.40% to 93.08% with the majority of RelativeStandard Deviation being lower than 20%. Limits of detection and quantification for all the pesticides were 2µg/kg and10µg/kg, respectively. The validated method seemed to be appropriate for the analysis of pesticide's residue in pistachio nuts.

Results: Identified pesticides included Fenitrothion, Carbaryl and Diazinon. Detectable pesticide's residue existed in 10% (5 samples) of the samples.

Conclusion: All the results were compared with the Iran's National Standards and the European Maximum Residue Limits. As compared to the acceptable daily intake, the calculated daily intake of each pesticide was much lower than the standard level, which could not cause any public health problem.

Keywords: Pesticide Residue, Pistachio, Risk Assessment, Strategic Crop.

INTRODUCTION

Due to the increasing growth of the world population and importance of health issues, demand for healthy food has increased. Pesticides are the most important chemicals consciously used by people to preserve agricultural commodity [1]. Residues of pesticides in crops may have harmful effects on consumer, classified into acute and chronic effects [2].

Thus, specification of pesticides' residue in farming crops and comparing the residues with the maximum residue limit (MRL) is necessary in the agriculture industry [3, 4]. MRL is the index for the maximum concentration of pesticides allowed in food, farming products and animal food. It also refers to the duration in which edibles may be preserved without posing a danger to human health. This is why MRL standards have been agreed on as controlling criteria of pesticides in crops. Pistachio nuts, *Pistacia vera L.*, are grown for economic purposes in countries such as Turkey and USA. The Iran is also one of the major producers and exporters of pistachio nuts in the world. Pistachio is considered as a rich source of antioxidant compounds such as phytoestrogens,

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and also as a strategic crop for export [5-7]. There is no database on the residue level of pesticides in the pistachio nuts in Iran. Although many scientists have analyzed the pesticide's residue level in various foods, the analysis of pesticides in nuts has been carried out only in few studies. Therefore, determining the pesticide's residue in this crop is essential for consumers, producers and the national supervisory authorities.

The main aim of this study was to assess the relative safety of Iranian pistachio based on the standard pesticides residue limits and the potential health risk to local inhabitants.

MATERIALS AND METHODS

Chemicals

All the organic solvents used in extraction processes were of liquid chromatography grades.The Primary Secondary Amine (PSA) was obtained from Supelco (Bellefonte, USA), and NaCl was provided from Merck (Darmstadt, Germany). Anhydrous $MgSO_4$ was purchased from SIGMA Aldrich CO (Japan). All selected pesticide standards were provided from Dr. Ehrenstorfer Co. (Augsburg, Germany).

Gas Chromatography-Mass Spectrometry (GC-MS)

An Agilent Technologies 6890 N Network GC System chromatographs (Wilmington, USA) with a SQ detector and equipped with an Agilent 7683B auto-sampler (Agilent technologies, USA) was used and an HP-5 capillary column (30 m ×0.25 mm I.D., 1 μm film thickness) was established for separation.

Helium was used as carrier gas at a constant flow of 1 ml/min. Injection port was adjusted at 250 °C and splitless mode was used. The oven temperature program for separating compounds was as follows: Initial temperature was 75 °C and remained so for 3 min; then it increased to 120 °C at 25 °C/min ramp rate and finally, increased to 300 °C at 5 °C/min ramp, holding at 300 °C for 10 min.

Sampling Procedure

Fifty samples of pistachios of different brands were collected from Tehran markets within a period from January until March 2015. The sample size for the analysis of pesticides residues in the pistachios was decided to be one Kg in accordance with the EU standard sampling rate [8].

Sample Preparation

The collected pistachio samples were analyzed to evaluate their pesticide's residue level and to prevent any loss of pesticides during blending (the samples were blended in a cryogenic state). The samples were prepared according to the following steps:

- Ten gram of homogenized pistachio sample was contaminated with the internal standard at a fixed concentration of 500 ng/g one hour before the analysis. After that, 5 ml deionized water and 12.5 ml acetonitrile were added to 10 g of milled pistachio. The mixture was then casted in a 50 ml falcon tube and blended for extraction. Then, 1 g NaCl and 2 g magnesium sulfate were added to the falcon tube, mixed for 1 min by shaker, and centrifuged at 3000 rpm, for about 5 min at -5 °C. The upper layer was moved to a 15 ml falcon tube containing 1 g magnesium sulfate and 0.1 g PSA and 0.1 g C18. The compound was mixed with vertex for 1 min and centrifuged at 3000 rpm, for about 5 min at -5

- Four milliliter of the material was poured to a vial and concentrated under nitrogen gas flow to 0.3-0.5 ml. The residue was reconstructed by toluene to 1 ml and ultimately, the vials were blended by vortex for 3 min. 2 μl of the solution was then injected into gas chromatograph.

Methods

The sample's matrix can make a change in the chromatographic response of pesticides [9-12]. These changes can be in the form of an increase or decrease in the rate of analytes recovery. Different techniques are used to decrease or eliminate the matrix effects. Accordingly, QuECHERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method, proposed by Anastassiades et al., (10) was applied to extract pesticides in a variety of foods. It involves extraction by acetonitrile and dewatering by magnesium sulfate at the presence of salt, after which the two phases of cleanup by PSA and C_{18} are carried out. In this study, a spiked calibration standard approach was employed in order to overcome matrix effects [13]. After spiking the pistachio samples using a standard mixture of 12 pesticides in different

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concentrations, a fixed concentration of internal standard (triphenyl methane) was also added and analyzed. Their standard curve was then designed using the values obtained by dividing the area under the curve of pesticides in the spiked samples by the area under the curve of internal standard. The spiked calibration curve for Propargite in pistachio samples is shown in Figure 1[13].

The study was the first attempt for monitoring of pesticides residue from different chemical groups in Pistachio nuts. The studied pesticides are fenthion, ethion, diazinon, fenitrothion, phosalone, propargite, carbaryl, cis- and trans-permethrin, alpha-endosulfan, beta endosulfan and chlorpyrifos in Iranian pistachio samples from different brands obtained during the year 2015.

The obtained curve was used to determine the concentration of the residual pesticides in unknown samples [13]. Applied method was validated with the related parameters [13]. Results of validation showed that the recovery of pesticides took place at five concentration rates (n=3) ranging from 81.40% to 93.08%. For the majority of samples, the method was repeatable with a relative standard deviation (RSD) lower than 20%. Limits of detection and quantification for all the pesticides were 2 µg/kg and 10 µg/kg, respectively. Results of validation were in accordance with the criteria set by SANCO Guideline. The validated method was suitable for the analysis of pesticides in pistachio nuts [13].

One QC sample was selected and studied on the side of the unknown samples at 250 ng/g level.

Firstly, the potential of pesticides was evaluated by comparing the chromatograms of the unknown samples with those of the spiked samples, and by identifying the type of pesticides (Figure 2). After confirming the presence of pesticides, the amount of their residue was calculated using a calibration curve.
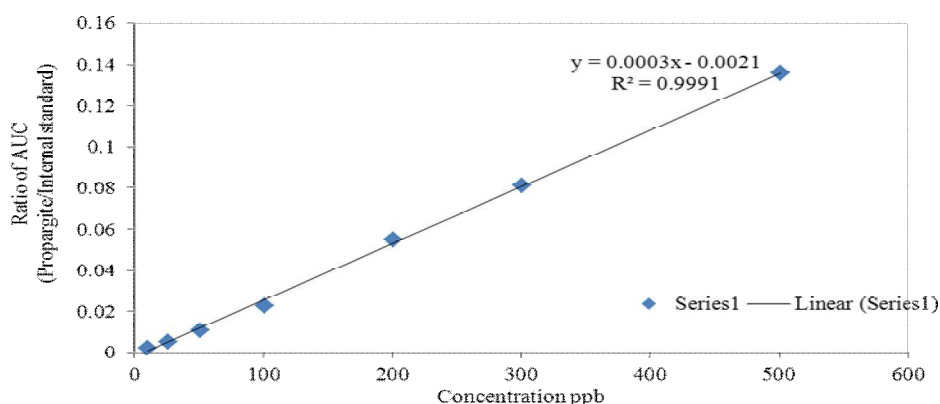

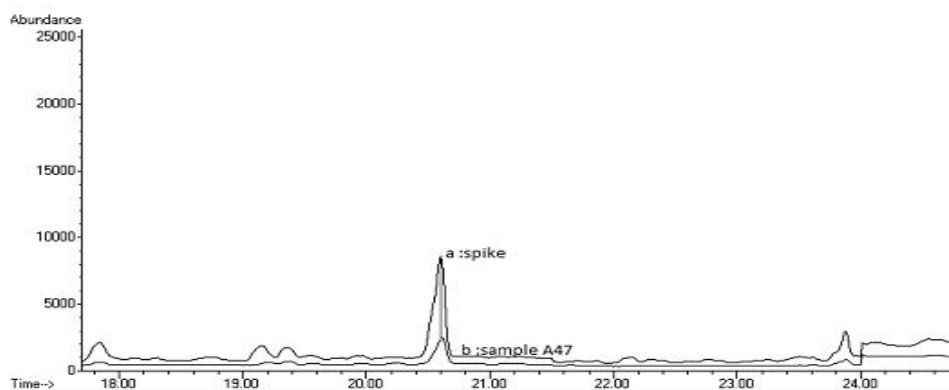**Figure 1.** Spiked calibration curve for Propargite in pistachio samples.

Figure 2. The chromatogram of (a) spiked sample of 500 ng/g with Carbaryl and (b) contaminated pistachio sample with Carbaryl.

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RESULTS

The real sample analysis indicated contamination with pesticides with different concentration values, presented in Table 1. Fenitrothion and carbaryl were detected in one sample at levels of 0.0486 mg/kg and 0.0973 mg/kg, respectively. Diazinon was detected in one sample at the level of 0.0432 mg/kg. Carbaryl was also identified in three samples at levels of 0.0780 mg/kg, 0.0610 mg/kg, and 0.0195 mg/kg. The detectable pesticide's residue was found in 10% of the samples (n=5).

Three samples had a contamination rate higher than the MRLs established by Institute of Standard and Industrial Research of Iran (ISIRI). In addition, four samples had a contamination rate higher than the MRLs defined by European Union (EU) (Table1).

The estimated daily intake of a pesticide (EDI) through food consumption was calculated using the data obtained by monitoring the samples. Measuring the residual pesticide is one way to calculate the amount of exposure to that pesticide and to help risk assessment studies. The consumption of pistachio nuts in Iran is 0.6 g per capita per day. The daily intake was estimated as mg pesticide/kg body weight per day, which is determined by the following equations. (Equation1a and Equation 1b)

-Equation 1a:

Average contamination = [1/2 LOQ× (number of non-contaminated samples) + (Total contamination of contaminated samples)]/Total number of analyzed samples

-Equation 1b:

EDI = (Average contamination × 0.6) /Mean weight of an adult

Based on the above equations, EDI of detected pesticides in the samples was determined for risk evaluation [14].

The corresponding rate of pesticide intake was compared to the acceptable daily intake value (ADI: mg/kg BW/day) of pesticides, the finding of which are reported in Table 2

Table 1. Contamination data for pesticides residue in pistachio samples marketed in Tehran, March 2015.

Sample	Pesticide	Concentration (mg/kg)	MRL (ISIRI)(mg/kg)	MRL (E.U)(mg/kg)
Sample A_{36}	Diazinon	0.0432	0.1	0.02
Sample A_{37}	Carbaryl	0.0195	0.05	0.02
Sample A_{38}	Carbaryl	0.0610	0.05	0.02
Sample A_{47}	Carbaryl	0.0780	0.05	0.02
Sample A_{49}	Fenitrothion	0.0486	0.05	0.02
Sample A_{49}	Carbaryl	0.0973	0.05	0.02

Table 2. ADI and EDI of detected pesticides by consumption of pistachios samples in adults in comparison to Hazard index.

Pesticide	ADI[1] (mg/kg BW)	EDI[2] (mg/kg BW)	Hazard index (%)[3]
Diazinon	0.002	0.000053	2.65
Carbaryl	0.008	0.000096	1.2
Fenitrothion	0.005	0.000045	0.9

1. Acceptable daily intake
2. The EDI was calculated from the average consumption per person per day and the pesticides residue data. The body weight for adults is assumed 60 kg.
3. Hazard index (%) =EDI/ADI×100

DISCUSSION

According to the results, detectable pesticide's residue was observed in 10% of the pistachio nuts' sample. In contaminated samples, the theoretical maximum daily intake and ADI had to be taken into account in order to assess the risk for the consumer. Risk assessment of pesticides was done by EDI and ADI. The information in Table 2 suggests that the calculated EDI, compared to the ADI calculated for each pesticide, is much lower than the ADI levels defined by JMPR (Joint FAO/WHO Meeting on Pesticides residue) [14-16]. The EDIs of pesticides ranged from 4.5×10^{5} to 9.6×10^{5} mg/day. The dangerous index for fenitrothion, carbaryl and diazinon were considered as 0.9%,

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1.2% and 2.65%, respectively, and the results exceeding 100% indicated a potential risk [17].

Thus, the detected levels signify that the EDI of the pesticides residue in the pistachio samples may not lead to serious public health problems. Although the results show a negligible risk associated with exposure via pistachio nuts consumption, a special precaution should be taken with the possible total exposure to these chemicals from various foods in the future.

In Nigeria, Aikpokpodion et al. showed the presence of pesticides residues (Alachlor, eendosulfan and chlordane) in kola nuts samples [18]. Dry fruit nuts have been analyzed for some organochlorine pesticide's residue (DDT, Endosulfan, HCH) in India. The results indicated very low levels of endosulfan (ND-0.091 mg/kg), HCH (0.007-1.328 mg/kg), and DDT (ND-0.140 mg/kg) in the studied samples [19]. In another study, residuals of thirty-seven pesticides were found in such agricultural products as nuts, cereals, potatoes, beans, fruits and vegetables in South Korea. In the present study, residual of pesticides were found in 23 samples (2.2%). Moreover, phosphine residuals were found in stored products such as pistachio nuts by gas chromatography and mass spectrometry. The results of the analysis showed very low levels of phosphine in the pistachio samples [20].

CONCLUSION

Although the results of the present study showed the safety of pistachio nuts, more regular and precise monitoring of pesticides is required. However, more studies on the potential risks of pesticides and their safe application are needed. The present study might be considered as a basis for the national supervisory authorities in Iran to take appropriate measures to assure that the pesticide's residuals in pistachio meets the permissible limits and,hence, is safe for consumption.

ACKNOWLEDGMENT

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