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Organophosphorus Pesticides Residues in Cooked *Capsicum annuum*

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Food is the basic necessity of life and food contaminated with pesticides is associated with severe effects on the human health. It is interesting to know the processing effects on pesticide residue, thus, this work aimed to evaluate the residual concentrations of organophosphorus in *Capsicum annuum* after different cooking times. The extraction of analytes was performed according to QuEChERS method and GC/FTD was used for the determination of OP pesticides. The reduction of OP concentrations demonstrated values in the range 4.8-70.4% but the most OP molecules showed 60% of reduction. The study showed the importance of the assessment of pesticide residues in plant foods thermally processed.

1. Introduction

Pesticide residues in vegetables are a major concern to consumers due to their negative health effects. The presence of their residues in foods can be a means to human exposure. It's important assuring the consumers food safety, thus the control of contaminating compounds is necessary (Jácome et al., 2014). Organophosphorus (OP) insecticides are widely used in different crops. These compounds have high toxicity and their indiscriminate and intensive use may lead residues in food and in environment (Kamanyire and Karalliedde, 2004). Toxic effects on humans are recognized as acute or chronic, the second one results from long-term exposure to low doses of a regular intake of pesticide residues in food and/or water.
Most of the commonly used organophosphate pesticides in Brazil have been included in this study based on their relevance in terms of residues found in sample and incidences of violating the maximum residue limit (MRL). The Brazilian monitoring programs for pesticide residues in food of vegetal origin shows important irregularities in many food commodities analyzed (Caldas et al., 2012).
According Consumer Expenditure Survey of Instituto Brasileiro de Geografia e Estatística (IBGE), o *Capsicum annuum* present relevant consumption data *per capita* in Brazil (IBGE, 2008). A Brazilian monitoring programs (Program on Pesticide Residue Analysis in Food - PARA), conducted by Agência Nacional de Vigilância Sanitária (ANVISA), identified intensive use of pesticides in *Capsicum annuum*, around 80% of the samples were unsatisfactory (ANVISA, 2010).
Application of heat to food commodities is commonly done through ordinary cooking, as well as others techniques of heating (Kaushik et al., 2009). Thus, this work aimed to evaluate the residual concentrations of OP in *Capsicum annuum* after different cooking times.

2. Material and methods

2.1 Materials and reagents

The solvents acetonitrile, methanol, acetone and ethyl acetate for preparation of stock solutions and extraction procedure were analytical and residue grades [Tedia Inc, Fairfield, OH, USA]. The sample preparation for

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multiresidues pesticides analysis uses the QuECheRS extractions salts anhydrous magnesium sulphate and primary second amine (PSA) [Restek Co., PA, USA]. Pesticide standards used in this study had high purity level (purity 98% and above) [Dr. Ehrenstorfer, Augsburg, Germany]. All reagents and standard were prepared using solvents with pesticide grade. The organophosphorus pesticides selected in this study were: phorate, methamidophos, parathion, pirimiphos, malathion, chlorpyrifos, terbuphos, phenthoate, ethion, triazophos and pyrazophos. The pesticides selected represent those most frequently analysed and detected in our routine analysis.The organic *Capsicum annuum* were purchased in a local market in Rio de Janeiro, Brazil.

2.2 Preparation of standards and analytical curve

Individual standard stock solutions at 10mg L^{-1} of pesticides (phorate, methamidophos, parathion, pirimiphos, malathion, chlorpyrifos, phenthoate, ethion, triazophos and pyrazophos) were prepared in 100 ml volumetric flasks with ethyl acetate. These stock solutions were divided into aliquots, sealed in ampoules and stored at -20 °C. Appropriated aliquots of all the stock solutions were diluted with ethyl acetate for the standard mix solution of 11 compounds containing 4µg mL^{-1} of each pesticide (1). This solution was used as spiking solution for recovery experiments and to prepare the standard solutions (ranging from 0,004 to 0,05 µg mL^{-1}) to obtain the calibration curves. All the standard solutions were stored at -20ºC.

2.3 Sample preparation and thermal processing

The *Capsicum annuum* were chopped using a Robot Coupe food chopper to homogenize the samples. Ten grams of homogenized sample were weighed into a 50 mL centrifuge tube and, stored at -20 ºC.
For thermal processing the samples tubes were fortified with 200µL of standard mixture solution (1) and left for twenty hours for pesticide to get in contact with the vegetable samples. Each sample was prepared in three replicates. The spiked samples were submitted of thermal processing for 0, 15, 45 and 60 minutes into a water bath at 100 °C.
For recovery studies, fresh vegetable samples were spiked with known amounts of pesticide standards in three replicates. Adequate concentrations of pesticide standards were spiked onto the samples to obtain different levels of concentrations.

2.4 Analysis of pesticides and instrumentation

The extraction was performed with organic solvents and salts ($MgSO_4$, NaCl), and clean-up sorbents used by dispersive solid phase extraction according to QuEChERS method that means ‘‘Quick Easy Cheap Effective Rugged and Safe’’. This method is a simple sample preparation methodology for pesticide multiresidues analysis that was first reported in 2003 (Anastassiades et al., 2003).
Ten mililiters of acetonitrile were added to ten grams of homogenized sample weighed in a 50 mL centrifuge tube. The samples tubes were shaken, vigorously, for 1 min, by hand, followed by vortex mixing for 1 min. Five grams of anhydrous magnesium sulphate and 1.5 g of sodium chloride were added and vortexed for 1 min. The extract was centrifuged at 5000 rpm for 5 min. An aliquot 1.5mL of the supernatant was transferred to a 2 mL tube, containing 0.2 g PSA and 3.0 g of anhydrous magnesium sulphate for clean-up. The extract was centrifuged once again at 3000 rpm for 3 min and the supernatant (purified extract) transferred to a vial for GC/FTD determination.
A Shimadzu Model 2010 GC equipped with a Flame Thermionic Detector (FTD) was used for the determination of OP pesticides. This instrument was equipped with a capillary column DB1701 (25.5 m x 0.25 mm; 0.25 µm) obtained from J & W Scientific, Folsom, California, USA. Helium was used as carrier gas at a flow of 1.1 mL min^{-1}, and the injection volume was 1.5 µL.
The injector temperature was 250°C and the analysis was carried out in splitless mode. The split time/valve position was on at the initial time, and off after 0.8 min. The oven column operated with a temperature program, starting at 80°C, followed by a 25°C min^{-1} to 130°C, 15°C min^{-1} to 210°C, 4°C min^{-1} to 270°C (held for 5 min). The total GC run time was 27.3 min. The FTD was maintained at 280°C and nitrogen was used as make-up gas at a flow of 15 mL min^{-1}. The air and hydrogen gas flows were set at 140 and 4 mL min^{-1}, respectively.

3. Results and Discussion

The analytical curve of each pesticide was constructed using seven different levels of the pesticide standard solutions. Analysis was performed in triplicates for each concentration. The method employed in this study exhibited limits of detection and quantification in the range of 0.006–0.030 and 0.021–0.102 µg. Kg^{-1}, respectively. The correlation coefficients (r^2 value) for OP pesticides were higher than 0.98 in all cases and the

relative standard deviation (RSD) values obtained ranged from 0,2 to 14% for all the pesticides investigated. Mean recoveries ranged from 70% to 101%, which is consistent with the SANCO (2012) recommendation, except for pirimiphos, which had 60% of recovery (Table 1). Based on these results, the methodology used demonstrated acceptable performance for OP analysis in *Capsicum annuum*.

PSA used in the clean up step was suitable for OP pesticides analysis using FTD detector. The colour intensity of the final extract of *C. annuum* samples was less intense than the extract before cleanup. No matrix interference peaks were observed in the chromatographic profile from FTD.

Table 1: Limit of detection, limit of quantification, correlation coefficient and recovery of OP in Capsicum annuum

Pesticide	Retention time (min)	Limit of detection (μg Kg^{-1})	Limit of quantification (μg Kg^{-1})	Coefficient of variation (R^2)	Recovery (%)
Phorate	6.64	0,009	0,031	0.9929	101,2
Methamidophos	9.57	0,030	0,102	0.9949	86,6
Parathion	10.33	0,021	0,068	0.9921	77,7
Pirimiphos	12.49	0,007	0,022	0.9977	60,0
Malathion	13.06	0,007	0,023	0.9954	76,6
Chlorpyrifos	13.27	0,008	0,026	0.9984	74,2
Terbuphos	13.60	0,022	0,074	0.9985	87,8
Phenthoate	15.15	0,008	0,027	0.9988	93,0
Ethion	18.53	0,006	0,021	0.9959	70,0
Triazophos	20.34	0,013	0,043	0.9815	74,6
Pyrazophos	25.45	0,014	0,048	0.9879	77,4

Figure 1 shows the GC/FTD analysis of the extract obtained from organic *Capsicum annuum*. It can be observed the absence of any OP molecules on its chromatographic profile. Organic samples were used in order to avoid any interference from pesticides originally present in the samples. For the evaluation of the effects of the thermal processing, the fortification of these samples were performed in concentrations close to Maximum Residues Limit (MLR) of each pesticide in this matrix. Figure 2 shows the OP pesticides analysis performed on GC/FTD system of *C. annuum* sample contaminated with standard mixture 1 containing the eleven pesticides. The OP peaks were identified by comparison of the retention times with those in the corresponding standards: 6.64 min (phorate), 9.57 min (methamidophos), 10.33 min (parathion), 12.49 min (pirimiphos), 13.06 min (malathion), 13.27 min (chlorpyrifos), 13.60 min (terbuphos), 15.15 min (phenthoate), 18.53 min (ethion), 20.34 min (triazophos) and 25.45 min for pyrazophos.

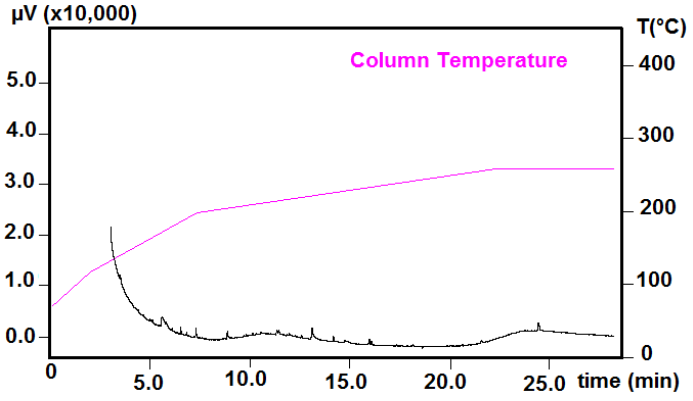

Figure 1: Chromatogram of the blank (organic sample).

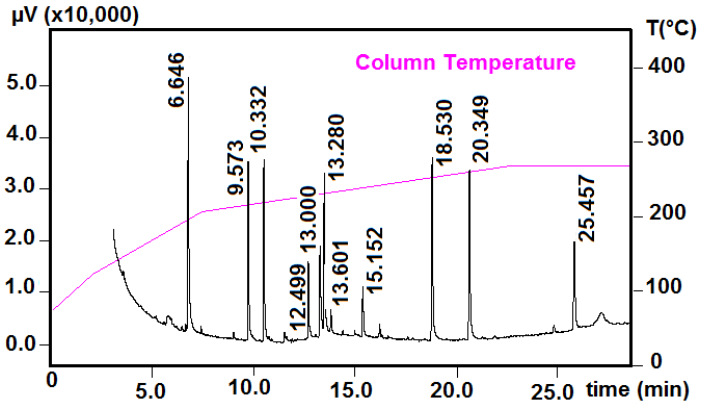

Figure 2: Chromatogram of the organophosporus studied (spiked sample).

The effects of thermal processing in the times 0, 15, 45 and 60 minutes on OP pesticides in *Capsicum annuum* are shown in Table 2. Heating had an effect in reducing methamidophos, parathion, pirimiphos, chlorpyrifos, terbuphos, phentoate, ethion, triazophos and pyrazophos. However, the concentration of phorate increased at 15, 30 and 45 minutes according to increasing time, suggesting a degradation in its metabolites or interference from matrix compounds formed during heating. The same behaviour was observed only at the last observation time of heating (60 minutes), when the increase of methamidophos, malathion, terbuphos and phentoate concentrations were remarked.

Table 2: Effect of heating on pesticide residues in Capsicum annuum.

Pesticide	Time (minutes)						
	0 (control)	15		45		60	
	concentration (µg Kg^{-1})	concentration (µg Kg^{-1})	variation* (%)	concentration (µg Kg^{-1})	variation* (%)	concentration (µg Kg^{-1})	variation* (%)
Phorate	0,043	0,049	13,9(+)	0,058	34,8(+)	0,065	51,2(+)
Methamidophos	0,081	0,044	45,7(-)	0,024	70,4(-)	0,033	59,2(-)
Parathion	0,082	0,048	41,5(-)	0,039	52,4(-)	0,050	39,0(-)
Pirimiphos	0,042	0,024	42,8(-)	0,026	38,1(-)	0,030	28,6(-)
Malathion	0,042	0,030	28,6(-)	0,036	15,3(-)	0,035	16,7(-)
Chlorpyrifos	0,041	0,026	36,6(-)	0,025	39,0(-)	0,025	39,0(-)
Terbuphos	0,086	0,053	38,4(-)	0,061	29,1(-)	0,051	40,7(-)
Phenthoate	0,042	0,037	11,9(-)	0,040	4,8(-)	0,048	14,3(+)
Ethion	0,038	0,025	34,2(-)	0,028	26,3(-)	0,032	18,8(+)
Triazophos	0,095	0,062	34,7(-)	0,066	30,5(-)	0,076	20,0(-)
Pyrazophos	0,084	0,069	17,8(-)	0,077	8,3(-)	0,096	14,3(+)

* The symbols (+) and (-) represent values that increase or decrease compared to the control.
** According ANOVA no significant difference between the treatments.

In this study, the thermal processing at 45 minutes caused the greatest loss on methamidophos concentration, while the phentoate presented the lowest loss in all times of heating.

The reduction of OP concentrations demonstrate values ranging from 4.8 to 70.4%. Most of pesticides had up to 60% reduction, not having 100% reduction. Wang et al. (2009) observed that different OP standard solutions did not show significant differences in peak areas over range of 0-70 seconds in a microwave oven. However, Liang et al. (2012) reported pesticides reduction after ultrasonic treatment, according with times of 5, 10 and 20 minutes.

Figures 3, 4 and 5 shows the gas chromatographic analysis of OP in matrix, according with heating time. We can observe a peak eluated close to the retention time 28 min that increases its concentration with the heating time.

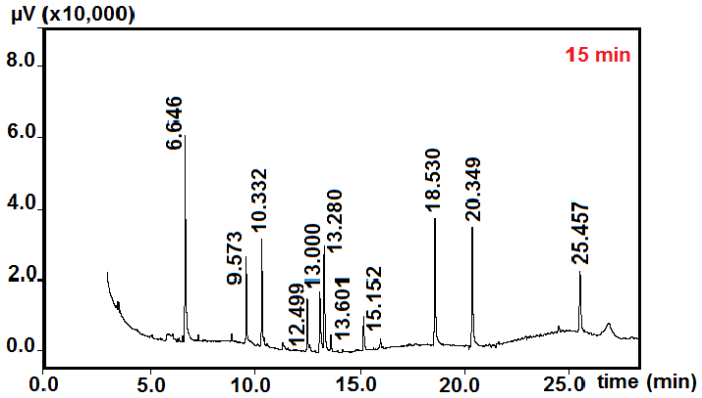

Figure 3: GC/FTD chromatogram of organophosporus after 15 minutes of heating.

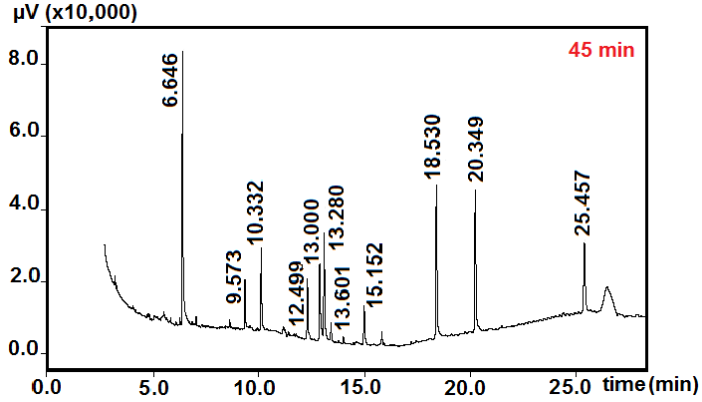

Figure 4: GC/FTD chromatogram of organophosporus after 45 minutes of heating.

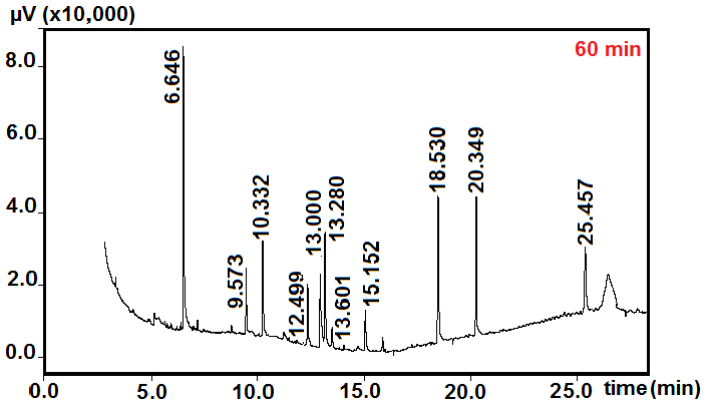

Figure 5: GC/FTD chromatogram of organophosporus after 60 minutes of heating.

4. Conclusion

QuEChERS method demonstrated be adequate for determination of 10 organophosphorus pesticides in *Capsicum annuum* samples. The clean up using PSA was effective for 10 OP molecules but it could be changed in order to optimize the pirimiphos recovery according with SANCO (2012). The OP multiresidues method using a GC/FTD system showed high sensitivity, good linearity, good selectivity, and good recoveries for almost all the pesticides evaluated.

The reduction of OP concentrations demonstrated values in the range 4.8-70.4% but the most OP molecules showed 60% of reduction. The study showed the importance of the assessment of pesticide residues in plant foods thermally processed, due to OP residues remained in the *Capsicum annuum* even after heating.

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References

Anastassiades M., Lehotay S., Stajnbaher D., Schenck F., 2003, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the Determination of Pesticide Residues in Produce, J. AOAC Int., 86, 412-431.

ANVISA (Agência Nacional de Vigilância Sanitária), 2010. Pesticides and Toxicology. Program on Pesticide Residue Analysis in Food (PARA). Available at: http://portal.anvisa.gov.br [Accessed August 05, 2014].

Caldas E., Jardim A., 2012, Brazilian monitoring programs for pesticide residues in food and results from 2001 to 2010, Food Control 25, 606-616.

IBGE (Instituto Brasileiro de Geografia e Estatística), 2009. Results and comments on the expenditure on food. Consumer Expenditure Survey, 2008-2009. Available at: http://www.ibge.gov.br [Accessed September, 2014]

Jácome S., Fonseca S., Pinheiro R., Todorov S., Noronha L., Silva J., Gomes A., Pintado M., Morais A., Teixeira P., Vaz-Velho M., 2014, Effect of Lactic Acid bacteria on quality and safety of ready-to-eat sliced cured/smoked meat products. Chemical Engineering Transactios 38, 403-408.

Kamanyire R., Karalliedde L., 2004, Organophosphate toxicity and occupational exposure, Occupational Med. 54, 69-75.

Kaushik G., Satya S., Naik S., 2009, Food processing a tool to pesticide residue dissipation – A review, Food Research International 42, 26-40.

Liang Y., Wang W., Shen Y., Liu Y., Liu XJ., 2012, Effects of home preparation on organophosphorus pesticide residues in raw cucumber, Food Chem. 133, 636-640.

Sanco, 2012, European Union Commission, Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed. Document Nº SANCO/12495/2011, 1th Jan.

Wang S., Yang S., Ren L., Qian C., Liu F., Jiang S., 2009, Determination of organophosphorus pesticides in leeks (Allium porrum L.) by GC-FPD, Chromat. 69, 79-84.