

## Influence of Heating on Residues of Organophosphates Added to Cowpea Samples

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The inappropriate use of pesticides can have a great impact on the environment and ecosystems. In human health, chronic organophosphate contamination can affect the renal, reproductive, respiratory and nervous systems. The presence of these active ingredients is more worrying considering the cumulative effect, which would be the sum of how much is consumed at each meal, and the contamination of soil, aquifers and groundwater. Mainly concern is the wide exposure subjected by rural workers and their families. The present work had as objective to identify and determine residues of ten organophosphates added to cowpea samples (*Vigna unguiculata*), and to observe the effect of the thermal treatment in these analytes. The QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) was used to the multiresidues extraction before and after heating. Gas chromatography coupled to the thermionic flame detector (FTD) was used in the identification and determination of the analytes. According to the results obtained in the work, it was possible to perceive that under the conditions in which the experiments were conducted, the organophosphates showed to be thermosensitive. Temperature and time of cooking used were under to the ones routinely used in the domestic kitchens for the preparation of beans.

Keywords: organophosphates; QuEChERS; thermosensitive

### 1. Introduction

The inadequate use of agrochemicals can have a great impact on the environment and ecosystems, contaminating groundwater, causing imbalances in the microbial populations of the soil, leading to the emergence of new parasites or inducing to the resistance of some pathogens which already existing (Schuwan-Estrada, 2002). In human health, the impact may be manifested by acute or chronic intoxication, which varies according to the chemical group of each active ingredient (AI) and their respective mechanisms of action. Acute organophosphate contamination has been shown to be extremely toxic, being lethal at low doses through different routes of exposure. In addition, chronic contamination may be associated with *diabetes mellitus* and affect the renal, reproductive, respiratory and nervous systems. Even though the concentrations of residues of these pesticides are within acceptable limits for a specific cultivate specie, the presence of these active ingredients is still worrying considering the cumulative effect by chronic exposure, which would be the sum of all the active ingredients consumed daily during each meal and the contamination of soil, aquifers and groundwater. Even more worrying is the wide exposure of rural workers and their families (Buratti et al., 2007). Suicides through pesticide self-poisoning are one-sixth to one-eighth of the world's self-murder and a third of this kind of deaths in rural Asia each year. Atropine is the main drug used forward treatment of OP poisoning with clear evidence of benefit if administered effectively. Oximes reactivate

cholinesterase enzymes and help to overcome even the nicotinic effects of OP poisoning (Bajracharya, Prasad and Ghimire, 2016).

Mostafalou & Abdollahi (2018) reviewed the epidemiological as well as experimental studies evidencing the association of exposure to OPs and incidence of neurodegenerative and neurodevelopmental disorders, including Alzheimer, Parkinson, amyotrophic lateral sclerosis (ALS), attention deficit hyperactivity disorder (ADHD) and autism. According with the authors, in addition, experimental studies have provided some evidence for involvement of cholinergic deficit, oxidative stress, neuro-inflammation, and epigenetic modifications due to the toxicity of the OP. Furthermore genetic mutations and polymorphisms of different variants of some genes like paraoxonase have been shown to be implicated in both susceptibility to OPs toxicity and neurological diseases.

Jokanovic (2018) reviewed the neurotoxic disorders appearing after acute and chronic exposure to organophosphates with emphasis on molecular mechanisms, clinical presentation, pathogenesis, and possibilities for prevention/medical treatment. Organophosphorus compounds cause four main neurotoxic effects in humans: the cholinergic syndrome, the intermediate syndrome, organophosphate-induced delayed polyneuropathy and chronic organophosphate-induced neuropsychiatric disorder. The author also approached the possible link between exposure to organophosphorus pesticides and neurodegenerative diseases as dementia, attention deficit hyperactivity disorder and Parkinson's disease in man.

The choice of the identification and quantification method for pesticides depends on the properties of the analytes. The main problems for analysis of pesticide residues are: the complexity and diversity of the matrices, and the low concentration of analytes in the samples. (Fenik et al., 2011). The QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) described by Lehotay et al. (2005) involves the liquid-liquid extraction (LLE) of the sample, followed by clean-up by extraction of the solid phase (SPE-Solid Phase Extraction). The clean-up extraction is the phase of isolation of analytes from the biological matrix, in order to remove interferents such as sugars, fats and some secondary metabolites. A salt, usually  $\text{Na}_2\text{SO}_4$ ,  $\text{MgSO}_4$  or  $\text{NaCl}$ , is added to the solution in order to increase ionic strength (Fenik et al., 2011). The identification and determination by gas chromatography (GC) can be applied to all classes of agrochemicals, since they are volatile compounds. The flame thermoionic detector (FTD), is a tool coupled to gas chromatography, with which the thermal energy is used to ionize the analyte, and the detection is made by incandescent flame. With this method, nitrogen and phosphorus molecules can be selectively detected, with a sensitivity that is approximately one hundred times greater than for carbon (Nollet, 2004).

During cooking, when subjected to substrate heating, loss of pesticide residues is expected due to its physicochemical properties, such as evaporation, co-distillation and thermal degradation, which may vary with the individual chemical nature of each active ingredient, as well as with the nature of the food matrix (Kaushik, Satya & Naik, 2009). The objective of the present work was to identify and determine residues of nine organophosphates added to cowpea samples using the QuEChERS method of multiresidue extraction and GC-FTD, and to observe the effect of the heat treatment on these analytes.

## 2. Material and Methods

The chromatographic analysis was performed on GC-2010 gas chromatography system (Shimadzu) equipped with a selective nitrogen and phosphorus detector. For the chromatographic separation, a DB5 capillary column (30 m x 0.32 mm x 0.25  $\mu\text{m}$ ), helium carrier gas, nitrogen make up gas, splitless injection (no flow division) at 250°C was used. Oven temperature was: initial temperature of 120°C followed by an increase of 70°C / min up to 182°C.

### 2.1 Identification of organophosphorus

With the identification of analyses goal, was used the method of retention time (RT) for each organophosphorus, so were prepared the standard solution of each one of organofosforados (OPs) with acetato de etila PA and injected on the GC, in the following concentrations (in  $\text{mL}^{-1}$ ): 0.051 phorate; 0.033 pirimiphos-methyl; malathion 0.0455; terbufos 0.051; chlorpyrifos 0.029; fentoate 0.031; etiona 0.049; triazophos 0.135 and pyrazophos 0.107.

### 2.2 Extraction method

The QuEChERS method of extracting pesticide multiresidues was adapted to recover the analytes added to matrix. In this study the technique involved the extraction of the sample with acetonitrile and liquid-liquid partition with 0.4 g of anhydrous sodium sulfate and 0.1 g of anhydrous sodium acetate per gram of matrix. The sample had been previously dehydrated at 40°C overnight and submitted to a laboratory mill (Perten, 3100). Extraction was performed by shaking a centrifuge tube at 5000 RPM for 20 minutes. The 1000  $\mu\text{L}$  portion of the supernatant extract was transferred to another tube containing 25 mg of a secondary primary

amine sorbent (PSA) plus 150 mg of magnesium sulfate ( $\text{Na}_2\text{SO}_4$ ) and again centrifuged at 3000 RPM (Rotations Per Minute) for 3 minutes. The extract was then transferred to flasks (vials) for GC-FTD injectors.

### 2.3 Heat treatment of cowpea

The evaluation of the heat influence on the organophosphorus added to samples was conducted through studies of addition of standard and recovery. This method of analysis was previously validated by the authors (Miranda et al., 2017). A triplicate of food matrix was doped with analytes, in the following concentrations ( $\mu\text{g}\cdot\text{mL}^{-1}$ ): 0.046 phorate; terbufos 0.045; pyrimidomethyl 0.044; chlorpyrifos 0.044; malathion 0.045; 0.045 fentoate; etiona 0.022; triazophos 0.058, and pyrazophos 0.050, and then was subjected to heating treatment in a water bath inside a 50 ml falcon tube at  $100^\circ\text{C}$  for three different time intervals 30, 60 and 90 minutes. A control aliquot was not doped and recovery of the added patterns was determined using the latter as a reference. The QuEChERS method was used for extraction. The percentages of degradation of the AIs were obtained by subtracting the concentrations found by chromatogram in each aliquot doped, by the concentrations obtained from an aliquot also doped at the same concentrations. The percentages of degradation of the AIs were evaluated using analysis of variance (ANOVA) by the software Graphpad Prism®.

### 3. Results and Discussion

The chromatograms obtained from samples of cowpea, doped and subjected to heat treatment at three different time intervals, are shown in Figure 1 below, where a represents the control sample (not doped), b, c and d express samples that were fortified and submitted to the water bath at  $100^\circ\text{C}$  for 90, 60 and 30 min., respectively and, e represents the sample that was doped but not heated (raw).

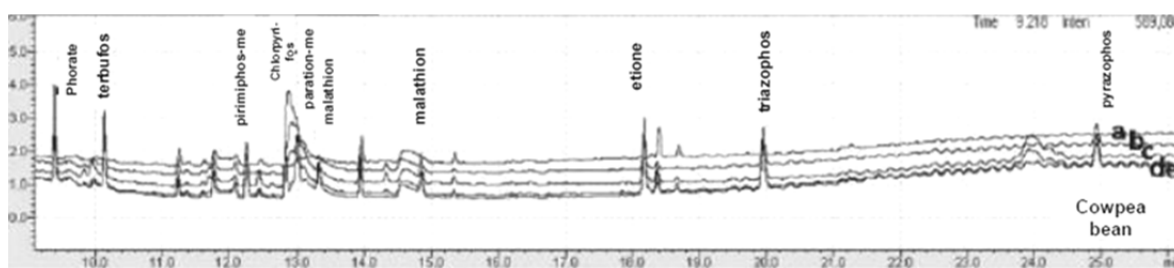


Figure 1 - Chromatograms of cowpea samples. a) control sample (not doped), b) samples that were doped and submitted to baking at  $100^\circ\text{C}$  for 90 min., c) samples that were doped and submitted to baking at  $100^\circ\text{C}$  for 60 min. and d) samples that were doped and submitted to baking at  $100^\circ\text{C}$  30 min., e) samples that were doped but not heated.

Observing the chromatograms of Figure 1 and being confirmed by Table 1 below it is possible to observe that there was decay in the recovery of the concentration of the added analytes as a function of the time of application of the heat treatment.

Table 1 - Organophosphorus residues of determined in cowpea ( $\mu\text{g}\cdot\text{mL}^{-1}$ )

AI	Cowpea <sub>Raw</sub>	Baking <sub>30min</sub>	Baking <sub>60min</sub>	Baking <sub>90min</sub>
Phorate	0.055	0.044	0.033	0.019
Terbufos	0.048	0.035	0.027	0.018
Pirimiphos-me	0.045	0.032	0.027	0.018
Chlorpyrifos	0.029	0.004	0.002	0.001
Malathion	0.021	0.014	0.009	0.007
Fentoate	0.079	0.049	0.024	0.020
Etiona	0.023	0.015	0.012	0.009
Triazophos	0.055	0.043	0.034	0.023
Pyrazophos	0.042	0.031	0.027	0.021

In almost all the analytes it was possible to observe a gradual decrease of the concentration recovery as a function of the baking time in which the sample was submitted to  $100^\circ\text{C}$ .

The Table 2 below expresses the percentage of recovery of OP obtained from the fortified samples that were not submitted to baking in water bath, in relation to the concentration of the added standards, and the percentage of decay in each time interval analyzed, in relation to the recovery of this raw sample.

Table 2- Percentage of recovery and decay of OP after heat treatment (%)

AI	Cowpea <sub>Raw</sub>	Baking <sub>30min</sub>	Baking <sub>60min</sub>	Baking <sub>90min</sub>
Phorate	119.43	80.00	60.00	34.53
Terbufos	106.59	72.92	52.25	37.50
Pirimiphos-me	101.51	71.11	71.11	40.00
Chlorpyrifos	65.48	13.79	13.79	3.44
Malathion	46.58	66.67	42.46	33.33
Fentoate	175.85	62.02	30.38	25.32
Etiona	104.31	65.22	52.17	39.13
Triazophos	94.31	78.18	61.82	41.82
Pyrazophos	83.59	73.81	64.29	50.00

Studies involving thermal treatments for the reduction or elimination of pesticides in agrochemicals food samples have been publishing since the end of the 1960s. Analyzing the dimethoate residue, added to potato puree and cabbage at the level of 2 ppm, which were submitted to cooking for 30 minutes after fortification, Askew et al. (1968) observed a percentage of decay in the range of 37-53% and 56-86% in each food, respectively.

Another old studies about the influence of heat on the degradation of pesticides added to various plant foods employ different heating operations and analysis techniques (Elkins, Farrow, & Kim, 1972; Habiba Ali & Ismail, 1992; Nakamura et al., 1993; Abou-Arab, 1999; Soliman, 2001).

Kaushik, Satya & Naik (2009) published a review on food processing operations along with the degree of residue removal. According to the authors an extensive review in the literature demonstrates that in most cases the food processing as baking, dairy manufacturing, drying, heat, fermentation, freezing, brewing, juice, malting, refrigeration, parboiling, peeling, storage, milling, washing, decoction, drying, and wine production leads to large reductions in the levels of residues in the matrices. According to the findings, washing with water and various chemical solutions for home and commercial use are necessary to reduce the intake of pesticide residues; peeling are necessary to remove the residues contained in the skins especially of fruits. Heat treatment helps to eliminate most of the residue. However, the levels of pesticides applied in post-harvest stored grains usually decrease very slowly. Thus, the removal of residues in food by processing is affected by the nature of food, type of active ingredient and kind of processing applied. Thus, a combination of techniques is best suited to eliminate as much of these harmful substances as possible.

Ling et al. (2011) also analyzed the effect of domestic processing on the removal of residues of chlorpyrifos and metabolites from different plant matrices, which were fortified at the level of 0.001 to 1.00  $\mu\text{g}\cdot\text{mL}^{-1}$ . Factors such as washing solutions, pH value, preparation operations and processing time were investigated. The studies were conducted by Gas Chromatography coupled to Triple Quadrupole Mass Spectrometry (GC/MS/MS). The washing operation decreased AI levels, and others treatments applied to food decreased further. In cabbage matrix braising, cooking and baking in microwaves reduced the waste by 93 %, 55 % and 60 %, respectively. In tomato, the reduction was 10%, 75% and 67%, respectively. In eggplant were reduced by 63%, 56%, 40%, respectively. In garlic shoots were 7.6%, 7.9%, 65.4%, respectively. The authors also observed that the metabolite 3,5,6-trichloro-2-pyridinol appears during cooking.

The main goal of Yang et al. (2012) study was to investigate the effect of washing and cooking on the levels of different pesticide residues in various food samples. Thirty-one food matrices and forty-four different pesticides were monitored using the QuEChERS method and Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS). Eight pesticides, including acetamiprid, azoxystrobin, fenobucarb, fosthiazate, iprobenfos, lufenuron, propiconazole and trifloxystrobin were detected in nine food samples, including brown rice, white rice, green pepper, ginger, butterbur, chi namul, spinach and leaf perilla. Residue levels ranged from 0.003  $\text{mg}\cdot\text{kg}^{-1}$  (trifloxystrobin) to ginger and 2.58  $\text{mg}\cdot\text{kg}^{-1}$  (azoxystrobin) in leaf perilla. The results indicated that residue levels in food products decreased substantially in the wash and baking sequence. However, the residual level of acetamiprid increased in peppers after boiling and braising.

A review about the effect of various industrial and domestic food processing on pesticides residues was published by Costa Cabrera et al. (2014). According to the authors, the heat is usually the shortest controlled parameter, due the time of reaction conditioning, the volatility of AI and the thermal degradation. These details are important for the quantitative indicators on residue levels. In addition, the reduction of agrochemicals by cooking depends on the physical-chemical characteristics of the AI.

Figueiredo et al. (2015) using CG/FTD analyzed a 4.8-70.4% reduction after bathing in a water bath for 15, 45 and 60 minutes at 100 °C of organophosphorus residues (phorate, methamidophos, parathion, pirimiphos-me, malathion, chlorpyrifos, terbufos, phentoate, etiona, triazophos and pyrazophos) added to *Capsicum annum* pepper samples. The authors observed that the highest decrease in the concentration of was to

methamidophos after 45 minutes of warming, while in the fentoate occurs the smaller loss in all the times. Most of the AI had a 60% reduction.

Eddleston & Chowdhury (2015) related that despite to be a alarming clinical and public health problem within the developing countries, responsible for at least 5 million deaths over the last three decades, the clinical care of patients with OP poisoning has little improved over the last six decades, being applied the same two antidotes: atropine and oximes. Novel antidotes such as nicotinic receptor antagonists, beta-adrenergic agonists and lipid emulsions are being studied in large animal models and in pilot clinical trials. According with the authors, it is possible that the only effective way to reduce deaths from OP poisoning will be a steady reduction in their agricultural use worldwide

According to Kaushik, Satya & Naik (2009) the advantages associated with the application of agrochemicals in increasing agricultural productivity should be balanced on the possible of health and environmental hazard. Above all, the application of such agents must comply with good agricultural practice. The authors also draw attention to a paradigm shift in world opinion from "chemical agriculture" towards "organic farming", in which a sustainable approach is intrinsic, aiming to minimize the damages caused by generalized contamination of the environment, among other advantages.

The selection of plant species tolerant to saline, water, and thermal stresses, the use of rhizobial inoculants, adoption of no-tillage, sowing green manure, and adoption of technologies to stock water to improve its efficiency and productivity. This kind of low-cost agricultural practices can contribute to build healthy and sustainable agroecosystems (Giongo et al., 2018).

The academic community has just started the research on the green pesticide selection behavior of peasants being according with the low-carbon environmental protection, which becomes the trend of this times and the living standard of consumers is constantly increasing. The reduction of the cost of green pesticides and the increase of penalties was conducive to the promotion of farmers' willingness to adopt green pesticides (Wang, 2018; Wu 2018).

#### 4. Conclusion

Using the QuEChERS method for extraction of the nine AIs and GC-FTD it was possible to perceive that the analytes were thermosensitive at the temperature and time conditions employed during this study. This information is important from the point of view of food safety, because in food preparations beans contain some substances which make it impossible to eat them raw. Thus, cooking of this food matrix decreases or eliminates not only the levels of this antinutrients as phytates, trypsin inhibitors, polyphenols and tannins, but also organophosphorus residues, which may be present.

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