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EVALUATION OF CHLORPYRIFOS RESIDUES DURING PROCESSING STEPS OF CANNED TOMATO

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ABSTRACT

Along with the development of agriculture, chemical industries also entered a constant flow of increased quality and quantity of agricultural products through pesticides. Among these, Organophosphate Pesticides such as Chlorpyrifos are largely used in agriculture, especially in the cultivation of tomatoes. In this study, Chlorpyrifos residues were determined based on two treatment methods (immersion and spray), pesticide exposure time (1 and 10 minutes), and tomato washing method in the final product. Insecticide residues were quantified using thin layer chromatography. The results showed that the residue level in the samples immersed with pesticide for 10 min was higher than in both samples immersed for 1 min and in sprayed ones. No significant difference ($P<0.05$) was observed in pesticide residue at two time levels of pesticide application for the immersion method but the results have significantly different between spray and immersion techniques. In comparison to the other methods, spraying left the least amount of Chlorpyrifos residue in samples. Regarding tests to evaluate the effects of chlorinated wash water on Chlorpyrifos residue, there was an increase in pesticide residue levels in samples during this treatment.

Keywords: Canned Tomato, Chlorpyrifos, Immersion, Pesticide, Thin layer chromatography

PRACTICAL APPLICATION

Food technologists have long sought to investigate the effects of industrial processing of agricultural products on pesticide residues in food. Food processing operations such as peeling, washing, blanching; extraction and thermal processing can reduce the extent of residue in crops like tomatoes, green beans, spinach, etc. The first step toward a decline in pesticide consumption is to determine the extent of residues in various agricultural products, so that less harmful executive programs can be presented to combat pathogens and pests based on more realistic inferences and a better understanding of the role of these chemicals in agricultural cycles.

INTRODUCTION

Mankind has always required food in his endless quest for survival and this need led to the creation and development of agriculture. Together with the advancement of farming, chemical industries embarked on the course of enhanced quality and quantity of agricultural products, and accomplished this task by providing chemical tools, currently referred to as pesticides. Despite the improvement and evolution of intensive farming conferred by pesticides, direct or indirect human contact with them may cause diseases such as cutaneous conditions, respiratory problems and even death [1].

Hence, scientists and food technologists have long sought to investigate the effects of industrial processing of agricultural products on pesticide residues in food. Recent reports have demonstrated that food processing operations such as peeling, washing, blanching; extraction and thermal processing can reduce the extent of residue in products like tomatoes, broccoli, green beans, spinach, etc. This emphasizes the necessity to detect pesticide residues in agricultural products serving as raw materials in food industry, because the first step toward a decline in pesticide consumption is to determine the extent of residues in various agricultural products, so that less harmful executive programs can be presented to combat pathogens and pests based on more realistic inferences and a better understanding of the role of these chemicals in agricultural cycles [2].

Organophosphate pesticides (OPPs) are widely utilized in agricultural production. These insecticides are chemicals synthesized by the reaction of phosphoric acid with alcohols, and are selected due to their greater biodegradability and lower persistence in the environment compared to organochlorine pesticides (OCPs) [3]. OPPs can enter the body in various ways such as inhalation, ingestion and dermal absorption. These compounds can later be distributed through fat in all organs of the body, especially in the nervous system and the liver, where they act as acetyl cholinesterase inhibitors [4]. Solid forms of organophosphates are less toxic than the liquid forms. Table 1 displays the toxicity of organophosphates for mice in both solid and liquid forms [5].

Table 1: Criteria for classification of organophosphate pesticides [5]

LD_{50*} for mice (mg/kg body weight)				Toxicity of pesticide	
Skin		Oral			
Liquid	Solid	Liquid	Solid		
<40	<10	<20	<5	Extremely hazardous	I_a
40-400	10-100	20-100	5-50	Highly hazardous	I_b
400-4000	100-1000	200-2000	50-500	Moderately hazardous	II
>4000	>1000	>2000	>500	Slightly hazardous	III

*** LD_{50} is an estimated value for mg amount of the toxic substance per kg body weight to kill 50% of the test population**

Based on this classification, the toxicity of Triazophos is placed in the first, Diazinon, Fenitrothion, and chlorpyrifos in the second and Malathion in the third category (highest toxicity). Therefore, prolonged use of these pesticides produces harmful effects on human health [6]. The tomato is a plant of the *Solanaceae* family, the main consumption of which is associated with *Lycopersicone sculentum*. The fruit weighs from 38 to 250 and sometimes up to 700 g. It has a dry matter content of nearly 6%, a pH of 5.4, and a moisture content of 94%. Pigments in tomatoes are fat-soluble, and most of these pigments are carotenoids [7]. From an economic standpoint, the tomato is considered the second most important garden produce after the potato, and also stands after the potato in terms of per capita consumption [8].

Canning is a preservation process which protects food from decay, consisting of a number of operations such as washing, removal of non-edible parts, size reduction, blanching or pre-heating, filling, seaming, heat treatment, cooling and storage. The temperature applied in thermal processing depends on the food microbial population; this temperature, as well as primary processing treatments, leads to a reduction in pesticide residues. Hamilton and Crossley [2004] conducted a study on pesticide residues in some canned products [9]. The results of this research showed that transfer factor (the ratio of the amount of pesticide residues in the treated product to the original value of the same pesticide remained) of Acephate is 0.5 in canned tomato, and 4 in tomato paste, and of Tiachlorpyrifos is 1 in tomato paste.

In this paper, the effect of canned tomato processing on the reduction of Chlorpyrifos levels is studied, and finally the best treatment is recommended for pesticide reduction.

MATERIALS AND METHODS

Chlorpyrifos is the most commonly used pesticide against harmful insects and invasive species (aphids, borers, etc.) for tomato all over the world [10]. As mentioned previously, the toxicity of Chlorpyrifos is moderate, highlighting the reason for selecting this insecticide for the present work. Raw and processed tomatoes are widely consumed in every corner of the globe, hence the significance of evaluating pesticide residues in these products.

Raw materials

The Sterling variety of tomatoes were purchased from wet markets in Mashhad, and stored in refrigeration until experimentation. Standard commercial Chlorpyrifos, acetone, dichloromethane, hexane, anhydrous sodium sulfate, Fluorescein, activated carbon, silica gel powder G, aluminum oxide, cotton, silica gel aluminum plates, and all chemicals were purchased from Merck and the solvents were prepared with the highest purity.

Preparation of poison

2 in 1000 solution of chlorpyrifos for application of pesticide (spray or immersion) was prepared as follows: 30 liters of tank water was prepared, to which 66.66 ml of the commercial pesticides Chlorpyrifos 60% was added. The mixture was stirred to full miscibility.

Sample preparation

Canned tomatoes were produced in 2 pesticide application time treatments (immersion for 1 min and 10 min). In addition, the pesticide was applied in another treatment by spray method. Firstly, 30 kg of tomatoes were prepared inside the pesticide solution, and immersed for 1 and 10 minutes (in two separate processes). After the immersion time (1 or 10 min), the tomatoes were emptied from the pesticide solution into plastic baskets, and were left for an hour so that Chlorpyrifos would have the opportunity to penetrate the skin and the inner parts of the tomatoes [11, 12, 13].
Secondly, 2-4 kg of these tomatoes was selected for pesticide measurement in the samples before washing. Then the remaining tomatoes were washed in a way similar to wash basin in terms of washing time and washing with water alone or with chlorinated water, and went through the rest of the canning steps. After production, sampling of canned tomatoes was performed.

Measurement of Chlorpyrifos residue

In this study, acetone was used along with dichloromethane as extraction solvents. First, the samples were crushed and homogenized with an electric blender. 50 g of the homogenized sample was mixed with

150 ml acetone, and stirred for a few minutes in a shaker at a high speed. Afterwards, it was filtered using an erlenmeyer flask, a buchner funnel, and a vacuum trunk. The residual material on filter paper was washed with 50 ml acetone. Then obtained extract was mixed with 100, 50 and 150 ml dichloromethane in a decantation funnel with sodium sulfate 4% solution, and extraction was fulfilled. The extract was exposed to 30 g anhydrous sodium sulfate for 40 min, depending on impurities and turbidity, in order to remove its water, impurities and turbidity. The resultant extract was evaporated by vacuum evaporation until drying [14].

In order to achieve the required sensitivity in pesticide residue analysis, extracts must be purified to remove any impurities and interfering substances. For this purpose, columns with adsorbent material were utilized. Purification columns were prepared as follows: 5 g anhydrous sodium sulfate (to remove even small amounts of moisture from the sample), 5 g Aluminum oxide (as bleach), 10 g activated carbon (as bleach), 2 g Fluorescein (fat absorbent), and 5 g silica gel (desiccant) were used [15]. Finally, the pure colorless extract was dried by vacuum evaporation to a concentration of 1 ml.

Thin layer chromatography is a universal approach used for pesticide residues assessment over the years. This technique has been regarded as a qualitative and quantitative method for its considerable speed and sensitivity. It is also very simple and cost-effective compared to other methods.

In this study, chromatography was performed as follows: spots of the prepared extracts (1 ml) to 5 µl with the same amount of standard samples (standard sample was prepared with a concentration of 1000 ppm, with the addition of 10 ml acetone to 10 mg standard Chlorpyrifos) were placed at a distance of 2 cm by an auto-spotter on aluminum plates (20 × 20 cm) coated with silica gel G (the spotting procedure was completed in 3 replications). After spotting, the plates were placed inside a chromatography tank (solvent tank) at a length of 20 cm, width of 10 cm, and height of 30 cm, in a rising solvent of 80:20 hexane: acetone. After the Ascent of spots to a height of 10 cm, the plates were removed from the solvent. After drying the plate in open air, the spots were visualized in a UV chamber, where they were exposed to ultraviolet radiation at a wavelength of 254 nm for 15 to 20 min [16]. The plates were placed in a TLC scanner (CAMAG) to determine the levels of insecticide on the spots. The device was able to determine the amount of insecticides with at a ppm scale.

Insecticide values were assessed using Pro CS4.

Toxin recovery tests to determine system accuracy

Toxin recovery tests were carried out by adding certain amounts of pesticides to fruit and vegetable samples and extraction, purification and quantification by the method used, like the rest of the samples. Toxin recovery test was performed in 3 replications as follows: 1 ml commercial Chlorpyrifos 60% was poured into 50 g homogenized tomato extract. Since Chlorpyrifos has a density of 1.117 g/ml at 20°C, and the pesticides used in the toxin recovery test were commercial with a purity of 60%, the amount of active ingredient of Chlorpyrifos in 1 µl was measured to be 670.2 µg. During spotting, dried vials containing the toxin were solved with 1 ml acetone. Due to the fact that 1 ml acetone representing the toxin in 50 g tomato extract was analyzed, toxin concentration should have been 670.2 µg in 1 ml acetone. Since 10 µl of 1 ml acetone in the vial was removed for spotting, the toxin should have been measured to be 6.702, but the results showed a toxin concentration of 5.73 µg. Calculations specified that chlorpyrifos recovery rate for the method used in this study was 85%.

Statistical method

In order to assess the effect of processing on pesticide residues in tomato canning, means of values were compared by Duncan's test at a significance level of 0.05. The software used for analysis of variance and mean comparison was MSTAT C, and Microsoft Excel was used for creating charts.

RESULTS

Chlorpyrifos residues in tomatoes after pesticide application

Data in this section revealed that pesticide levels in samples immersed for 10 minutes prior to washing were higher than samples immersed for 1 minute prior to washing. In both cases, pesticide levels were higher than samples under spraying treatment prior to washing. In view of the fact that the pesticide used in industry as well as this research is usually commercial and emulsion form, it is soluble in water. Thus, it is expected that a certain extent of the pesticide, not yet absorbed by fruit tissue, dissolves in water and leads to a reduction in level of pesticide residues. Figure 1 illustrates Chlorpyrifos residues in tomato samples after washing treatment in different methods of pesticide application.

Effect of drinking water washing treatment and immersion in chlorinated water on survival of Chlorpyrifos in tomato samples

Chlorinated washing treatment was only performed for the 10-minute immersion method. Test results (Figure 2) estimated Chlorpyrifos residues for samples washed with drinking water to be about 16.6 µg/g, and 24.96 µg/g for samples immersed in chlorinated water.

The effect of peeling on Chlorpyrifos residues in tomatoes

Figure 3 shows the effect of peeling on Chlorpyrifos residues

The effect of thermal processing on Chlorpyrifos residues in canned tomatoes

Thermal processing (pasteurization) of canned tomatoes was done at 90°C for 40 min. As is evident in figure 4, the pesticide residue levels in canned tomatoes decreased. In this case, toxin residue levels were higher in samples obtained from immersion method than from spray treatment, and 10 min treatment time left more pesticides than did the 1 min treatment.

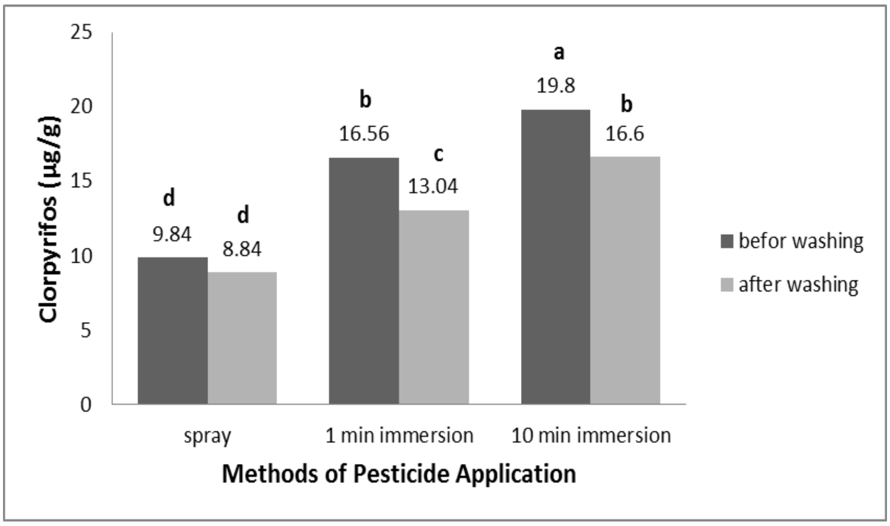

Figure 1: Chlorpyrifos residues in tomato samples after pesticide application in 2 in 1000 Chlorpyrifos solution

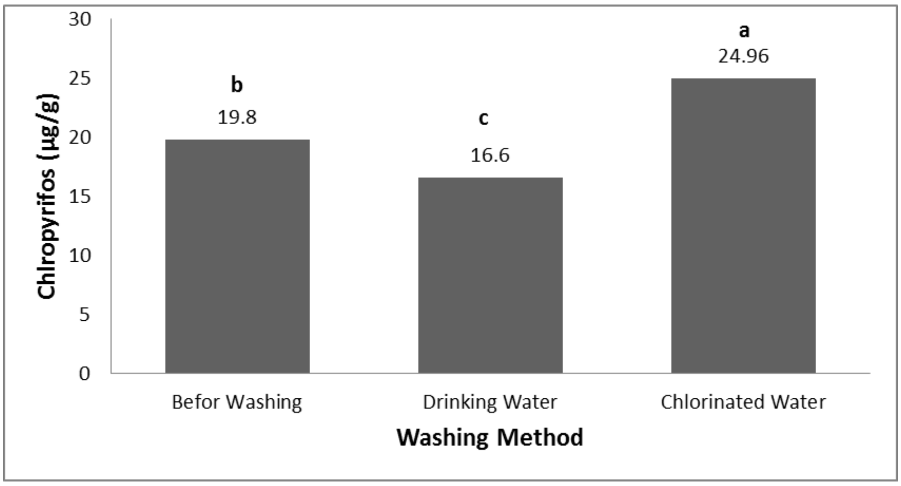

Figure 2: Effect of drinking water and chlorinated water washing treatment (2.5 mg/L) on survival of Chlorpyrifos in tomato samples in 10-minute immersion method

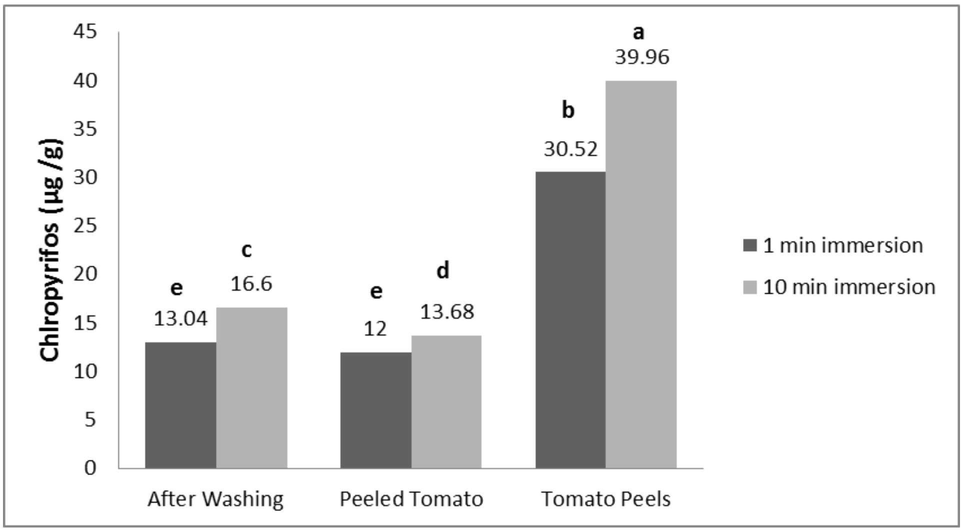

Figure 3: Effect of peeling on Chlorpyrifos residues in tomato samples

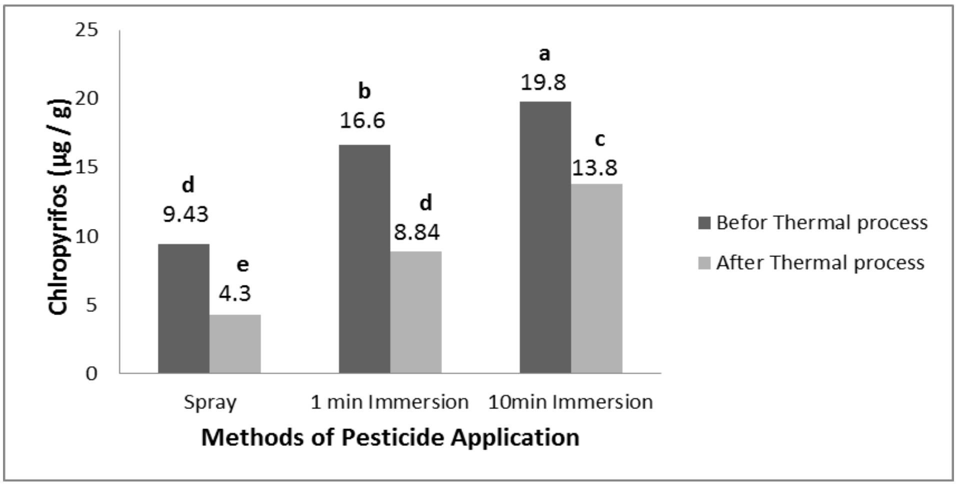

Figure 4: Effect of thermal processing on Chlorpyrifos residues in canned tomatoes in 10-minute immersion treatment

DISCUSSION

Removal of pesticides during washing treatment depends on a variety of factors such as water temperature and type of washing. Hot water washing treatment and blanching proved to be more effective than cold washing [17, 18, 19]. Systemic pesticides, compared to non-systemic ones, are removed more effectively, because non-systemic pesticides are absorbed into tissues [20, 21, 22, 23]. Kamel [2009] evaluated the stability of 10 organophosphate pesticides in chlorinated waters [24]. Their results revealed that all studied pesticides in proximity to free chloride form axons, which last for at least 72 hours in chlorinated water. In such studies, Chlorpyrifos pesticide transforms to its axon in presence of chloride.

As can be seen (figure 3), the peeling process managed to reduce Chlorpyrifos residues. This illustrates the fact that the surface effect of Chlorpyrifos on tomato skin is greater than its effect on the inner parts. Due to the lipophilicity of the skin, residual pesticides such as Parathion lipophilic, Folate 7, Captan and synthetic Pyrethroids gather in the skin, and seldom enter the extract [25, 26]. Keikotlhaile [2010] showed that organophosphate pesticides decrease further than organochlorine pesticides during the canning process [27].

CONCLUSION

The current study indicated that various processes during the production of canned tomatoes had a decreasing effect on Chlorpyrifos residue. However, the remaining residue in the final product is mostly hard to avoid. In virtue of the fact that toxins have cumulative effects in fatty tissues of living organisms, negligible amounts of toxin residues in food can lead to numerous diseases in the future, including cancer, fetal malformations, etc. Thus, controlling the amount and frequency of spraying crops and orchards with pesticides is a necessity in order to minimize the risk of hazardous chemicals entering bodies of living organisms. Therefore, farmers must obey a series of regulations and rules on application of pesticides. Moreover, this study demonstrated that Chlorpyrifos pesticide residue in samples immersed for ten minutes was higher than samples immersed for one minute, under spraying method treatment. In immersion methods, variations in the amount of pesticide residue in two levels of one and ten minutes of pesticide application showed no significant difference at the 5% level of probability. Nevertheless, variations were significantly different between spraying methods and immersion methods. Among these methods, the spraying method of pesticide application left minute amounts of Chlorpyrifos residues in samples. Tests for the assessment of wash water chlorination effects on Chlorpyrifos residues revealed that toxin residues increased during treatment with chlorinated water.

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