

Influence of Ripening Stages of Tomatoes in the Analysis of Pesticides by Gas Chromatography

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Parâmetros de maturação de frutos de tomate como pH, °Brix, acidez e teores de licopeno e β-caroteno foram avaliados. Cinco agrotóxicos foram quantificados em extratos orgânicos de tomate em diferentes fases de maturação. A técnica de extração sólido-líquido com partição em baixa temperatura (ESL-PBT) foi utilizada para a obtenção desses extratos orgânicos. O efeito da matriz tomate foi calculado a partir dos resultados da análise por cromatografia gasosa com detector de captura de elétrons (CG-DCE). Correlações entre os parâmetros de maturação e efeito matriz foram estudadas. A correlação entre o teor de carotenoides e o efeito matriz foi elevada para todos os pesticidas estudados. Os resultados indicam que o efeito produzido pela matriz tomate está correlacionada com a presença de licopeno e β-caroteno em extratos orgânicos. A análise de componentes principais (PCA) mostrou que o licopeno, caroteno e a mistura de ambos têm efeitos significativos sobre a resposta cromatográfica de pesticidas.

Some parameters of tomato fruits ripening such as color, pH, °Brix, acidity and lycopene and β-carotene content were evaluated during the ripening of fruits. Five pesticides were quantified in organic extracts derived from tomatoes at different stages of maturation. The solid-liquid extraction technique with partition at low temperature (SLE-PLT) was used to obtain these organic extracts. The matrix effect of tomato was calculated from the results of analysis by gas chromatography with electron capture detector (GC-ECD). Correlations between maturation parameters and matrix effect were performed. The correlation between the content of carotenoids and matrix effect was high for all pesticides studied. The results indicate that the effect produced by the tomato matrix is correlated with the presence of lycopene and β-carotene in organic extracts. The principal component analysis (PCA) showed that lycopene, carotene and the mixture of both have significant effects on the chromatographic response of pesticides, separating them into distinct groups.

Keywords: matrix effect, tomato, GC-ECD, PCA

Introduction

The tomato (*Lycopersicon esculentum* Mill) is one of the vegetable with a large market in Brazil and is much appreciated for its high nutritional value. It is rich in vitamin A and C, phosphate and potassium, besides presenting about 95% of water in its composition.¹

Tomato production in Brazil is approximately three million tons *per* year. Sixty-five percent of this production is destined for fresh consumption and 35% for industrial processing.² Tomato fruits are highly susceptible to pests

so, in order to avoid crop losses, a lot of different pesticides are used to control these pests.³ The successive application of pesticides and non-observance of grace periods thereof make herbicide residues remain in the fruits after harvest. Then, it becomes necessary to analyze pesticide residues in these fruits to ensure the quality of the food being consumed.

The analysis of trace organic contaminants in foods typically consists of the following consecutive steps: (i) extraction of analytes from the sample, (ii) removal of co-extractives from crude extract and (iii) identification and quantification of target analytes.⁴ The gas chromatography (GC) is the most used analytical technique in the quantification

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of pesticides in different foods and environmental matrices. During chromatographic analysis, the matrix components contained in the organic extracts may influence the quantification of analytes of interest generating inconclusive results.⁵ The change in the chromatographic response due to the presence of co-extractives is named matrix effect. The matrix effect is observed when a considerable difference of response is obtained between standards prepared in the solvent and those prepared in the matrix extract.⁶

In samples of tomato the chemical composition of the matrix extracts varies according to the stage of the fruits ripening and the extraction method used. The green color of unripe tomato fruits is due to the presence of chlorophyll. With the onset of maturation, occur the degradation of chlorophyll and the synthesis of yellow pigments, especially β -carotene and xanthophylls. The reddish color is characteristic of ripe fruit, due to the accumulation of lycopene. The colors of the tomato species differ from yellow to orange red depending on the ratio lycopene/ β -carotene the fruit has.⁷ The sugar and organic acid content also varies during maturation. Sugars (sucrose and fructose) constitute about 65% of total soluble solids and accumulate in the final stage of fruit ripening.¹

The presence of tomato co-extractives in the organic extracts obtained in the solid-liquid extraction with partitioning at low temperature (SLE-PLT) cause significant effects on the chromatographic response of pesticides analyzed by GC.⁸⁻¹⁰ In this study, it was attempted to correlate the observed matrix effect in analysis of five pesticides with certain physicochemical properties of the tomato. Some parameters of tomato fruit ripening such as color, pH, °Brix, acidity and lycopene and β -carotene content were evaluated during the ripening of fruits. These data were correlated with the observed matrix effect on the analysis of five pesticides by gas chromatography with electron capture detector (GC-ECD).

Experimental

Samples

Samples of unripe hydroponic tomato of the salad kind, free of pesticides, were acquired in the market of Viçosa-MG and stored at room temperature during the ripening process.

The samples were classified into five stages of maturation during the storage period. (1) Pinkish green: between 0% and 10% of the whole surface with reddish or yellowish color; (2) greenish pink: between 10% and 30% of the whole surface with reddish, pinkish or yellowish

color; (3) pinkish: between 30% and 60% of the whole surface with reddish or pinkish color; (4) light red: between 60% and 90% of the whole surface having reddish pink or red and (5) red: more than 90% of the fruit surface having intense red coloration.²

Characterization of tomato samples

The tomato color was measured with colorimeter "Tristimulus" Minolta® (Ramsey, USA) model CR-400 (illuminant C and 10° viewing angle and color space CIELAB) with direct reading of reflectance of the coordinates "L*" (luminosity), "a*" (green to red) and "b*" (yellow to blue). Four readings were made at the middle region of the fruits in the longitudinal direction.¹¹

The acidity was determined by titration of the pulp with standardized solution of NaOH 1.00×10^{-1} mol L⁻¹ and 3 drops of phenolphthalein indicator.¹²

The pH measurement was performed by direct immersion of the electrode in the tomato pulp, according to the Instituto Adolfo Lutz method.¹² The determination of the pH of the pulp was performed using digital benchtop pH meter Homis® (São Paulo, Brazil), model MPA 210.

The soluble solids content of the pulp of tomatoes was determined by a manual refractometer model 107-0 to 90% BRIX-Biobrix® (São Paulo, Brazil), according to the Instituto Adolfo Lutz method.¹² The results were expressed as °Brix.

The level of lycopene and β -carotene were estimated spectrophotometrically and expressed as level of lycopene.

Standard solutions

Standard lycopene was obtained from tomato samples in advanced ripening stage according to the method proposed by Nunes and Mercadante.¹³ The obtained crystals (75% m/m)¹⁴ were solubilized in petroleum ether and analyzed by GBC Cintra 20 spectrophotometer (Honey, WV, USA) at a wavelength of 470 nm.²

The solution of β -carotene was prepared by dissolving Sigma standard (Seelze, Germany) in methanol at a concentration of 600 mg L⁻¹. A solution at a concentration of 10 mg L⁻¹ was prepared by diluting in acetonitrile.

Stock standard solutions of chlorothalonil (99.3% m/m), iprodione (99.3% m/m) and deltamethrin (99.7% m/m), purchased from Sigma Aldrich (Seelze, Germany); chlorpyrifos (99.0% m/m), purchased from Chem Service (West Chester, USA) and permethrin (92.2% m/m) acquired from Syngenta (São Paulo, Brazil) were prepared in acetonitrile at a concentration of 1000 mg L⁻¹ and stored at 4 °C. From the dilution of stock solutions, solutions were

prepared containing five pesticides at concentrations of 10 mg L⁻¹ in the same solvent.

Obtaining matrix extracts

The organic extracts from tomato samples in each stage of maturity were obtained from the technique of solid-liquid extraction with partition at low temperature (SLE-PLT).⁸ Thus, 4.00 g of crushed tomato samples were transferred to a 22 mL vial and added to an extracting mixture comprising 0.5 mL of water, 6.5 mL of acetonitrile and 1.5 mL of ethyl acetate. The system was homogenized and allowed to stand in a freezer at -18 °C for 6 h. The organic liquid phase was passed through 1.5 g of sodium sulfate and collected into a 10 mL volumetric flask with acetonitrile. The extracts were stored at 4 °C until the time of analysis by gas chromatography and spectrophotometry.

Quantification of pigments in the matrix extracts

The quantifications of lycopene and β-carotene in matrix extracts, in several stages of maturation, were performed in spectrophotometer with wavelength of 470 nm.² Analytical curves were obtained at concentrations between 0.25 and 2.5 mg L⁻¹ in acetonitrile. Concentrations of two carotenoids in tomatoes organic extracts were calculated by linear regression equation obtained from the analytical curve and were expressed in terms of the concentration of lycopene.

Analysis by GC-ECD

A gas chromatograph Shimadzu (Kyoto, Japan) model GC-2014 equipped with an electron capture detector (ECD), auto injector AOC-20i was used for analyzes. The chromatographic separation of the analytes was performed on a HP-5 capillary column from Agilent Technologies (Palo Alto, USA) with a stationary phase consisting of 5% diphenyl and 95% dimethyl siloxane (30 m × 0.25 mm d.i., 0.1 μm film thick), and nitrogen (Air Products, São Paulo, Brazil) with 99.999% of purity as the carrier gas at a flow rate of 1.2 mL min⁻¹. The programming of the oven temperature was 150 °C (2 min), heated at 40 °C min⁻¹ to 210 °C (1 min), then again heated at 40 °C min⁻¹ to 260 °C (1 min). Finally the temperature was raised at 30 °C min⁻¹ to 290 °C (5 min). The temperatures of the split/splitless injector and detector were 280 and 300 °C respectively. It was injected 1.0 μL of the sample into the chromatograph at a split ratio of 1:5. The total analysis time was 12.75 min.

Matrix effect

Effect of the components of tomato

Standard solutions containing the five pesticides in the concentration of 100 μg L⁻¹ in acetonitrile and in organic extracts of the matrix were analyzed by GC-ECD. The five organic extracts used were obtained from the SLE-PLT of tomato samples at different stages of maturation. The areas obtained for the analytes in pure solvent were related to areas obtained from the same compounds in organic extract of the matrices using equation 1:

$$\text{Matrix effect(\%)} = \left(\frac{X_1 - X_2}{X_2} \right) \times 100 \quad (1)$$

where X₁ is the average of the areas of the pesticide prepared in extract of the matrix and X₂ is the average of the areas of the pesticide prepared in pure solvent.

Effect of lycopene and β-carotene

In order to evaluate the influence of lycopene and β-carotene in the chromatographic responses of the pesticides, to the standard solutions containing the five pesticides at concentrations of 100, 300 and 500 μg L⁻¹ were added solutions of lycopene, β-carotene and mixtures of both pigments in concentrations of 0.5, 1.0, 2.0, 3.0 and 4.0 mg L⁻¹. These concentrations were chosen based on the concentration of carotenoids found in fruits of tomato, which was approximately 2.0 mg L⁻¹. The percentage of matrix effect was calculated by equation 1. The results were explored using principal component analysis.

Principal component analysis

The principal component analysis was performed in order to find patterns in distributions of the five pesticides and to verify the effect of lycopene, β-carotene and mixtures of these two carotenoids on each pesticide with the purpose to extract relevant information about this system.

A 15 × 15 matrix was built for the multivariate treatment of data. The five pesticides in three concentrations (100, 300 e 500 μg L⁻¹) were defined as samples and were therefore placed in the rows. Lycopene, β-carotene and mixtures of these two carotenoids were defined as variables and therefore were placed in columns. The response used as information of the matrix effect was calculated by equation 1.

The data were imported by Software MATLAB 7 (The MathWorks, Inc.) and subjected to chemometric treatment using the PLS_Toolbox 6.2 (Eigenvector Research, Inc., USA). The columns of the matrix were autoscaled and then PCA was carried out.

Results and Discussion

Quantification of pigments in the matrix extracts

Previous works have shown the influence of the tomato components in chromatographic responses of the pesticides during the analysis by GC.^{9,10} Pigments are among the major components of the tomato. These pigments, when present in the organic extract, can be deposited into the chromatographic system during the injections or, depending on the conditions of analysis, can be decomposed in the injector forming volatile compounds.¹⁵ The estimate of the amount of pigments in the extracts obtained from samples of tomato was performed by UV-Visible spectrophotometry. Absorption spectra of standard solutions of lycopene and β -carotene were obtained in the range 380-600 nm. Figure 1 shows these spectra.

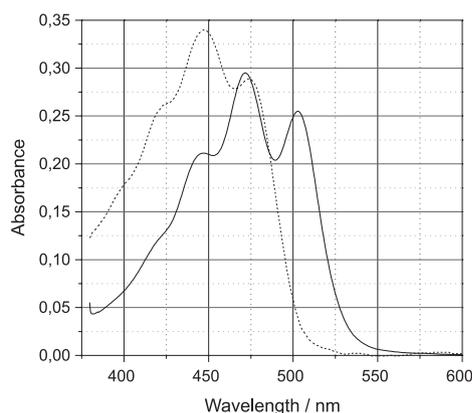


Figure 1. Absorption spectra of lycopene standard solution (—) and β -carotene (-----) at a concentration of 1.0 mg L⁻¹.

The levels of these carotenoids in tomato organic extracts, expressed in mg L⁻¹ of lycopene were 0.79, 1.56, 1.60, 2.01 and 2.07 for the stages pinkish green, greenish pink, pinkish, light red and red, respectively (see Table 1).

The levels of these carotenoids present in organic extracts confirm the assessment of the maturation stages

Table 1. Characterization of tomato fruits throughout maturation

Parameter	Stage of maturation				
	Pinkish green	Greenish pink	Pinkish	Light red	Red
Solubles solids (°Brix)	5.60 ± 0.00 ^a	5.60 ± 0.00 ^a	5.0 ± 0.82 ^a	5.70 ± 0.14 ^a	6.00 ± 0.03 ^a
pH	4.25 ± 0.00 ^a	4.17 ± 0.04 ^a	4.14 ± 0.15 ^a	4.26 ± 0.04 ^a	4.25 ± 0.04 ^a
Titratable acidity (g citric acid/100g)	0.30 ± 0.00 ^a	0.32 ± 0.05 ^a	0.37 ± 0.04 ^a	0.32 ± 0.01 ^a	0.33 ± 0.03 ^a
Color (a*/b*)	0.08 ± 0.01 ^a	0.10 ± 0.06 ^a	0.40 ± 0.30 ^{ab}	0.77 ± 0.20 ^b	1.11 ± 0.10 ^b
Carotenoides / (mg L ⁻¹)	0.79	1.56	1.60	2.01	2.07

Mean values (triplicate) ± standard deviation; ^{a,b}the same letters in the same row do not differ by Tukey test ($p < 0.05$).

performed by the relationship of color (a*/b*). The main information acquired is that as the fruit matures, the concentrations of lycopene and β -carotene increase in organic extracts obtained by the technique SLE-PLT, as expected.

Characterization of tomato samples

From tomato fruits at each stage of maturation, it was obtained soluble solids data (°Brix), pH, total titratable acidity and color parameter (a*/b*). Table 1 presents the mean of three replicates obtained for these parameters.

Statistically, by Tukey's test ($p < 0.05$) it was not observed changes in the values of °Brix, pH and total titratable acidity during the period of fruit maturation. Similar results were found in the literature for these parameters.¹⁶⁻¹⁹ The relations a*/b* and (a*/b*)² have been commonly used as an index of fruit maturation and present high correlation with the different foreseen maturation stages.¹¹

Analysis by GC-DCE

The chromatographic conditions optimized for simultaneous analysis of the five pesticides allowed a good separation of compounds as can be seen in the chromatogram presented in Figure 2A. The retention time of peaks for each compound was used for identification in the organic extract of tomato. Quantitation of the compounds permethrin and deltamethrin that have more than one peak was performed by summing the areas of the peaks.²⁰

It is observed that the presence of tomato co-extractives causes changes in the baseline of the chromatograms and the responses of pesticides are also changed (Figure 2B). However, no interference peak was detected with the same retention time of the pesticides.

Matrix effect

Table 2 shows the chemical structure of the five pesticides studied. In previous work it was observed the influence of

different matrix components in chromatographic response of these five compounds for analysis by GC-ECD.^{10,21,22}

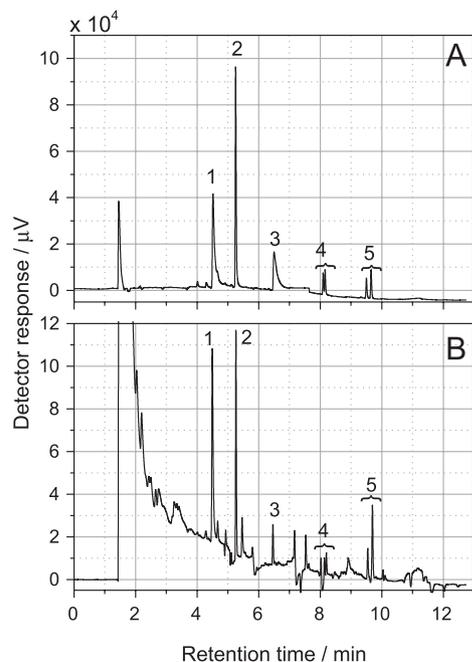


Figure 2. Chromatogram of a standard solution of pesticides in acetonitrile (A) and in the organic extract of tomato (B) at a concentration of 100 μg L⁻¹, where: 1: chlorothalonil; 2: chlorpyrifos; 3: iprodione; 4: permethrin and 5: deltamethrin.

Table 2. Chemical structure of pesticides

Compound	Formula
Chlorothalonil	
Chlorpyrifos	
Iprodione	
Permethrin	
Deltamethrin	

However, a systematic analysis of the influence of different stages of ripening of the fruit has not been reported in the literature.

The percentages of matrix effect were evaluated on the difference of the five pesticides responses when analyzed in pure solvent (acetonitrile) and in tomatoes organic extracts at different stages of maturation. The results are shown in Figure 3.

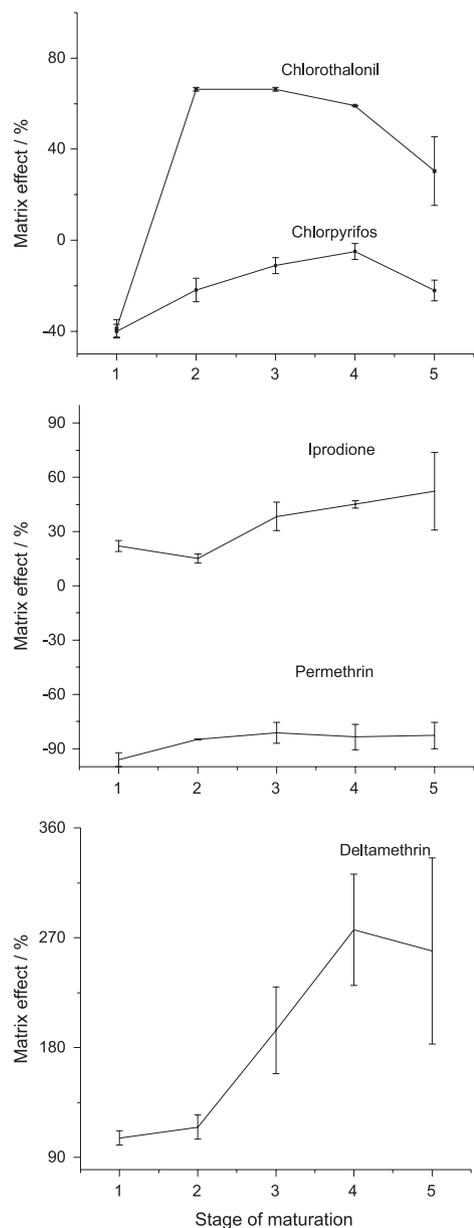


Figure 3. Matrix effects for pesticides at 100 μg L⁻¹ in different stages of maturation, where: pinkish green (1); greenish pink (2); pinkish (3); light red (4) and red (5).

It was observed that for the pesticides chlorothalonil (Figure 3A), iprodione (Figure 3B) and deltamethrin (Figure 3C) there is a positive matrix effect for almost all stages of

maturation. For the pesticides chlorpyrifos (Figure 3A), iprodione and permethrin (Figure 3B), the matrix effect over the period of maturation were statistically identical.

For the pesticides deltamethrin and chlorothalonil it was observed significant changes in the matrix effect due to the maturation stage. These results suggest that the matrix effect shown by the two pesticides has a correlation with the color of the fruit.

The matrix effect data calculated for each pesticide according to the maturation stage were correlated with the values of °Brix, total titratable acidity, pH, color and carotenoid concentration. Table 3 presents the results of these correlations.

The matrix effect variable in the analysis of pyrethroids permethrin and deltamethrin presents a linear relationship with color. That is, as the fruit matures, the color increases and the matrix effect to that pesticide is greater. The color of tomatoes is directly related to the concentration of carotenoids. The lycopene accumulation in the fruits is responsible for the appearance of the red color and the presence of β -carotene gives rise to the yellowness.

The correlations obtained between the matrix effects observed for pesticides in different maturation stages and the concentrations of carotenoids in these stages were quite

Table 3. Correlation between the matrix effect in different stages of maturation and the physical and chemical characteristics of tomatoes and concentrations of carotenoids

Pesticide	°Brix	TA ^a	pH	Color	Carotenoids
Chlorpyrifos	-0.27	0.54	-0.22	0.45	0.75
Iprodione	-0.20	0.78	-0.43	0.58	0.86
Permethrin	0.07	0.45	0.24	0.87	0.75
Chlorothalonil	-0.19	0.67	-0.50	0.39	0.81
Deltamethrin	0.57	0.18	0.43	0.97	0.76

^atitratable acidity

high. Observing the data obtained so far it can be inferred that as the fruit ripens the color increases, increasing the concentration of lycopene and β -carotene in tomato fruits. The level of 95% confidence the concentration of carotenoids is correlated positively with the effect of all pesticides studied matrix, being significant only for iprodione, as verified by the data of Table 3.

Principal components analysis

Figure 4 shows the PCA results considering the matrix effect observed for the five pesticides in solutions

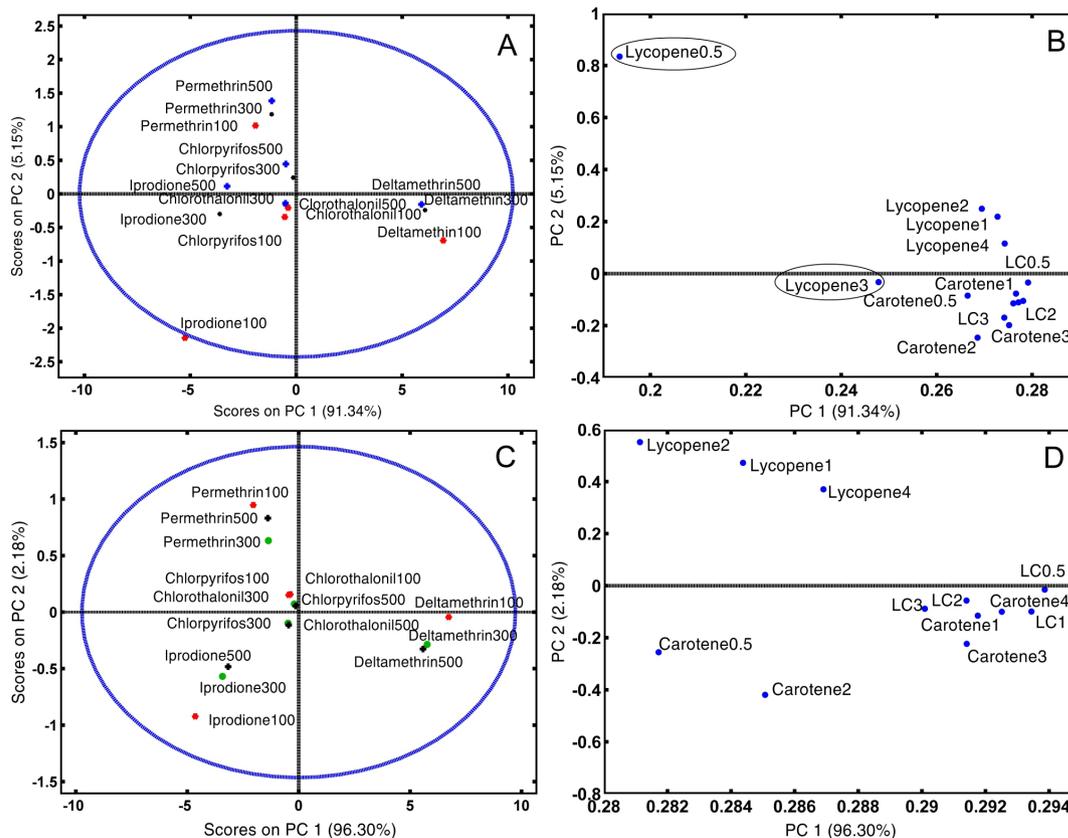


Figure 4. Principal components analysis using the effect of the components of the matrices in the chromatographic response of five pesticides. (A) and (C): pesticides in different concentrations (scores); (B) and (D): components of tomato (loadings). LC: mixture of the two carotenoids, lycopene and β -carotene.

containing the lycopene and β -carotene pigments. The results presented in Figures 4A and 4B show that 96.55% of the variance of the data is explained by the first two components. The graph of scores shown in Figure 4A indicates that pesticides suffer the matrix effect produced by carotenoids and this effect is independent of the concentration of pesticide in the range studied (100, 300 and 500 $\mu\text{g L}^{-1}$). However, iprodione (Figure 4A) has a different behavior. The loadings of the variables lycopene 0.5 mg L^{-1} and lycopene 3 mg L^{-1} were responsible for this anomalous behavior, as outlined in Figure 4B. By removing these variables, it was observed a more homogeneous and interpretable distribution of the scores of the samples (Figures 4C and 4D).

An interpretation of Figures 4C and 4D indicates that the first component (96.30% of variance) is responsible for the separation of deltamethrin of other pesticides. The mixture of carotenoids, lycopene and carotene showed a greater positive effect on the chromatographic response of deltamethrin, while lycopene and carotene with concentrations of 2 mg L^{-1} and 0.5 mg L^{-1} had higher negative effect on the chromatographic responses of the other pesticides. The second component (2.18% of variance) shows that lycopene presents a great effect on the chromatographic response of the permethrin, while carotene concentrations of 2 mg L^{-1} and 0.5 mg L^{-1} negatively influenced on response of iprodione. The responses of chlorpyrifos and chlorothalonil pesticides suffered slight negative effect of both, carotene and lycopene, when pure. The second component confirms that the response of deltamethrin suffers positive effect principally from the carotene and lycopene mixture. The results indicate that the concentrations of carotene showed no interpretable behavior of the effect on responses.

Conclusion

The responses of the analysis by gas chromatography with electron capture detection (GC-ECD) of the studied analytes were affected by components of tomato matrix. These changes could be related to the presence of carotenoids in different stages of maturation. It was observed that, as the fruit matures, there are an increase in concentrations of β -carotene and lycopene in organic extracts obtained by SLE-PLT. This fact made the chromatographic responses of pesticides were significantly changed. The matrix effect in this study was independent of analyte concentration. The principal component analysis (PCA) showed that chromatographic responses of pesticides are affected differently by the presence of lycopene, carotene or a mixture of both. The concentrations of carotenoids in the

system did not show linear behavior on the matrix effect on chromatographic responses. When there is matrix effect is recommended to prepare the analytical curve using the extract of the matrix.

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