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Heating, Identification, and Determination of Pesticides Residues in Black Bean Samples

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The advantages associated with pesticide application in increasing agricultural productivity must be weighed against the possible danger to health and the environment. Above all, the application of these agents must be in accordance with good agricultural practice. The presence of pesticide residues above acceptable daily intake values and maximum residue limits is of concern from a food safety point of view. The removal of food residues by processing may be affected by the nature of the food, type of active ingredient and specificity of the processing applied. Chronic organophosphate contamination can affect the renal, reproductive, respiratory and nervous systems. The present work aimed to identify and determine the residues of ten organophosphates (chlorpyrifos, etione, malathion, methamidophos, parathion-methyl, phenthoate, phorate, pirimiphos-methyl, terbuphos, pyrazophos, and triazophos) added to common bean samples (*Phaseolus* vulgaris) and to observe the effect of heat treatment on these analytes. The QuEChERS method (Quick, Easy, Cheap, Effective, Robust and Safe) was used for the extraction of multiple waste before and after heating. Gas chromatography coupled to the thermionic flame detector (FTD) was used to identify and determine the analytes. Moreover, in this study, the methodology was also validated by determining the ten organophosphates previously mentioned, and metamidophos by studies of selectivity, linearity, intermediate precision, detection limit, quantification limit and precision study by addition and recovery. According to the results obtained in the work, it was possible to notice that, under the conditions in which the experiments were conducted, the organophosphates were thermosensitive. The temperature and cooking time used were lower than traditional beans dishes. The method was selective for all analytes, as there was no interference greater than 30% of the quantification limit. The limits of detection were between 1.60 and 52.96 $ng.kg^{-1}$ and the limits of quantification were between 3.30 and 176.0 $ng.kg^{-1}$. Regarding intermediate precision, the differences in area values were ≤ 15 % at different concentrations except for phorate. The correlation coefficients (r) of the linearity curves were greater than 0.99 for all active ingredients within the applied working range. The recovery percentage was between 70 and 120 %, except phorate. Thus, the methodology was satisfactory for the identification and determination of nine of the eleven organophosphates analyzed in beans.

1. Introduction

The inadequate use of pesticides can cause a great impact on the environment and ecosystems, contaminating groundwater, soil, inducing to the resistance of some pathogens, and in human health the impact may be manifested by acute poisoning or chronic intoxication, varying 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 (Figueiredo et al., 2015). The extensive use of pesticides in agriculture is a major cause of contamination of surface and ground waters, especially due to the action of rain

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and irrigation waters that cause leaching, drainage and spreading. The active ingredients belonging to the group of organophosphates (OP) are widely used as insecticides, miticides, nematicides and fungicides, and poisoning by these products is similar to other acetylcholinesterase inhibitors (Liu et al., 2008). High performance liquid chromatography (HPLC) and gas chromatography (GC) techniques are traditionally used in pesticide residue analysis. These procedures allow the separation and quantification of various compounds with high resolution, and include the possibility of multiresidues analysis (Collins, 1997; Li et al., 2007). Legumes are a rich source of protein, carbohydrates, B-complex vitamins and minerals such as potassium, phosphorus, iron, calcium, zinc and magnesium; at relatively low costs to the consumer. The common beans (*Phaseolus vulgaris*) are one of the most consumed foods in many countries around the word, and the most legume consumed in Brazil. However, the digestibility of the grains is limited due to cell wall structure of the cotyledons to protein structure and the presence of antinutritional factors / anti-nutrients such as tannins, phytates, trypsin inhibitors and polyphenols. The latter can also cause toxicity if consumed in the raw food (Miranda et al., 2015). The objective of the present work was to identify and determine residues of nine organophosphates added to common bean variety black using the a multiresidue method of pesticides extraction was adapted from QuEChERS method (Quick, Easy, Cheap, Effective, and Safe Ruged) described by Lehotay, Maštovská and Lightfield (2005) and gas chromatography coupled to the thermionic flame detector (GC-FTD), and to observe the effect of the heat treatment on these analytes (Miranda et al., 2017). In order to identify and determine the presence of eleven organophosphorus pesticides, in black-bean samples, a multiresidue technique of extraction was applied, and the analyses were carried out by gas chromatography coupled to a thermoionic flame detector (GC-FTD). This study aimed identify and determine the residues of ten organophosphates (OP) added to common blackbean samples (*Phaseolus*), after and before a heating treatment, as well as to validate a methodology of identification and quantification of OP multiresidue added to beans samples, applying studies of selectivity, linearity, intermediate precision, limit of detection, limit of quantification, addition and recovery. This work also includes studies of selectivity, intermediate precision, limit of detection (LD), limit of quantification (LQ), and recovery. LD was obtained considering the signal-to-noise ratio of 3 and 10. LQ was calculated by multiplying LD by 3.33 (Ribani et al., 2004).

2. Methods

2.1. Analyte Preparation

Standards were purchased from Dr. EhrenstorferGmBH® (Augsburg, Germany) with the following purities: chlorpyrifos (98.5 %), etione (98.0 %), malathion (99.0 %), methamidophos (98.0 %), parathion-methyl (98.5 %), phenthoate (96.5 %), phorate (99.0 %), pirimiphos-methyl (99.5 %), terbuphos (93.0 %), pyrazophos (98.5 %), and triazophos (81.0 %). The standards solutions were prepared in ethyl acetate pesticide grade, from Tedia® (Fairfield, USA), purity: 99.9 %. The multiresidue extraction employed acetic acid HPLC grade, purity: 97.9 %; acetonitrile HPLC grade, purity: 99.9 %; all from Tedia® (Fairfield, USA); magnesium sulfate and sodium acetate ACS grade, purity: 99.0 %, from Sigma-Aldrich® (St. Gallen, Switzerland); and Bondesil® PSA 40 UM, from Agilent Technologies® (Saint Clara, USA). Purified water was from Advantage A10Millipore® system, resistivity 18.2 MΩ.cm a 25 ºC.

2.2. Detection method

For chromatographic separation a capillary column DB5 (30 m x 0.25 mm x 0.25 µm) was employed, using helium as carrier gas, nitrogen gas as make up, with splitless injection at 250 °C. The temperature program was started at 80 °C, followed by a 25 °C min^{-1} increase to 130 °C, 15 °C min^{-1} to 210 °C, and 4 °C min^{-1} to 270 °C (held for 5 min). The FTD was maintained at 280 °C and make-up gas at 15 $mL.min^{-1}$ flow. The air and hydrogen gas flows were set at 140 and 4 $mL.min^{-1}$, respectively.

2.3. Linearity

An analytical curve was constructed with triplicate injections of the standard solutions of the eleven OP (chlorpyrifos, etione, malathion, methamidophos, parathion-methyl, phenthoate, phorate, pirimiphos-methyl, terbuphos, pyrazophos, and triazophos) in eight different concentrations: 0.006 $mg.kg^{-1}$, 0.008 $mg.kg^{-1}$, 0.018 $mg.kg^{-1}$, 0.027 $mg.kg^{-1}$, 0.036 $mg.kg^{-1}$, 0.045 $mg.kg^{-1}$, 0.06 $mg.kg^{-1}$, 0.07 $mg.kg^{-1}$.

2.4 Intermediate precision

Intermediate precision was determined from the estimated relative standard deviation (RSD) of triplicate injections of three dilutions.

2.5 Addition and recovery analysis

Accuracy was evaluated by recovery studies, performed by adding standard solutions containing a pool of OP in three different dilutions a = 0.016 $mg.kg^{-1}$, b = 0.022 $mg.kg^{-1}$ and c = 0.44 $mg.kg^{-1}$. A fourth aliquot was unfortified, and recovery of added standards was determined using the latter as a reference. Recovery percentages were obtained by subtracting the concentrations found in doped aliquots of samples minus the concentrations of the unfortified. The QuEChERS multiextracton method was applied, and the analyses were also performed by GC-FTD.

2.6 Heating treatment analysis

An aliquot of the matrix was added to a pool of OP standards at the following concentrations (in $\mu g.mL^{-1}$) phorate 0.046; terbuphos 0,045; pyrimiphos-methyl 0.044; chlorpyrifos 0.044; parathion methyl 0.045, malationa 0.045; 0.045 phenthoate; etione 0.022; triazophos 0.058 and pyrazophos 0.050. Due to the absence of a standard solution, metamidophos could not be included in this study. A control aliquot was not doped and the recovery of the added standards was determined using the latter as a reference. Were applied the same methods of multiextraction and analysis of the tests cited earlier. The degradation percentages of the OP were obtained by subtracting the concentrations found in each doped aliquot, by the concentrations obtained from a doped aliquot at the same concentrations, and which had been heated in a water bath in a 50 mL falcon tube. at 100 ºC for three different time intervals 30, 60 and 90 minutes, respectively. The OP degradation percentages were evaluated using analysis of variance (ANOVA) by Graphpad Prism® software, and means were compared by Tukey test at 5 % error probability.

3. Results and discussion

3.1 Heating treatment

Chromatograms obtained from black bean samples, fortified and subjected to heat treatment at three different time intervals, are expressed in Figure 1 below, where a represents the control (non-fortified) sample, b, c and d express samples which were fortified and submitted to the water bath at 100 °C for 90, 60 and 30 min respectively, and represents the sample which was fortified and extracted without being heated.

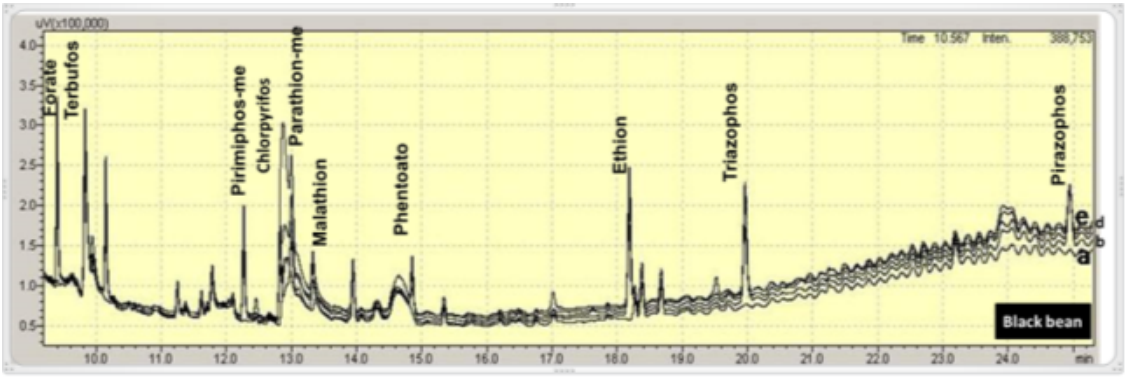

Figure 1: Chromatograms of bean samples. a) control sample, b), c) and d) samples that were fortified and submitted to baking at 100 ^{o}C for 90, 60 and 30 min, respectively, e) unheated fortified sample.

It was possible to observe a gradual decrease of the recovery of the concentration as a function of the time in which the sample was submitted to 100 ^{o}C.

3.2 Method validation

In Figure 2 below, the plotted chromatograms of the eight OP standard injections at different concentrations are observed. Where A corresponds to the concentration of analytes at point 0, B corresponds to concentration at point 1, C corresponds to point 2, D to 3, and point 4, F corresponds to point 5, G corresponds to 6 and H to point 7.

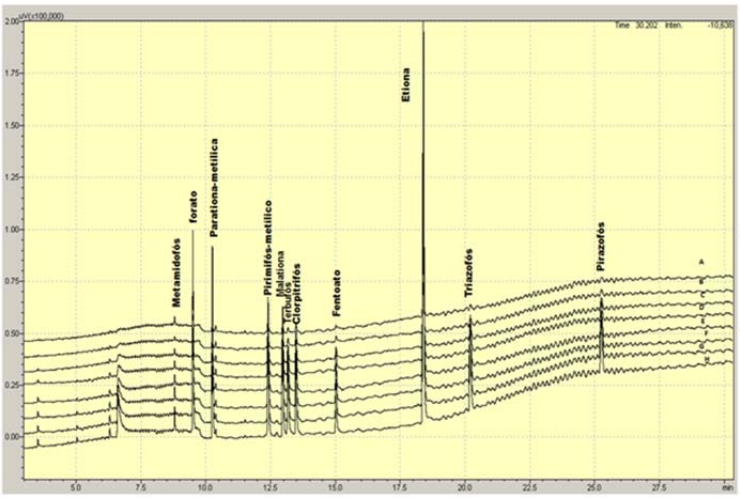

Figure 2: Chromatograms of OP standard solutions at eight different concentrations.

According to Figure 2, was possible to observe the retention time of each analyte, as well as the increasing response of the areas obtained at each time corresponding to the dilutions of the added standards. To forato and terbuphos it was possible to build the curve with respective values of r = 0.9969 and r = 0.9916, by injecting standards at six different dilutions. For the other organophosphates it was possible to obtain a value of r close to 1.0 with the injection of eight different dilutions.
Table 1 below shows the intermediate precision results according to their RSD (%) for the eleven organophosphates analyzed at three different concentrations and triplicate injections.

Table 1: Intermediate precision in three different concentrations and RSD

	Concentration (mg Kg^{-1})			RSD (%)		
Organofosforados	a	b	c	a	b	C
Metamidophos	0.017	0.026	0.043	15.47	16.73	7.65
Phorate	0.017	0.025	0.042	7.51	15.42	10.47
Parathion-me	0.017	0.025	0.042	7.35	15.16	11.43
Pyrimiphos-me	0.017	0.026	0.044	9.90	13.94	7.38
Malathion	0.017	0.026	0.043	8.64	11.15	4.71
Chlorpyriphos	0.018	0.027	0.046	11.52	12.67	7.96
Terbuphos	0.018	0.027	0.045	4.25	13.01	13.33
Phenthoate	0.017	0.026	0.044	19.29	11.09	21.56
Etione	0.020	0.029	0.049	9.07	8.20	4.50
Triazophos	0.019	0.029	0.049	11.67	8.26	10.82
Pyrazophos	0.018	0.027	0.045	20.50	14.08	10.05

The results obtained are within the limit accepted by the chromatographic method validation guide, for area values ≤ 15 %, with exceptions: phenthoate at concentrations of 0.017 and 0.044 $\mu g.mL^{-1}$; pyrazophos at a concentration of 0.018 $\mu g.mL^{-1}$ and metamidophos at a concentration of 0.026 $\mu g.mL^{-1}$. Gobo et al. (2004) using GC-FTD had obtained intermediate precision with satisfactory results for organophosphates, except for metamidophos.
Regarding sensitivity, there were no intrinsic interferences from black bean samples in retention times close to those of the analytes, according Table 2.
As observed in Table 2, the recovery percentage of methamidophos, chlorpyrifos, etione and pyrazophos is inside the limits recognized by the Brazilian laws that varied from 80 to 120 % with all three dilution of standard solutions of OP added to the common beans samples. Parathion-methyl, phenthoate and triazophos had good recoveries only with the addition of 0.016 $mg.kg^{-1}$. Pirimiphos-methyl and malathion had good recoveries for addition of 0.022 $mg.kg^{-1}$. Terbuphos had acceptable recovery at 0.016 and 0.044 $mg.kg^{-1}$. Gobo et al. (2004) had satisfactory recoveries for all concentrations in study of OP residues added to tomatoes. Bastos et al. (2012) obtained satisfactory recovery values for all pesticides added to milk samples with exception of dichlorvos.

Table 2. Percentage of recovery of OP

OP	a (%)	b (%)	c (%)
Metamidophos	92.42	102.55	119.62
Phorate	130.93	188.45	126.98
Parathion-me	115.76	212.7	160.53
Pyrimiphos-me	75.49	90.02	76.78
Malathion	64.35	103.5	61.72
Chlorpyriphos	96.96	126.55	99.7
Terbuphos	90.12	98.45	87.14
Phenthoate	76.87	119.21	81.34
Etione	83.84	100.09	80.97
Triazophos	118.74	32.72	122.96
Pyrazophos	120.05	119.67	116.03

The Limits of Detection and Quantitation calculated for the organophosphates are presented in Table 3.

Table 3: Limits of Detection and Quantification

IA	LD ($ng.mL^{-1}$)	LQ ($ng.mL^{-1}$)
Methamidophos	202.4	607.2
Phorate	3.15	9.44
Terbuphos	3.22	9.65
Pyrimiphos-me	2.07	6.22
Chlorpiriphos	3.32	9.89
Parathion-me	3.61	10.83
Malathion	3.87	11.62
Phentoate	6.27	18.81
Etione	0.69	2.07
Triazophos	4.97	14.91
Pyrazophos	5.29	15.88

According to Schenck & Lehotay (2000), pesticides prepared in pure solvents may have higher LD and LQ concentration values than those obtained from solutions prepared in the matrix extract. When injected in pure solvent the pesticides undergo the highest adsorption in the injection system, while when injected into the matrix extract, they undergo matrix effect and a larger amount is transferred to the column.
Bastos et al. (2012) observed a regression varying from 0.01 to 0.05 $\mu g.mL^{-1}$ in 40 of the 53 organophosphates evaluated. The LD values varied from 1.6 to 62.93 $ng.L^{-1}$, and the LQ values ranged from 3.31 to 176.26 $ng.L^{-1}$, and regarding the selectivity studies, the samples are appropriate for the method evaluation. The results of intermediate precision for the eleven OP are presented according to relative standard deviation percentage (RSD) in Table 1. The results are within the accepted limits by the validation script chromatographic methods to area values ≤ 15 %, with the exception of phenthoate at 0.017 and 0.044 $mg.kg^{-1}$, pyrazophos at 0.018 $mg.kg^{-1}$ and methamidophos at 0.026 $mg.kg^{-1}$. Gobo *et al*. (2004) using GC-FTD obtained satisfactory results for intermediate precision of organophosphates, with the exception of methamidophos. The percentages of OP recovery are expressed on Table 2. In column a, the concentration of OP which was added to the common bean aliquot was 0.016 $mg.kg^{-1}$, in column **b** the concentration of OP added was 0.022 $mg.kg^{-1}$ and in **c** it was 0.44 $mg.kg^{-1}$.

4. Conclusions

The analytes were thermosensitive under the conditions employed during this study, according to the results obtained in the work. This information is important from the point of view of food safety, since beans aren´t already consumed in raw form due to the presence of anti-nutrients intrinsic to this food matrix. The methodology was satisfactory for identification and determination of nine from the eleven analyzed

organophosphates added to beans samples. The temperature and cooking time used were lower than the traditional beans dishes. The method was selective for all analytes, as there was no interference greater than 30% of the quantification limit. The limits of detection were between 1.60 and 52.96 $ng.kg^{-1}$ and the limits of quantification were between 3.30 and 176.0 $ng.kg^{-1}$.

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