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EFFECTS OF SOME FOOD PROCESSING ON THE REMOVAL OF SOME INSECTICIDE RESIDUES FROM TOMATOES FRUITS AND POTATOES TUBERS AND EFFECTS OF THESE RESIDUES ON SOME ENZYME ACTIVITIES

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ABSTRACT

Different pesticide residues (carbofuran, carbosulfan, methomyl, malation, silycron and cypermethrin) were extracted from treated samples of tomatoes fruits and potatoes tubers before and after food processing. GLC, spectrophotometric and enzymatic methods were used to determine pesticide residues in different samples. Pesticide residues for all pesticides were detected with the previous methods with some variation in results depending on the methods sensitivity. The pesticide residues concentration in potatoes tubers were ranged between 8.74 and 7.24 ppm for cypermethrin and malathion with using spectrophotometric methods, while these residues concentrations were ranged between 8.407 and 7.79 ppm for carbofuran and malathion by using enzymatic methods. Silycron residues was 8.3 ppm with using GLC. Also the same parameters was used with tomatoes fruits. The detected concentrations for malathion was ranged between 8.22 and 0.534 ppm with used spectrophotometric methods before and after food processing. The detected pesticide residues of methomyl and malathion were used to determine their effects on some enzymes activities (in vivo).Results showed that all concentrations were affected gradually on the alanine amino transaminase (ALT), aspartate amino transaminase (AST), acid phosphatase, alkaline phosphatase and acetyle cholinestrase (AchE) activities.

INTRODUCTION

Pesticides are used in a large scale throught the world as a major mean for pest control. Althought pesticidal chemicals provide numerous benefits in terms of increased production and quality of the product, their residues in the environment are of concern.

The wide spread use of pesticides in food production has resulted in the occurance of residues in agricultural crops (vegetables and fruits) and in processed food. Waiting periods between application and havashing should be decreased to sure that, at time of markting, the residues are below the Maximum Residue Limits (MRL's). The important point should be taken in consideration to enssure that pesticide levels on the tested vegetables (Potatoes and tomatoes) are not over the established MRL's of applied insecticides after elapsing the recommended waiting period. It is also of great importance to minimyze such levels of insecticide residues remaining in crops to safety tolerence for consumers protection. (Mayburg, B.R, 1989).

Pesticide concentrations in different kinds of food production have already been reported in different part of the world (El-Shemy *et al*. 1992; El-Tantawy *et al*. 1992; Willy *et al*. 1996a and 1996b Zidan *et al*. 1991 and 1997; Ambrus 1996; Ambrus *et al*. 1996; FAO/WHO 1995 and 1996 and FAO 1997).

In Egypt a great interest was directed to study the effect of different contaminates and pesticide residues on the human health.

In this study potatoes tubers and tomatoes fruits was selected as one of the important kinds of food consumed by the Egyptian peoples. Therefor in this study we determined some detected concentrations (cypermethrin, carbofuran, carbosulfan, methomyl, malathion and silycron) in treated samples of potatoes tubers and tomatoes fruits. Also we studied the effect of detected concentration of malathion and methomyl residues on some enzymes activities (alanine amino transaminase, aspartate amino transaminase, acid phosphatase, alkaline phosphatase and acetyl cholinestrase) activities in albino rats to avoid their side effects on the consumers of such polluted diets.

MATERIALS AND METHODS

I- **Insecticides used**

Six insecticides belonging to different groups were used in this investigation as follows:

1-Carbamate insecticides

a- Carbosulfan, [2,3 - dihydro-2, 2-dimethyl (benzofaran – 7 -yl (dibutylaminothio) methyl carbamate] 925% WP and 99% a.i.)

b- Carofuran, [2,3-dihydro-2,2-dimethyl benzo-furan-7-yl methyl carbamate] (10% granules and 99% a.i.)

c- Methomyl, [-S-methyl-N-(methyl carbamoyloxy)thio-acetimidate] (90% WP and 99% a.i.

2-Pyrethroid insecticides

a-Cypermethrin, [-(RS)-α-cyano-3-phenoxybenzyl (IRS)-cis-trans-3-(2,2-dichlorovenyl)-2,2-dimethyl cyclopropane caroxylate] (30% EC and 96% a.i.)

3-Organophosphorus insecticides

a- Silycron, [O-4-bromo-2-chlorophenyl O-methyl S-propyl phosphorothioate] (72% EC. and 96% a.i.)

b- Malation [S- 1, 2-bis (ethoxycarbonyl) ethyl-O, O-dimethyl phosphorodithioate] (5% powder, 57% EC. and 96 a.i.)

II-Vegetables and fruits used

a- Potatoes tubers (*Solanum tuberosum)*

Spunta potatoes grown in North El-Tahrir were used in this study. Potatoes were manually harvest from untreated area, 16 weeks following

planting and stored at room temperature with 65% relative humidity in the dark until treated with different pesticides used.

b-Tomato fruits (*Lycopersicum esculentum*)

Fruits of Castlerock tomato grown in North El-Tahrir farm were used in this study. Fruits were collected from the untreated area, stored on refrigerator at 4 °C until treated with different pesticides used.

III-Animals

Male white mature albino rats, *Rattus norvegicus* (average weight of 200 – 220 gm each) obtained from the faculty of medicine, Tanta University were used in the current study. Animals were fed daily on the mixture of equal parts of crush wheat, flour bread and crush maize. Milk powder was mixed with water and fed one time weekly. Rats were administrated orally with the extracted pesticides from different samples of potatoes and tomatoes for one time. These groups of control rats were used, the first group received normal food, the second and the third ones administrated with non poisoned potatoes or tomatoes extract.

V-Treatment of potato tubers

Tuber of size (3.4 – 4.0 cm in diameter) was randomly selected peeled and dipped in an aqueos solution of 10 ppm. of methomyl, malathion, silycron, carbofuran, carbosulfan and cypermethrin for 30 minutes and then dried by air for 20 minutes, control tubers were dipped in water only.

VI-Treatment of tomato fruits

Tomatoes fruits were randomly selected, peeled and macerated in a high speed blender. The juice was used to prepare the spiked samples of different pesticides to form the concentration of 10 ppm. Of all tested insecticides. Spiked samples were used for analysis procedures.

VII- Cooking procedures

a - For potatoes tubers:

Two methods of cooking were used to prepare the potatos tubers samples by using the boiling water, by fryzing the tubers in corn oils.These samples were taken and were prepared for the extraction methods used.

(1) - Boiling potatoes.

Potatoes were cooked in boiling water for 30 minutes at 100 °C. After draining and cooling, the tuber samples were analyzed.

(2) - Fried potatoes.

Potatoes were cut to a small rood and friedin in a pure corn oil. For each treatment four replicates of fried potatoes.

b - For tomatoes juice

Two mode of cooking were used:

(1)- Juice was concentrated by direct heat treatment for 15 minutes to form a paste of tomatoes.

(2)- Juice was concentrated in presence of 10% of natural butter on direct heat treatment and then paste refrigerated and separated from natural butter. For each treatments four replicates were analyzed.

VIII- Extraction procedures:

a- From potatoes and tomatoes:

Pesticides in different samples of potatoes and tomatoes were extracted as follows:

- For malathion and methomyl, 50 gm of each sample were hemogenated well in high speed blender with 150 ml methylene chloride at three times. The combined homogenates were collected and shaken for 30 minutes in high speed blender then the extractes were filtrated on an anhydrous sodium sulphate layer, the combined filtrated were evaporated to dryness using a vaccum rotary evaporator. The residue was dissolved in 5 ml acetone.
- For carbosulfan, silycron, carbofuran and cypermethrin samples were extracted with 150 ml chloroform at three times using the same procedure of extraction of malathion and methomyl.

b-From waste water:

According to the method of Bayoumi *et al*., 1983, polluted water spiked samples were filtrated on a whatman glass filter G_4, 25 ml of filtrated water were extracted using 75 ml of chloroform at three times for different tested pesticides. The samples were shaken well in a separatory funnels. The organic solvent was then drained on an anhydrous sodium sulfate layer, which was washed twice with 25 ml of suitable solvent. The combineds filtrate were evaporated to dryness under vaccum in a rotary evaporator and recovered in 10 ml acetone.

c- From oil:

50 gm of oil spiked samples were extracted three times with 100 ml acetonitrile in a separatory funnel. The acetonitrile extracts were drained on an anhydrous sodium sulphate layer which was washed twice with 25 ml of acetonitrile. The combined filtrates were evaporated to dryness and the residues were quantitatively dissolved in 2.5 ml acetone.

XI-Clean up

The clean up of different pesticides used were made using Thin-layer chromatography (according to Bayoumi, 1982 and 1986) and florisil column (according to Bruce *et al.*, 1983).

XII-Determination methods

a- Gas liquid chromatography analysis

Shimad 24 chromatographic GLC – 4CM equipped with flame photometric detectors (FPD) with phosphorus filter was used to determine the organophosphorus insecticides, Malathion. The calibrated conditions of the gas chromatographic were carefully checked before injecting any samples.

The working conditions for the gas chromatographic GC-4CM were as follows:

Column: Packing OV-17, column temperature: 240 ºC, flow rate: 40 ml/min. atm., chart speed: 5 mm/min., carrier gas: N_2 with flow rate 0.8 ml/min., air flow rate: 1.0 L/min.

b- **Colorimetric determination**

The tested insecticides, carbofuran and carbosulfan were determined according to the method of Rangaswamy *et.al.,* 1976. The other insecticides, methomyl, malathion, and cypermethrin were determined according to the method of Meagher *et.al.,* 1967; Hill *et.al.*, 1967; Hodgson and Casida, 1962 and Eiduson, 1961.

c- **Determination of enzyme activities**

The activity of acetyl cholinestrase (AchE) was determined according to the method of Ellman *etal.* (1961). Phosphatases activity determined by Kits according to the method of Kind and King (1954). The method of Rietman and Frankel (1957) was used for determination serum AST and ALT activity. The method carried out by Gornall *et.al.*, (1949) was used to determine the total protein.

RESULTS AND DISSCUSION

Results represented in table (1) showed that different analytical methods used to determine the different pesticide residues (methomyl, silycron , carbosulfan, carbofuran,cypermethrin and malathion) and their residues in polluted potatoes tubers and tomatoes fruits at the level of10 ppm. From the same table it was evident that all methods were successfully used to determine a small amount of the pesticide residues in all samples; but as shown from the depected results in this table, the detected concentrations were slightly changed depending on the sensitivity of the determination methods and the pesticide groups.

Table (1): Determination of pesticide residues in spiked samples (at 10 ppm level) of potatoes tubers and tomatoes fruits using different analytical methods

Pesticide	Cypermethrin	methomyl		Carbosulfan		carbofuran		malathion			Cilycron	
Method	S	S	E	S	E	S	E	S	E	GLC	E	GLC
Potatoes	8.74	8.01	8.102	7.421	8.01	8.272	8.407	7.24	7.79	----	9.36	8.3
Tomatoes	7.55	7.28	8.22	7.68	7.68	7.28	8.76	8.22	7.84	8.86	7.26	9.4

S = Spectrophotometric methods & E = Enzymatic methods & GLC = Gas liquid chromatography

The detected concentration of malathion or silycron residues and their metabolites in treated samples [(fresh potatoes, boiled potatoes, fried potatoes, fresh tomatoes, paste of tomatoes (with and without butter), water and oil used in the potatoes preparations] using GLC were presented in table (2). From the previous table it was quite obivious that all analyzed samples

contains remarkable amounts of pesticide residues. In addition some unknown metabolites were found in the case of tomatoes paste prepared in the presence of butter, with retention time of 2.4 min that for malathion insecticide, while another unknown metabolites were found with silycron in most prepared samples after food processing with different retention times compared with the original compounds. These previous results may explain the reduction of pesticide residues in the different treated samples.

Table (2): Retention time and concentrations of malathion and silycron residues in different samples of polluted potatoes tubers and tomatoes fruits using (GLC) method

Compounds and Samples	Silycron		Malathion	
	Rt	Conc ppm	Rt	Conc ppm
Silycron	5	-----	----	----
Malathion		-----	6	-----
Treated potato	5	-----	6	0.8
Boiled potato	5	o.206	6	0.198
Waste water		0.25	-----	0.49
Fried potato	2.3, 5	-----	6	0.094
Oil of fried		0.06	-----	0.24
LSD *0.05*		0.05	-----	0.052
Tomatoes fruitts	2.0, 5	----	6	0.052
Paste	4.0, 5	0.354	6	2.529
Paste*	2,5, 5	0.13	2.4, 6	0.524
LSD 0.05		0.665	----	0.228

Also, the effects of cooking procedure on the pesticide residues of (carbofuran, carbosulfan, cypermethrin and methomyl) were also studied using spectrophotometric and enzymatic methods with all experimental samples (tables 3 and 4). Results in tables (3 and 4) showed that all tested samples contains a remarkable concentrations of previous insecticide. The detected concentrations using the spectophotometric methods were 1.35, 1.142, 0.748 and 0.727 ppm in the fried potatoes for carbofuran, carbosulfan, methomyl and cypermethrin, respectively. On the other hand the obtained results with boiling processes resulted in significant losses in the residues of all tested pesticides, that may reduce the hazard on human health, however the detected concentrations were 1.025, 1.367, 0.872 and 0.540 for carbosulfan, carbofuran, cypermethrin amd methomyl in boiled potatoes, respectively. In the case of fried potatoes the detected concentrations were 0.796, 0.509 and 0.426 ppm for crbosulfan, carbofuran and methomyl, respectively with using enzymatic methods and these residues were 1.464, 0.777 and 0.590 ppm for methomyl, carbosulfan and carbofuran, respectively in the boiled potatoes, while the detected concentrations with tomatoes paste prepared with butter using spectrophotometric methods were 1.544, 0.27, 0.474 and 0.394 ppm for cypermethrin, methomyl, carbosulfan and carbofuran, respectrively. In the case of prepared paste without butter the residues concentrations were 2.789, 0.534, 1.693 and 0.893 ppm for

cypermethrin, methomyl, carbosulfan and carbofuran. The data in table (4) also showed that the detected concentration using enzymatic methods in the case of tomatoes paste prepared with butter were, 0.027, 0.271 and 1.154 ppm for methomy, carbosulfan and carbofuran, respectively, but these concentrations in tomatoes paste prepared without butter were, 0.366, 1.221 and 2.654 ppm for the same pesticides, respectively.

The distribution of pesticide residues in different items used in food preparation (water and oils) were analyzed using the suitable method for detecting the pesticide residues (spectrophotometric methods). The detected concentrations in waste water were 3.89, 3.324, 2.264 and 1.38 ppm for cypermethrin, carbofuran, carbosulfan and methomyl, respectively, while for oil samples the detected concentrations were, 0.383,0.174,0.087and 0.056 ppm for carbosulfan, cypermethrin, carbofuran and methomyl, respectively.

Table (3): **Determination of pesticide residues in spiked samples at (10 ppm level) after food processing of potatoes tubers and tomatoes fruits using colorimetric methods**

Pesticides Treated	Methomyl (ppm)	Carbosulfan (ppm)	Carbofuran (ppm)	Cypermethrin (ppm)
Fried potato	0.727	1.142	1.35	0.727
Boiled potato	0.872	1.025	1.367	0.872
Waste water	3.89	2.264	3.324	3.89
Oil of fried	0.174	0.383	0.087	0.174
Paste	2.789	1.693	3.24	2.789
Paste*	1.544	0.474	2.297	1.544
LSD *0.05*	0.287	0.129	0.351	0.287

***Prapared in 10% natural butter**

Table (4): Determination of pesticide residues in spiked samples at (10 ppm level) after food processing of potatoes tubers and tomatoes fruits using enzymatic methods

Pesticides Treated	Methomyl (ppm)	Carbosulfan (ppm)	Carbofuran (ppm)
Fried potato	0.426	0.796	0.509
Boiled potato	1.404	0.777	0.590
Waste water	1.38	2.06	2.867
Oil of fried	0.074	0.306	0.366
Paste	0.366	1.221	2.654
Paste*	0.027	0.271	1.154
LSD *0.05*	0.290	0.348	0.531

***Prapared in 10% natural butter**

These previous findings were in agreement with different findings obtained by (El-Tantawy *at al* 1992, El-Shemy *et al* 1992, Monday *et al* 1992, Galoux *et al* 1992,Ramadan *et al* 1992, Yukari *et al* 1991 and Willy *et al* 1996a&b) who reported that food processing operation such as washing,blanching and cooking remove major portions of the pesticide residues.Also the previous

results were in agrement with that obtained by Zidan *et al.(*1997), who reported that, juice extraction by peeling followed by cooking resulted in removal of insecticidal residues showing 87.84, 81.68, 81.17, and 89.83% loss with priimiphos-methyl, malathion, fenitrothion and fenpropathrin, respectively. Combination of peeling process and without washing increased the removal efficiency of residues to 96.98, 96.07, 95.50 and 98.32% loss of pirimiphos-methyl, malathion, fenitrothion and fenpropathrin, respectively. Also our results were in harmony with those of Powell *et al* (1970), Zidan *et al.* (1991),Ramadan (1991) and Ismail *et al.* (1993).

Data in table (5) revealed that rats showed normal behaviour without any toxicity symptoms during the experimental period, except that treated with methomyl and its extracts which showed some convulsion after treatment and disappeared after one hour later. The analysis of the enzymes activities in treated rats showed that, pesticide residues extracted from fried potatoes showed in general moderate effect on alanine amino-transaminase phosphatase (AKP) and acid phosphatase (ACP) activities. These results confirms with the findings of Ambrose *et al. (1970), Tag El-Din et al. (1996), Lykasova and Rabinovich (1988) and Agrowal and Sultana (1993).* Total protein content was not affected by the pesticides and their extracts. The results showed that residues of methomyl and malathion were significantly decrease the acetylcholinestrase activity at different degree during the experimental period.

Generally, it was clearly stated that the cooking does not destroy the pesticides tested present in the potatoes tubers and / or tomatoes fruits. There was a high decrease in the concentration of the pesticides tested after cooking, generally more significant after cooking of potatoes tubers with the friying method for silycron and cypermethrin, while this reduction in the pesticide concentration were obtained with boiling process in the case of organophosphorus compound (malathion) and the carbamate compounds (methomyl, carbosulfan and carbofuran) . Simalar results were found after cooking the tomatoes juice in the presence of natural butter with all tested pesticides.So,these reduction in the pesticides concentration may reduce the hazard of these pesticides residues on human health.

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تاثير بعض العمليات الغذائية على التحلص من بعض متبقيات المبيدات فى درنات البطاطس وثمار الطماطم وتأثير هذه المتبقيات على نشاط بعض الانزيمات

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استخلصت متبقيات مبيدات (الكربوفيوران ، الكربوسلفان ، الميثوميل ، الملاثيون ، السليكرون والسيبرمثرين) من ثمار الطماطم ودرنات البطاطس قبل وبعد العمليات الغذائية (الطهى أو القلى أو السلق .....الخ) وقدرت فيها تركيزات متبقيات المبيدات السالفة الذكر باستخدام طرق التقدير الكيميائية بواسطة طرق التحليل الكروماتوجرافى (GLC) وطرق التحليل اللونى (SPECTROPHOTOMETRY) و الطرق الإنزيميه.

تم تقدير المتبقيات فكل العينات بإستخدام الطرق السابقه و كان هناك بعض الإختلافات فى النتائج المتحصل عليها تبعا لحساسية الطريقه المستخدمه فى التقدير.

تراوحت تركيزات المتبقيات فى درنات البطاطس بين ٧٤و٨ جزء/ مليون لمبيد السيبرمثرن و ٢٤و٧ جزء/ مليون لمبيد الملاثيون وذلك بإستخدام الطرق اللونيه بينما تراوحت هذه القيم بين ٤٠٧و٨جزء/ مليون لمبيد الكربوفيوران و٧٩و٧ جزء/ مليون لمبيد الملاثيون و ذلك بإستخدام الطرق الإنزيميه . و بإ ستخدام الطريقه الكروماتوجرافيه الغازيه لتقدير متبقيات مبيد السليكرون كان المتبقى هو ٣و٨ جزء/ مليون فى درنات البطاطس و ٤و٩ فى ثمار الطماطم .أما فى حالة إستخدام الطرق اللونيه لتقدير متبقيات مبيد الملاثيون فى ثمار الطماطم فقد تراوحت هذه التركيزات بين ٢٢و٨ جزء / مليون قبل إ جراء عمليات التصنيع الغذائيه و ٥٣٤و٠ جزء/ مليون بعد إ جراء عمليات التصنيع الغذائيه عليها .

إستخدمت التركيزات و التى تم تقديرها من مبيدات الميثوميل و الملاثيون لقياس تأثيراتها على فعالية بعض الإنزيمات فى الفئران البيضاء (داخليا) و قد أوضحت النتائج المتحصل عليها أن كل التركيزات المستخدمه لها تأثير على فاعلية غالبية الإنزيمات المختبره مثل الألانين أمينو ترانس أمينيز و الأسبرتيت أمينو ترانس أمينيز و الفوسفاتيز الحامضى و الفوسفاتيز القاعدى و الأستيل كولين إستريز.

Table (5): Effect of a single administration of tested insecticides in different samples on serum transaminase, posphatases , AChE activity and total protein content after 48 hours and 21 days of treatments.

Treatment*	Transaminases (U/L)				Phosphatases (U/100 ml)				AchE		Total protein	
	AST Mean ±SE		ALT Mean ±SE		Acid phos. Mean ±SE		Alk. phos. Mean ±SE		Umole/min/mg protein*10^{-3}		(g%)	
	48 hours	21 days	48 hours	21 days	48 hours	21 days	48 hours	21 days	48 hours	21 days	48 hours	21 days
Methomyl[1]	13.88±1.71	19.4±0.28	12.9±1.18	9.24±0.31	3.76±0.33	3.21±0.11	43.11±0.85	39.14±0.23	0.95±0.57	0.906±0.21	8.93±0.14	8.1±0.22
Fried(P)	39.67±0.99	22.03±0.6	23.05±0.58	17.83±0.34	29.88±0.19	13.4±0.19	27.08±1.31	40.72±0.26	1.7±0.29	1.83±0.12	9.36±0.13	7.39±0.07
paste*(T)	47.0±1.46	15.12±1.0	27.78±0.84	17.67±1.76	4.89±0.35	9.11±0.82	36.52±0.564	31.41±0.29	1.067±0.04	1.92±0.54	8.62±0.8	8.8±0.13
Malathion[2]	23.25±1.6	7.41±0.53	22.53±0.08	4.21±0.23	3.35±0.01	4.21±0.52	18.87±1.44	0.217±0.017	0.28±0.05	0.55±0.03	6.34±0.09	6.42±0.27
Fried(P)	64.78±1.02	11.5±0.12	79.49±0.93	4.35±0.46	38.09±3.43	9.42±0.71	58.34±0.05	0.16±0.07	0.97±0.5	0.663±0.24	7.39±0.08	6.9±0.11
paste • (T)	54.89±0.84	8.75±2.13	28.28±0.75	5.67±0.24	39.84±4.29	8.89 ±2.39	16.52±0.36	0.109±0.039	0.75±0.15	1.02±0.09	6.85±0.06	6.67±0.05
Control	52.42±1.7	50.24±2.1	40.3±5.94	50.2±0.17	14.32±0.2	17.62±073	72.36±2.25	69.9±0.02	2.531±0.28	2.42±0.25	8.86±0.165	5.62±0.15
Control (P) ••	53.56±0.1	51.07±1.9	37.6±5.7	49.2±0.72	18.57±0.6	18.16±0.24	70.93±0.45	71.7±0.19	2.15±0.29	2.38±0.27	10.03±0.28	6.53±0.23
Control(T)•••	46.31±3.7	49.62±0.2	51.6±8.8	52.6±0.21	31.87±0.8	19.4±0.27	71.69±0.42	70.4±0.32	3.087±0.23		9.16±0.22	6.9±0.8

*** Concentration used for 1-(fried(P)=0.748, 0.426 ,--) ,(boiling(P)=0.54,1.464,--),(paste(T)=0.534,0.366,--),(paste •(T)=0.27,0.027,--)**
2-(fried(P)=0.826,0.743,0.094 ,(boiling(P)=0.397,0.557,0.198 (paste(T)=0.893, 2.75,2.529),(paste •(T)=0.394,1.301,0.as obtained from the colorimetric, enzymatic and GLC analysis ,respectively
• in natural butter •• (P)Potatoes •••(T) Tomatoes