

**„BABEŞ-BOLYAI” UNIVERSITY CLUJ-NAPOCA  
FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING**

**Florina-Dorina Tuşa (Covaciu)**

**APPLICATIONS OF CHROMATOGRAPHY AND MASS  
SPECTROMETRY IN QUALITY CONTROL OF VEGETABLES AND  
FRUITS COMMERCIALISED ON THE ROMANIAN MARKET**

**- PhD thesis summary -**

**Scientific supervisor  
Prof. Dr. Mircea Vlassa**

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**Key words:** pesticides, vegetables and fruits, isotopic ratio, micro-macro elements content, chemometry, QuEChERS, GC-ECD, GC-MS, ICP-MS, IRMS

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The abstract of the PhD thesis presents some of the results of its own experimental researches, the general conclusions and the selective bibliography.

The thesis brings relevant original contributions to the field of pesticide residue determination in the food sector (by significantly increasing sensitivity and selectivity, while shortening analytical time), as well as in the use of complementary techniques for the purpose of highlighting the differences between berries (wild vs. crop) and vegetables (outdoors vs. greenhouse) and testing of commercial samples to authenticate them against geographic origin.

This thesis presents the first isotopic fingerprinting of berries from Transylvania region and the initiation of the first database on their isotopic content.

The PhD thesis "*Applications of Chromatography and Mass Spectrometry in Quality Control of Vegetables and Fruits commercialised on the Romanian Market*", is structured in two parts: **Part I - Theoretical Considerations** and **Part II -Experimental Results and Personal contributions**. The thesis includes: list of abbreviations, objectives and original contributions, background, introduction, 11 chapters, 3 annexes, scientific activity and bibliography.

**Part I** "*Theoretical Considerations*" is comprised of four chapters:

**Chapter 1** "*Pesticides*" brings together information from the literature on the presence of pesticides in vegetables and fruits, pesticide classification, maximum residue limits for pesticide residues in vegetables and fruits, and statistics on pesticide use at national level.

**Chapter 2** "*Methods of processing samples for the determination of pesticides in vegetables and fruits*" presents a literature study on pesticide residue extraction methods and "clean-up" methods of the obtained extracts used in complex matrices such as fruits and vegetables.

**Chapter 3** "*Analytical methods for the detection of pesticides in complex matrices*" presents the techniques commonly used in food control in regards to pesticide residue content.

**Chapter 4** "*Quality Control of fruits and vegetables based on Isotopic and Elemental Measurements*" describes analytical techniques used in authentication, using mass spectrometry for isotopic ratios combined with elemental analysis in determination of the geographical origin

of vegetables and fruits and correlating the elemental and isotopic composition profile of the food to its geographical origin, using statistical methods.

**Part II** of *"Experimental Results and Personal Contributions"* is structured in seven chapters as follows:

**Chapter 5** *"Validation of the QuEChERS extraction method for pesticide residue determination in complex matrix by gas chromatography and mass spectrometry"* presents a simple, fast and inexpensive method for the determination of pesticide residues in carrots and other possible complex matrices. Evaluation of the performance parameters (selectivity, working domain, linearity, detection and quantification limit, precision and accuracy) for validation of the optimized QuEChERS extraction method of pesticides from carrots using chromatography and mass spectrometry.

**Chapter 6** *"Application of optimized QuEChERS extraction method for the determination of pesticide residues in different categories of vegetables"* presents the obtained results on the level of pesticide residues in vegetables commercialized on the Transylvanian market by GC-ECD and GC-MS using optimized QuEChERS extraction method validated in chapter 5.

**Chapter 7** *"Elemental, isotopic and pesticidal analysis of berries from Transylvania region"* provides data on isotopic fingerprinting of berries from Transylvania region.

**Chapter 8** *"Investigation of differences in isotopic, elemental and pesticidal content of berries grown wild versus cultivated"* presents the correlations between isotopic and elemental determinations and pesticide residue content of berries from Transylvania (wild vs. cultivated) using chemometric methods .

**Chapter 9** *"Testing of commercial strawberries and their authentication against geographic origin"* presents a study on the content of pesticides, minerals and toxic metals found in strawberries from Transylvania and other countries sold in supermarkets and farmers markets in Romania.

**Capitolul 10** *"Evaluation of elemental, isotopic and pesticidal content in tomatoes grown outdoors and in greenhouses"* presents the evaluation of the tomatoes grown outdoors and of those grown in greenhouses using as differentiation markers the content of pesticides, isotopic, micro and macro elements, essential elements, toxic metals and rare-earth elements.

**Chapter 11** *"Conclusions and Recommendations"* summarizes the overall conclusions and recommendations and contains a synthesis of the results obtained.



The analysis of fruits and vegetables for the evaluation of pesticide residues is an important quality control procedure established to reduce the misuse of pesticides and to ensure the quality and safety of food for human consumption [1]. Rapid, efficient, precise and accurate analytical methods are needed to allow for the simultaneous determination of multi-class and multi-residue pesticides. High selectivity, sensitivity, low cost, large numbers of samples, a less tiring manipulation and a correct quantification of the residue is needed. Vegetable and fruit control activities ensure consumer protection by checking that all these products for human consumption are safe and meet the quality requirements [2], [3].

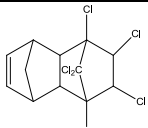
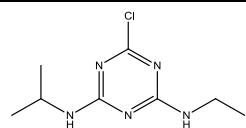
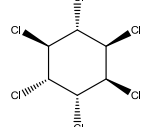
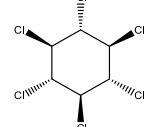
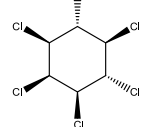
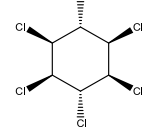
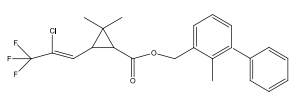
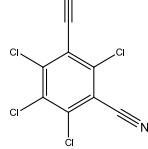
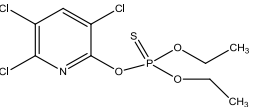
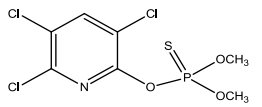
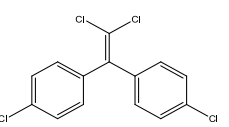
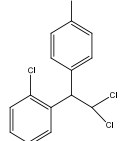
Determination of pesticide residues in vegetables and fruits is necessary to ensure that people's exposure to contaminants, especially through food intake, does not exceed an acceptable level for health. In this area, reproducible analytical methods are required to allow effective separation, selective identification and exact quantification of analytes at low trace levels in vegetables and fruits. Therefore, robust analytical methods need to be validated in order to achieve both objectives of research and monitoring. The greatest analytical challenge in food safety is to present reliable results as quickly as possible without affecting the method such as recovery, accuracy, sensitivity, selectivity and specificity.

## Validation of the QuEChERS extraction method for pesticide residue determination in complex matrix by gas chromatography and mass spectrometry

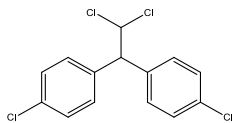
The development of analytical methodologies for the monitoring of pesticide residues in vegetables and fruits is an important subject of research in quality assessment [4]. The complete procedure for the determination of pesticide residues in these matrices is complex and consists of several stages. It is very important that the extraction and analysis steps are validated to ensure compliance with the requirements that define the procedure and to assess its utility [5].

This chapter aims at the optimization and validation of the QuEChERS extraction method and development of a multi-residue separation method for the detection of the 30 pesticides studied (commonly used in spraying vegetables and fruits). The compounds selected for this study are shown in Table 1-1.

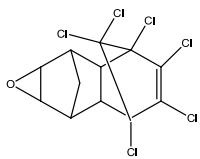
**Table 1-1.** Chemical structure of studied pesticides

Name Class CAS Number	Chemical structure	Name Class CAS number	Chemical structure
Aldrin Insecticid 309-00-2		Atrazine Erbicid 1912-24-9	
$\alpha$ -Hexachlorbenzene ( $\alpha$ -BHC) Insecticid 319-84-6		$\beta$ -Hexachlorbenzene ( $\beta$ -BHC) Insecticid 319-85-7	
$\delta$ -Hexachlorbenzene ( $\delta$ -BHC) Insecticid 319-86-8		$\gamma$ -Hexachlorbenzene ( $\gamma$ -BHC) Insecticid 58-89-9	
Bifenthrin Insecticid piretroid 82657-04-3		Chlorothalonil Fungicid 1897-45-6	
Chlorpyrifos Insecticid 2921-88-2		Chlorpyrifos methyl Insecticid 5598-13-0	
1,1-Dichloro-2,2-bis(4-chlorophenyl)-ethene (4,4'-DDE) Insecticid 72-55-9		2,4'-Dichloro-diphenyl-dichloro-ethane (2,4'-DDD) Insecticid 53-19-0	

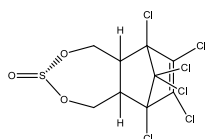
4,4'-Dichloro-diphenyl-  
dichloro-ethane  
(4,4'- DDD)  
Insecticid  
72-54-8



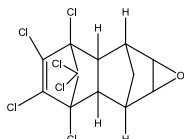
Dieldrin  
Insecticid  
60-57-1



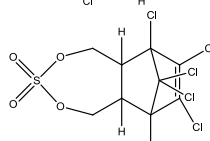
Endosulfan I  
Insecticid  
959-98-8



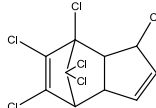
Endrin  
Insecticid  
72-20-8



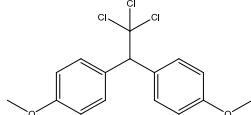
Endosulfan sulfate  
Insecticid  
1031-07-8



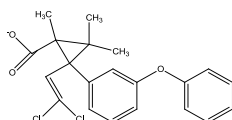
Heptachlor  
Insecticid  
76-44-8



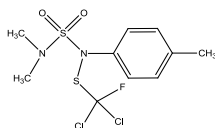
Methoxychlor  
Insecticid  
72-43-5



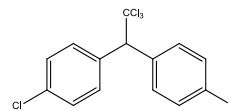
*cis*-Permethrin  
Insecticid  
61949-76-6



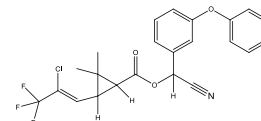
Tolyfluanid  
731-27-1



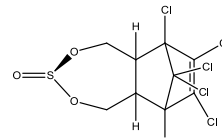
4,4'-Dichloro-diphenyl-  
trichloro-ethane  
(4,4'-DDT)  
Insecticid  
50-29-3



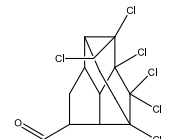
*lambda*-cyhalothrin  
Insecticid  
91465-08-6



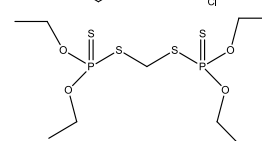
Endosulfan II  
Insecticid  
33213-65-9



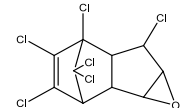
Endrin aldehyde  
Insecticid  
7421-93-4



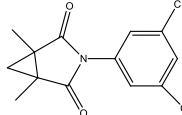
Ethion  
Insecticid  
563-12-2



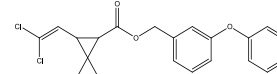
Heptachlorepoxide  
Insecticid  
145213-12-3



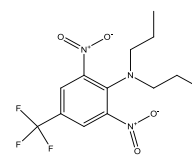
Procymidone  
Fungicid  
32809-16-8



*trans*-Permethrin  
Insecticid  
54774-47-9

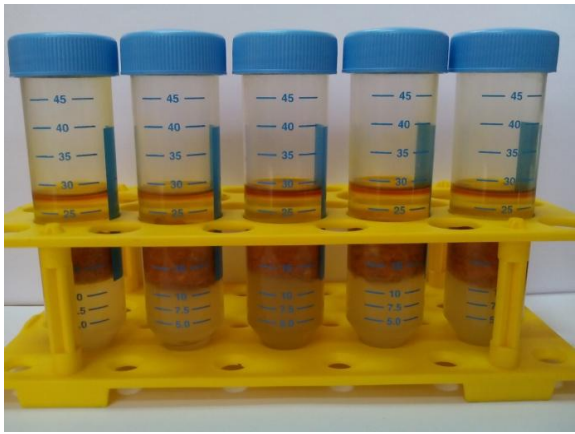


Trifluralin  
Erbicid  
1582-09-8



## 1.1. Extraction procedure

The main disadvantage of the QuEChERS extraction method is the obtaining of relatively "dirty" extracts. In order to improve the outcome over the last decade numerous changes and variations of the original method have been reported in the literature [6]. Taking into account that the "clean-up" step is one of the most important steps in the elimination of unwanted interferences from the extracts, before carrying out the analysis we optimized the QuEChERS extraction method by adding n-hexane to the first extraction step (Figure 1-1).



a).



b).

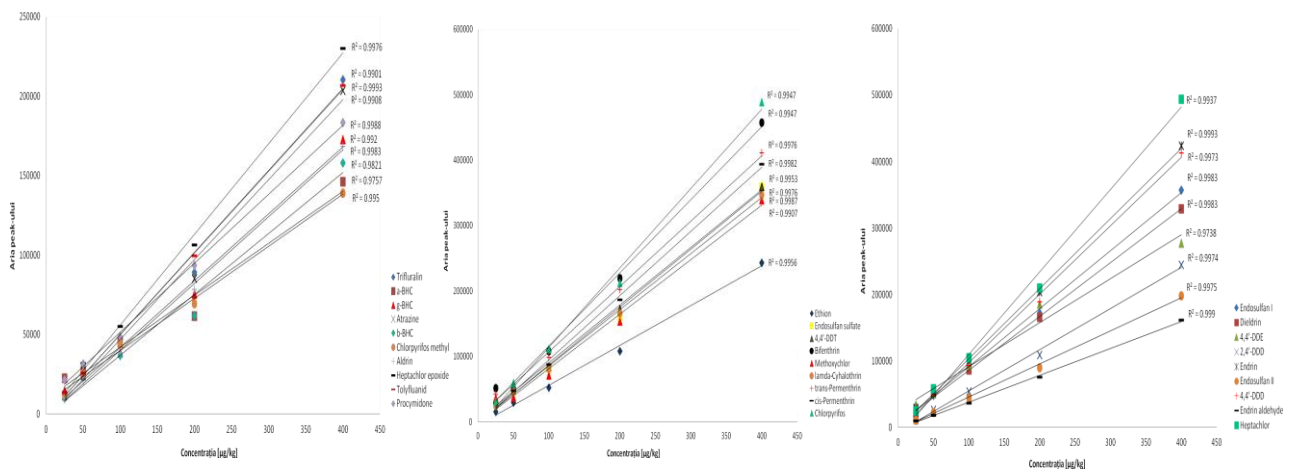
**Figure 1-1.** The separation step after the extraction process  
a). before removal of n-hexane b). after removal of n-hexane

## 1.2. Validation of the optimized QuEChERS extraction method for pesticides from carrots

The analysis was performed in less than 30 minutes on carrot samples using a gas chromatograph equipped with two detectors: ECD and FID. The identification of the compounds was confirmed by GC-MS. In order to confirm the functionality of the method, the following performance parameters were assessed: work domain and linearity, selectivity, detection and quantification limits, precision and accuracy.

### 1.2.1. Work domain and Linearity

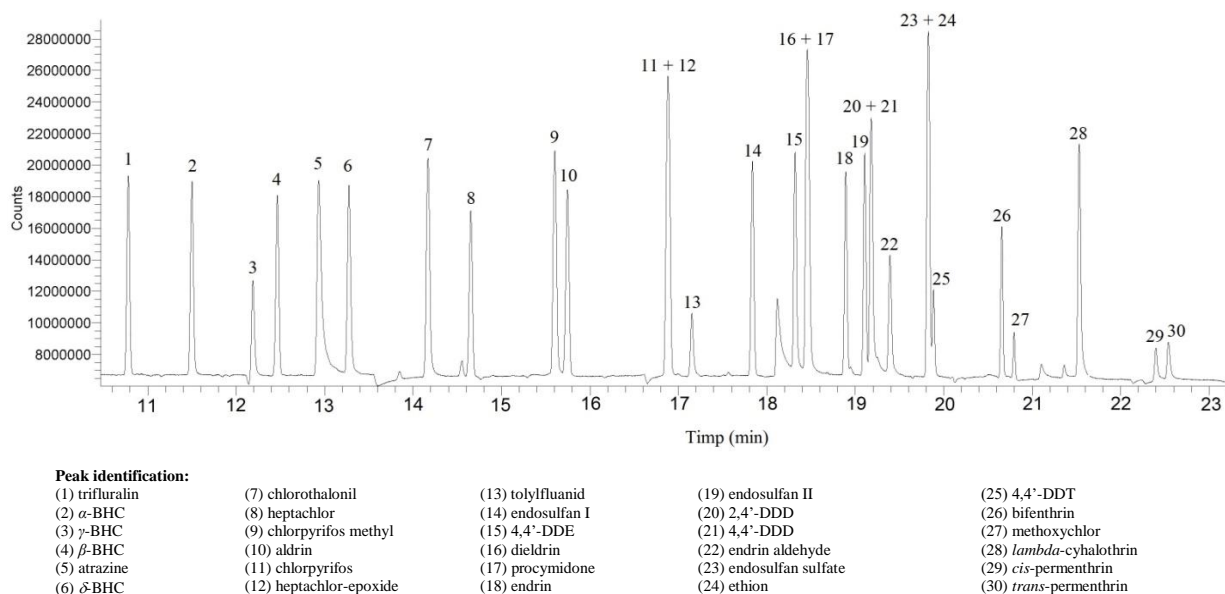
A calibration curve of 5 points was formed. Five working solutions were prepared containing the 30 pesticides at concentrations of 10, 25, 50, 100 and 150 µg / kg. The calibration curves obtained can be seen in Figure 1-2.



**Figure 1-2.** Calibration curves for studied compounds

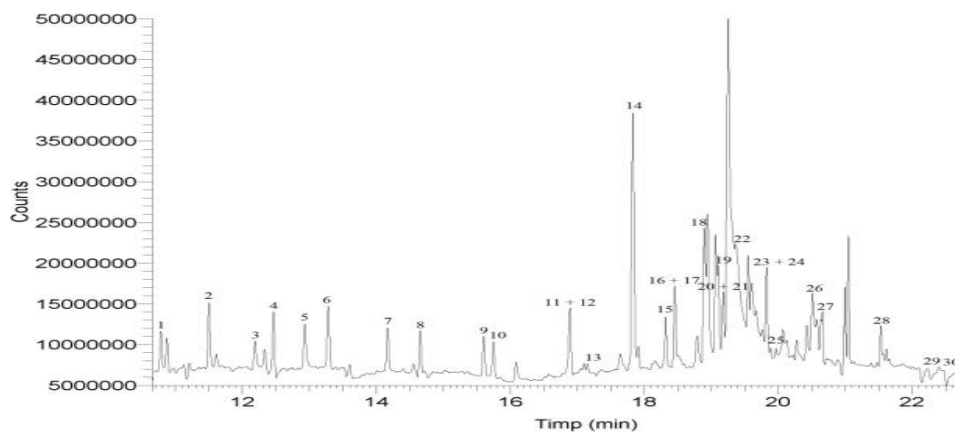
### 1.2.2. Selectivity of the method

Figure 1-3 shows the typical GC-ECD chromatogram of the standard mixture (400 ng / ml of each compound) within 11-23 min. The separation of all pesticides was generally good, with sharp peaks, with the exception of 6 non-separated pesticides.



**Figure 1-3.** The GC-ECD chromatogram of the standard solution of the 30 pesticides (400 ng/ml)

The response for all pesticides was linear in the concentration range (10-150  $\mu\text{g}/\text{kg}$ ), with a correlation coefficient ( $R^2$ ) greater than 0.9920 based on the measurement of areas of the analyte peaks using the electron capture detector, with the exception of two compounds: 4,4'-DDE and  $\alpha$ -BHC, with a coefficient of 0.9738 and 0.9757, respectively. Figure 1-4 shows the GC-ECD chromatogram of an extract from a fortified carrot sample at the concentration of 50  $\mu\text{g}/\text{kg}$  of the pesticides studied.



**Figure 1-4.** The GC-ECD chromatogram of the extract from a carrot sample fortified at the concentration of 50  $\mu\text{g}/\text{kg}$  of the 30 pesticides

### 1.2.3. Limit of detection and quantification

The detection limits obtained by the optimized extraction method for the studied pesticides were from 0.34 to 3.08 µg/kg for GC-FID, 0.23-1.68 µg/kg for coupled system GC-MS and 0.18 to 0.92 µg/kg for GC-ECD, with the exception of *cis* and *trans*-permethrin for which it was 2.71 and 2.89 µg/kg, respectively (Table 1-2).

### 1.2.4. Accuracy and precision

The results obtained on the recovery efficiency for the 30 pesticides are in the range of 22.21-98.35% (Table 1-2). The three repeated recovery determinations indicated a good reproducibility in the case of these pesticides (standard deviation (RSD) being for all compounds <11, with the exception of trifluralin and β-BHC, for which it was around 12.5. Peaks interfering with or overlapping with the studied pesticides can lead to low recovery rates [7].

**Table 1-2.** Validation parameters for optimized extraction method and maximum allowable pesticide residue in carrots

Pesticides	GC-ECD T <sub>R</sub>	Linearity (R <sup>2</sup> )	Recovery Factors GC-ECD (%)	RSD (n=3) GC-ECD (%)	LOD GC-FID (µg/kg)	LOD GC-MS (µg/kg)	LOD GC-ECD (µg/kg)	MRL (µg/kg)
Trifluralin	10.54	0.9901	64.35	12.20	0.71	0.52	0.23	100
α-BHC	11.25	0.9757	85.08	3.90	1.66	1.23	0.35	10
γ-BHC	11.96	0.9920	52.79	4.40	0.93	1.28	0.92	10
β-BHC	12.20	0.9821	72.57	12.20	2.10	1.14	0.39	10
Atrazine	12.22	0.9908	87.79	8.70	2.85	1.28	0.33	100
δ-BHC	13.04	0.9992	98.35	10.03	3.08	1.68	0.48	10
Chlorothalonil	13.89	0.9980	43.66	4.11	1.29	0.91	0.27	50
Heptachlor	14.35	0.9937	51.31	1.10	0.62	0.94	0.35	10
Chlorpyrifos methyl	15.31	0.9950	82.02	12.50	1.29	1.52	0.25	50
Aldrin	15.43	0.9983	46.87	3.80	2.85	0.86	0.31	20
Heptachlorepoxide	16.6	0.9976	58.19	6.80	0.85	0.64	0.22	20
Chlorpyrifos	16.62	0.9947	62.75	3.30	0.45	1.00	0.20	50
Tolyfluanid	16.89	0.9993	52.14	4.60	0.85	0.82	0.62	20
Endosulfan I	17.58	0.9983	63.69	1.30	2.00	0.66	0.29	50
4,4'-DDE	18.08	0.9738	37.04	1.20	1.14	0.89	0.27	50
Dieldrin	18.21	0.9983	33.36	8.70	0.62	0.66	0.19	10
Procymidone	18.23	0.9988	42.71	10.80	0.70	0.66	0.18	20
Endrin	18.66	0.9993	30.41	1.20	2.22	0.23	0.30	10
Endosulfan II	18.89	0.9975	85.79	1.50	2.86	0.97	0.27	50
4,4'-DDD	18.96	0.9973	22.21	2.90	0.62	0.91	0.25	50
2,4'-DDD	18.98	0.9974	23.17	3.40	0.57	0.68	0.18	50
Endrin aldehyde	19.19	0.9990	41.47	1.30	0.62	1.00	0.39	10
Endosulfan sulfate	19.61	0.9953	31.66	2.80	1.67	0.40	0.31	50
Ethion	19.63	0.9956	97.40	3.10	1.43	0.40	0.57	50
4,4'-DDT	19.69	0.9976	32.05	4.64	0.47	1.10	0.52	50
Bifenthrin	20.47	0.9947	43.83	1.60	0.34	0.60	0.51	50
Metoxychlor	20.62	0.9907	59.19	2.10	3.07	1.39	0.89	10
λ-Cyhalothrin	21.33	0.9987	23.17	4.30	0.37	1.33	0.66	20
trans-Permethrin	22.16	0.9976	76.78	1.80	1.00	1.10	2.22	50
cis-Permethrin	22.3	0.9982	67.89	4.20	1.33	1.33	1.84	50

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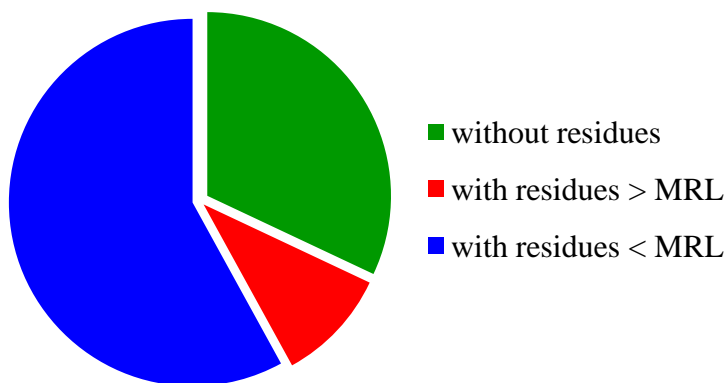
**Application of optimized QuEChERS extraction method for the determination of pesticide residues in different categories of vegetables**

---

In this chapter the level of pesticide residues in the vegetables commercialized on the Transylvanian market and having different proveniences was evaluated. For this purpose, a total of 50 vegetable samples (carrot, potato, parsley, parsnips, green beans, courgette, eggplant, cabbage, salad, cucumber, cauliflower, pepper and kapia pepper) were investigated. QuEChERS and GC- ECD was used for the separation, detection and quantification of pesticides, as described in Chapter 1.

The results showed that more than half of the samples (68%) contain pesticides and 10% contain pesticide residues above the maximum residue limits.

The percentage of the vegetable samples with non-detectable residues and with a below and above MRLs are shown in Figure 2-1.

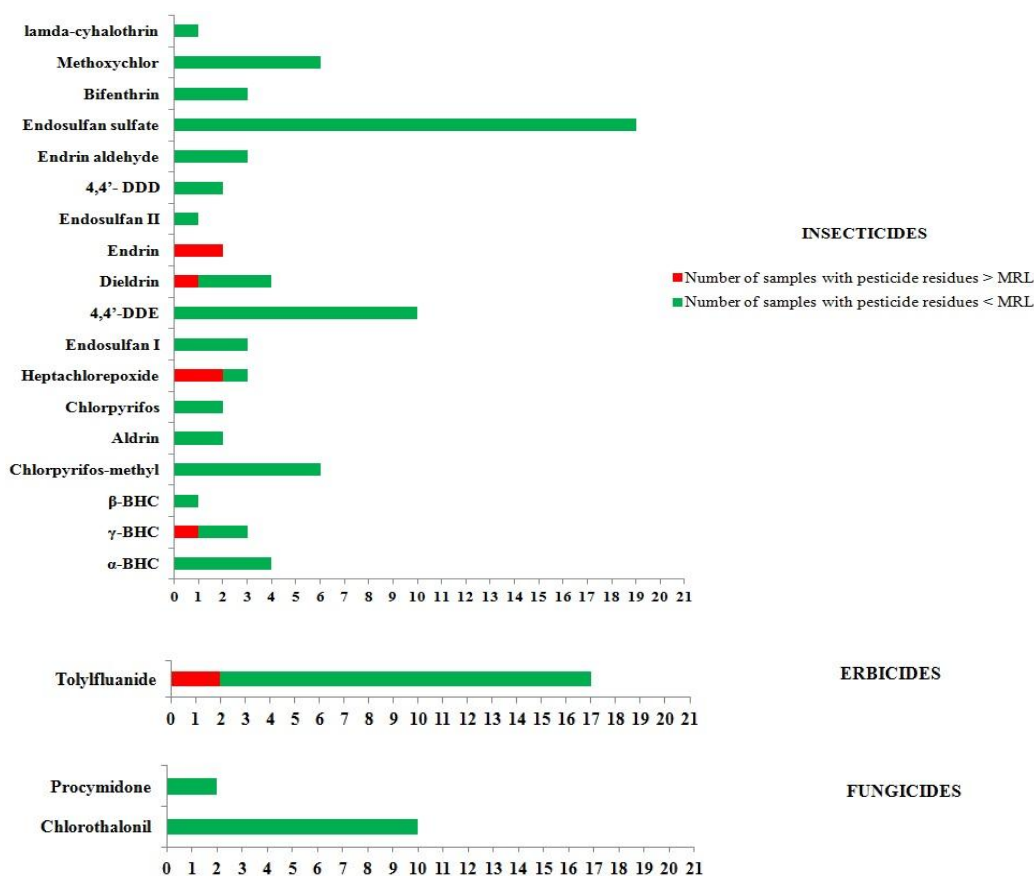


**Figure 2-1.** Percent of vegetable samples with no detectable residues, with residues below and above MRL

Out of the samples containing residues, 85.3% (29 samples) contain pesticide residues under MRL, while 14.7% (5 samples) have values above the MRLs. The distribution of pesticide residues in the analyzed vegetable samples is shown in Figure 2-2. 21 pesticides (including 18 insecticides, 2 fungicides and one herbicide) out of the 30 targeted pesticides were detected in the investigated vegetables.

Regarding pesticides detected in carrots, 6 pesticides were detected in 9 samples and all concentrations were below the MRLs set for carrots. Residues of chlorpyrifos methyl were detected in two samples (1.06  $\mu\text{g} / \text{kg}$  in the sample from Poland and 3.44  $\mu\text{g}/\text{kg}$  in the sample

from Italy). In a carrot sample from Spain, the presence of 4,4'-DDE in the concentration of 1.97 µg/kg was observed. Three samples (two from Poland and one from Italy) showed endosulfan sulphate, in two samples from Portugal and Romania, aldrin was detected, and in two samples from Italy bifenthrin was detected. For methoxychlor, five samples showed residues under MRL. Concentrations determined were 0.91 µg/kg in the sample in England, 1.85 and 1.49 µg/kg in samples from Poland, 1.09 µg/kg in the sample from Spain and 1.43 µg/kg in the sample from Romania [7].



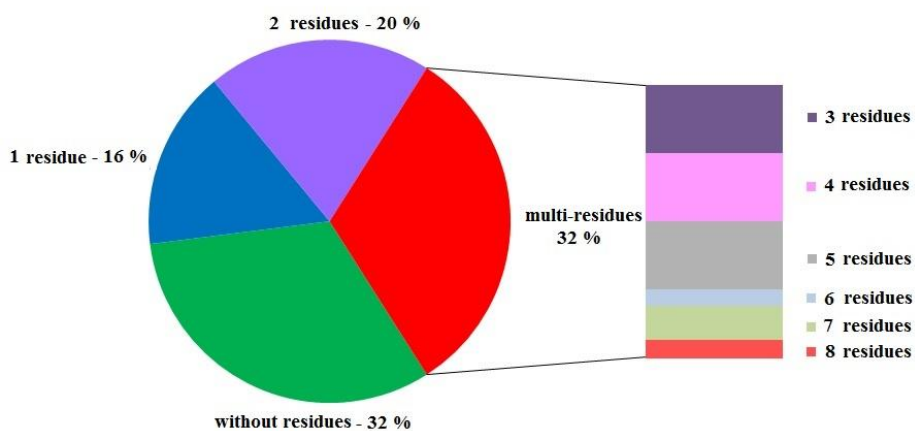
**Figure 2-2.** The frequency occurrence of pesticides in vegetable samples

Pesticide residues were found in all the potatoes, courgette, green beans, eggplant, white cabbage, tomatoes, cucumber, green salad, cauliflower, pepper and kapia, parsley and root pasta. A total of 9 pesticides were detected in potatoes with all the concentrations being below MRL, and with the most common compounds detected being: chlorothalonil, tolyfluanide and endosulfan sulfate. The pesticide residue with the highest concentration was however tolyfluanide in sample C<sub>2</sub>. A high level of tolyfluanide can be observed in the sample of the



cucumber and green salad analyzed, of 8.39  $\mu\text{g}/\text{kg}$  and 4.08  $\mu\text{g}/\text{kg}$  respectively. Out of the three tomatoe samples analyzed, the highest amount of tolylfluanide was determined in the sample R<sub>2</sub> from Spain (18.09  $\mu\text{g}/\text{kg}$ ) and R<sub>3</sub> from Romania (12.14  $\mu\text{g}/\text{kg}$ ). All of these concentrations are below MRL (20  $\mu\text{g}/\text{kg}$ ). However, the highest amount of Tolylfluanide was determined in the sample of white cabbage V<sub>a2</sub> (185.45  $\mu\text{g}/\text{kg}$ ) and eggplant V<sub>1</sub> (28.48  $\mu\text{g}/\text{kg}$ ), both being above MRL. The detected pesticides that exceed MRLs, are part of the old-generation pesticides and the persistent pesticide class:  $\gamma$ -BHC, was detected slightly above the MRLs in the sample of kapi red pepper A<sub>k2</sub> (10.23  $\mu\text{g}/\text{kg}$ ), heptachlor epoxides in two samples of parsley and root of parsley (93.21  $\mu\text{g}/\text{kg}$  and 31.76  $\mu\text{g}/\text{kg}$ ), dieldrin in the root parsley sample (37.74  $\mu\text{g}/\text{kg}$ ) and endrin in parsley and root parsnips (26.76  $\mu\text{g}/\text{kg}$  and 12.45 mg/kg).

Among the tested vegetables 8 samples contained a single pesticide (16%) and 26 samples contained multiple pesticides (52%, from two to eight pesticides) (Figure 2-3). The most frequently detected residues were a combination of two pesticides (20%). And multiple residues of 3, 4 and 5 pesticide residues were also found in 4 samples, a total of 12 samples (24%). The sample with most pesticides detected (8) was the root parsley sample. All pesticides present in this sample are insecticides and 3 of them: heptachlor epoxides (93.21  $\mu\text{g}/\text{kg}$ ), dieldrin (37.74  $\mu\text{g}/\text{kg}$ ) and endrin (26.76  $\mu\text{g}/\text{kg}$ ) are above MRL. These multi-residue pesticide present a greater risk to the health of consumers.



**Figure 2-3.** Multi-residue pesticides in samples

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### Elemental, isotopic and pesticidal analysis of berries from Transylvania region

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In Transylvania there is a great monetary potential in the picking of berries. Their commercial value is even greater as these fruits come from isolated or non polluted places, therefore the development of analytical methods capable of differentiating the region they come from is particularly important. The best way to authenticate products against their geographical origin, recognized and successfully applied at EU wide level, it is the so called isotopic fingerprinting.

It is important to know that each plant retains in its "memory" the specifics of the place where it grew and developed, which is reflected in the isotopic reports of the "bioelements" ( $^2\text{H}/^1\text{H}$ ,  $^{13}\text{C}/^{12}\text{C}$  și  $^{18}\text{O}/^{16}\text{O}$ ) that make up the composition of that plant. Geographic conditions (altitude, distance from the sea or ocean) and weather, along with other factors such as year of production, water used for irrigation, soil type, are the main elements influencing the isotopic ratios of plants. The determination of the isotopic ratios:  $^2\text{H}/^1\text{H}$  și  $^{18}\text{O}/^{16}\text{O}$  can provide information on the geographical origin of food (berries) while determining the  $^{13}\text{C}/^{12}\text{C}$  isotope ratio gives information about the botanical origin of the products and less about the geographical location [8].

The effect of heavy metal contamination of berries can not be underestimated because these foods are important components of human diet. Heavy metal contamination of food is another important aspect in ensuring food quality [9], [10].

This chapter describes the sample preparation and the analytical methods used to determine the elemental, isotopic and pesticide residue composition. The first isotopic fingerprinting of berries in Transylvania is presented. The novelty of this study is to combine several complementary methods of analyzing food quality and safety, with particular reference to berries.

The mean isotopic values of berries, in order to see the naturally occurring isotopic variations, are shown in Table 3-1.

**Table 3-1.** Variation of isotopic content of berries fruit

Sample	$\delta^{13}\text{C}$ (‰)		$\delta^2\text{H}$ (‰)		$\delta^{18}\text{O}$ (‰)	
	min	max	min	max	min	max
Zmeură	-27.9	-22.3	-56.6	-25.0	-4.8	2.9
Mure	-31.1	-23.4	-36.2	-16.8	-2.3	3.5
Merișoare	-29.3	-26.3	-37.2	-19.5	-3.2	1.5
Afine	-32.0	-25.3	-34.4	-17.0	-1.2	8.9
Cătină	-29.1	-25.8	-38.2	-24.1	-3.5	4.3
Măcieșe	-27.9	-26.4	-59.5	-44.1	-11.3	-2.8
Păducel	-29.5	-25.4	-56.2	26.9	-0.9	43.8
Porumbe	-28.5	-27.4	-68.0	-20.9	-6.8	7.7
Alune de pădure	-30.0	-28.7	-85.1	-43.2	-21	-8.6
Căpșuni	-27.2	-24.6	-41.1	-1.5	-4.3	12.5

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### Investigation of differences in isotopic, elemental and pesticidal content of berries grown wild versus cultivated

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Considering the genetic differences between wild and cultivated berries, a potential variability of the elemental composition can be observed. The mineral content depends on many factors: variety, maturity, soil conditions, environmental factors, time and methods of harvesting and agricultural practices [11], [12].

In this chapter, the isotopic composition of carbon, oxygen and hydrogen was determined in order to distinguish between wild and cultivated berries, and ICP-MS techniques have been used for complementary elemental content measurements. Correlations between isotopic and elemental determinations were performed using chemometric methods (ANOVA test and linear discrimination analysis (LDA)).

Isotopic values of the cultivated fruits are higher than of those of wild fruits (eg, isotopic mean values  $\delta^{13}\text{C}$ : cultivated raspberries (- 25.4 ‰), wild raspberries (- 26.3 ‰), cultivated blackberries (- 25.5 ‰), wild blackberries (-28.6 ‰), cultivated blueberries (- 27.1 ‰), wild blueberries (- 29.0 ‰)). This can be explained by the differences in altitude, the wild fruits growing at a higher altitude than the cultivated ones. Precipitation at high altitudes has a lower isotopic composition ( $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$ ) compared to lower altitudes, (eg. cultivated raspberry from Campia-Turzii  $\delta^{18}\text{O} = - 1.3$  ‰ and  $\delta^2\text{H} = - 32.1$  ‰, and the wild raspberry from Băișoara has  $\delta^{18}\text{O} = - 3.4$  ‰ and  $\delta^2\text{H} = - 43.6$  ‰). Also,  $\delta^{13}\text{C}$  is more negative for plants/fruit growing in areas characterized by lower temperatures and higher humidity (eg high altitudes) [13].

#### 4.1. Differentiation of wild versus cultivated berries using the ANOVA test

A first proposed classification was made using the following discriminant markers:  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , Na, Mg, P, Ca, Mn, Co, Ni, Zn, Rb, Sr and Ba. One-way analysis of variance (ANOVA) was performed to identify significant differences in isotopic and elemental content of different berry species.

Significant differences ( $p < 0.05$ ) were found in isotopic and macro/micro elements in different berry species. Differentiation of the investigated fruits based on isotopic content can be explained by the fact that the degree of isotopic enrichment also depends on the type of fruit due

to the particularities of each plant, thus resulting in differences in the isotopic and elemental profile of the fruits coming from the same geographical area.

Among the studied macro-minerals, the ANOVA results showed significant differences in Na, Mg, P and Ca content. Different mineral content may be due to the differential capacity of the different berry species to absorb soil ions and/or specific distribution capacity throughout the plant [13]. Taking into account the results of the content of macro nutrients in the berry samples, we can observe the highest concentration of Na and the lowest content of Ca in sea buckthorn samples compared to raspberries, blackberries and cranberries. Samples of raspberries and blackberries contain higher levels of Mg and P compared to cranberry and blueberries, while sea buckthorn has higher P levels than blueberries.

Among the studied micro-elements, the ANOVA results showed significant differences in Mn, Co, Zn, Ni, Ba, Rb and Sr content. Cranberries exhibited the highest Mn concentration compared to the other berries studied. Concerning the Zn and Ni content, a higher level was recorded in raspberries and sea bass compared to cranberries and blueberries. In our study, the determined Ni concentration was within the acceptable range. The analysis carried out has determined that blackberries are a good source of Co as compared to sea buckthorn and blueberries. The ANOVA test indicated higher Ba values in cranberries and blueberries compared to other berries. Rb concentrations found in raspberries were statistically lower than those in cranberries, while the Sr content in blackberries was statistically higher than the other studied berries. These differences may be due to the geochemical and geopedological characteristics of the soil where these fruits have been grown. [13].

#### **4.2. Differentiation of berries by species using linear discriminatory analysis (LDA)**

In order to obtain a more comprehensive classification of fruit samples according to the type of berry, the LDA chemometric method was used. In this case, the strongest discrimination markers ( $p < 0.05$ ) were: Ba, Na, P, Ca, and Zn. The first two discriminating DF functions (DF1 and DF2) provide sample separation (Figure 4-1). The classification results showed that 87.5% of the original cases were correctly classified and the percentage of the cross-validation procedure was 72.5%.

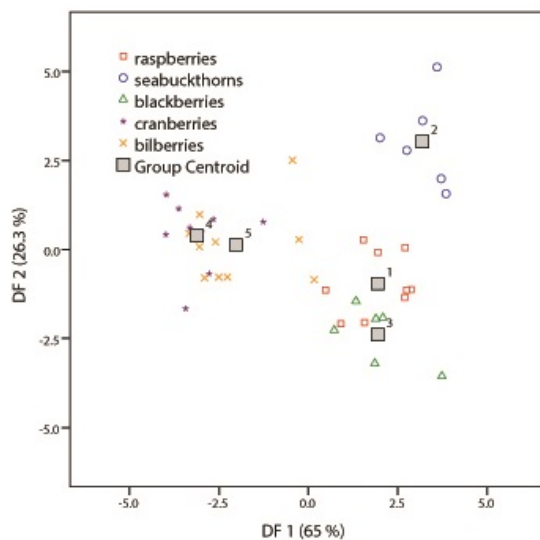


Figure 4-1. Graphical representation of berries after LDA

### 4.3. Differentiation of wild and cultivated berry samples using linear discriminatory analysis (LDA)

LDA test was performed to differentiate wild berries from the cultivated blueberries. The results obtained showed a total separation of the two categories and the samples tested have been 100% correctly classified, both by initial classification and by cross validation. The discriminant function obtained explains 100% obtaining the total variation and the Wilks lambda value of 0.002. This function has as variables the following parameters: Rb, Pb, Co,  $\delta^{18}\text{O}$ , Na, Mg.

The significant function obtained ( $p = 0.01$ , Wilks lambda 0.553) was tested and the same percentage was obtained both initially and by cross-validation. LDA test was performed to differentiate wild berries from cultivated blueberries. The results obtained showed a total separation of the two categories with a classification of the samples tested being 100% correct both originally and by cross-validation. The value of the lambda Wilks obtained was 0.001. This function has as variables the following parameters: Ag, As, Mg,  $\delta^2\text{H}$ , and Cd.

### 4.4. Determination of pesticide residues in sample of cultivated berries

Two pesticides ( $\alpha$ -BHC and chlorpyrifos methyl) have been identified and confirmed in the analyzed cultivated berries from the Transylvania area. The concentration of  $\alpha$ -BHC determined in blackberries is 0.28  $\mu\text{g}/\text{kg}$ , which is below the MRL for berries. The presence of chlorpyrifos methyl was observed in a single raspberry sample, the concentration (2.96  $\mu\text{g}/\text{kg}$ ) being also below MRL.

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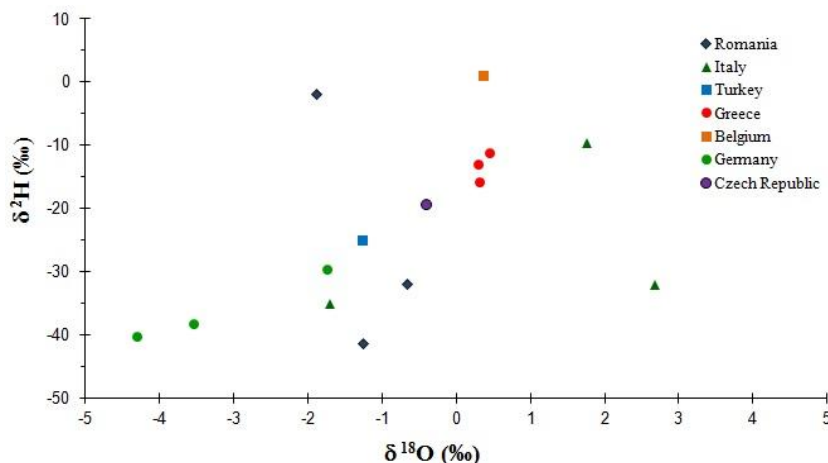
### Testing of commercial strawberries and their authentication against geographic origin

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In this chapter the content of pesticides, minerals and toxic metals present in strawberries sold on the Romanian market was evaluated. The Pearson correlation and main component analysis (PCA) were used to determine whether there are similarities in minerals and metal content in strawberry samples. In addition, to highlight the differences based on geographic origin of the strawberries studied, stable isotope ratios ( $^2\text{H}/^1\text{H}$ ,  $^{18}\text{O}/^{16}\text{O}$ ,  $^{13}\text{C}/^{12}\text{C}$ ) were determined.

Figure 5-1 shows the isotopic values of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  corresponding to the water extracted from the investigated samples. It can be seen that the lowest isotopic values were obtained for strawberries from Germany while the highest values were obtained for the two samples from Italy. These differences were to be expected and explained by the climatic and meteorological differences between these two countries in terms of isotopic footprint, average temperature and precipitation. Significant differences in  $\delta^{18}\text{O}$  are also observed between samples from Italy, one of them having a lower value, around -1.7 ‰. Differences between the Italian samples may be due to the origin of the fruit, the different climatic and meteorological conditions, or the use of irrigation water with different isotopic compositions. For fruit samples from Greece, the results were very close, indicating that the crops were most likely grown under the same climatic and meteorological conditions. Strawberry samples from: Romania, Belgium, the Czech Republic and Turkey show the isotopic values of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in between of those from Germany and Italy, in a direct correlation with their climatic conditions [13]. The isotopic variation  $\delta^{13}\text{C}$  was approximately 2.6 ‰, the variation range being between -24.3 ‰ (sample S<sub>9</sub>) and -27.2 ‰ (sample S<sub>10</sub>). These small differences indicate that  $\delta^{13}\text{C}$  is not a good indicator of geographic origin but rather gives information about plant species.

The results showed: strawberries are rich in essential minerals, such as: K P, Ca and Mg respectively. Cr, Mn, Fe, Ni, Cu, Zn, Ca, Cd and Pb were some of the other trace elements detected. Pb and Cd were found in small amounts. Recorded values for Fe and Ni concentrations were lower than 133.1 mg/kg and 8.7 mg/kg, respectively.



**Figure 5-1.** Correlation of isotope values of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  from water extracted from strawberry samples

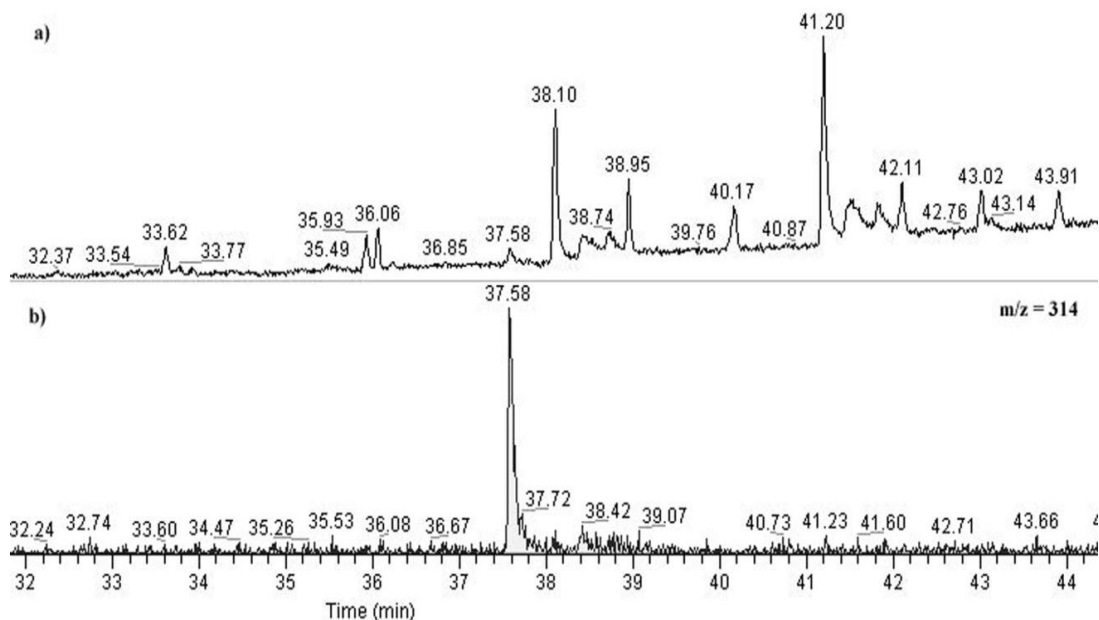
Of all the macro-minerals found in the analyzed samples, K was the most abundant one. It was present in the largest quantity in strawberries from Belgium and in a sample from Romania. Strawberries from Italy and Belgium have a higher content of Ca compared with fruits from Greece, which had an average of 1124.7 mg/kg. The results for concentrations of P indicated that strawberries produced in Greece and Belgium have higher mean values compared with those from other countries. In the case of Mg, the mean value for strawberries from Germany and the Czech Republic are close, and the ones from Turkey are characterized by the lowest concentration of this element. Pb and Cd content in the analyzed strawberries was relatively low and did not exceed the maximum allowed level, with the exception of two samples from Belgium and Romania and a sample from Greece. It can be observed that all concentrations of Zn in strawberries exceed the acceptable levels. Apart from this, 7 out of 17 strawberry samples have a high Cu content, maximum acceptable level being also exceeded.

Significant correlations between the elements in the 17 samples of strawberries were highlighted using the Pearson correlation. The correlations obtained were as follows: Mg with P (0.570), K (0.866), Cu (0.553), Zn (0.592) and Cd (0.640), P was correlated with K (0.574) and Zn (0.788) K with Zn (0.610) and Cd (0.551), Cr with Fe (0.824), Ni (0.693) and As (0.500), Fe with Ni (0.784), Cu with Cd (0.665) And finally Cd with Pb (0.689). No negative correlations were observed. Mn and Ca are not correlated with other elements. These associations between different elements could suggest the same place of origin [14].

In the case of pesticides, only one compound was detected and identified in sample  $S_2$  from Greece. Figure 5-2 shows the chromatogram obtained on GC-MS of sample  $S_2$  over the 32-

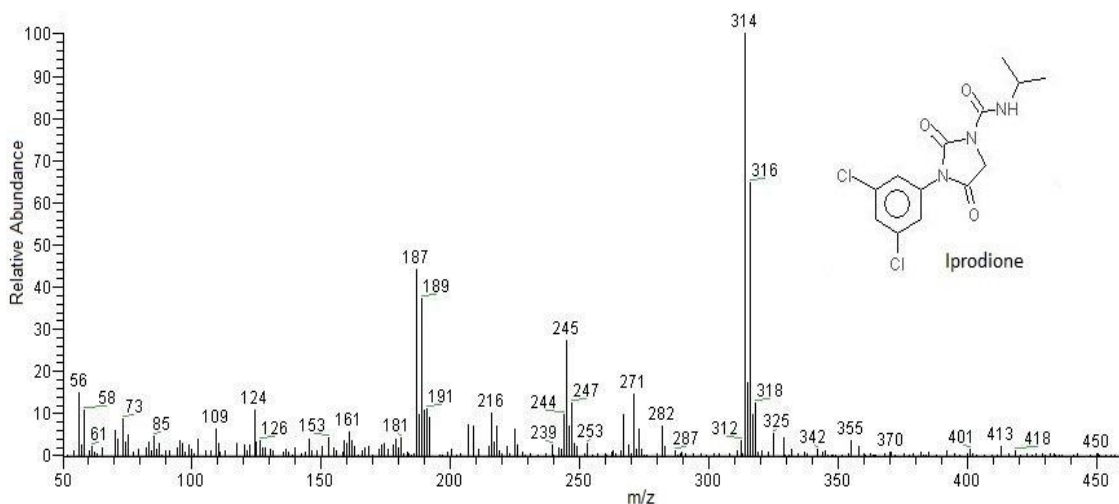


44 minute time interval. By selective monitoring of ion  $m/z = 314$ , it can be seen that a visible peak appears at 37.58. The mass spectrum of this compound is shown in Figure 5-3. Based on the mass spectrum, the compound was identified as a common fungicide called iprodione. To determine the quantity of Iprodione in the sample attributed to the chromatographic peak, a comparison with the area of the external standard 2,4,6-Tri-chlorobiphenyl (PCB 30) was made. The quantity of the identified compound calculated this way was 8.6  $\mu\text{g}/\text{kg}$ . Other pesticides were not detected in the other strains of strawberry analyzed [14].



**Figure 5-2.** GC-MS chromatogram of sample  $S_2$  ranging from 32-44 min.

a) Chromatogram of total ionic current (TIC), b) Chromatogram with selective ion monitoring  $m/z = 314$  (SIM)



**Figure 5-3.** Mass spectrum of the identified compound from  $T_R = 37.58$  min

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### Evaluation of elemental, isotopic and pesticidal content in tomatoes grown outdoors and in greenhouses

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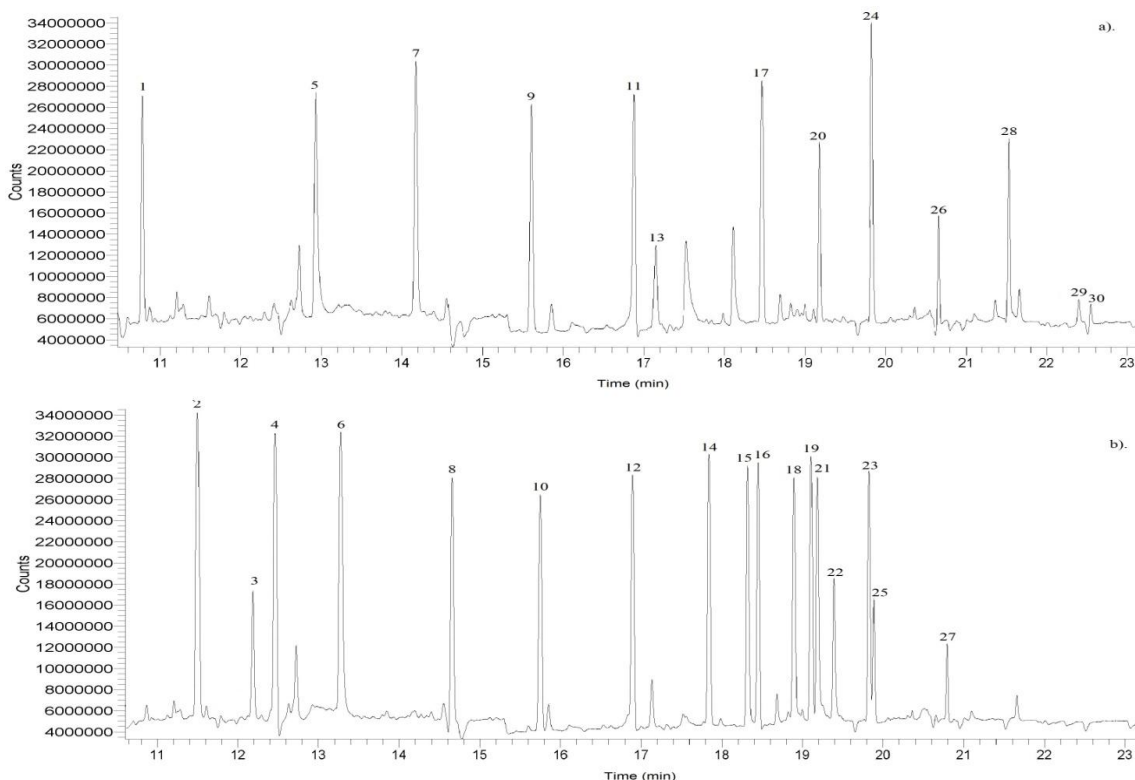
Tomatoes (*Lycopersicon esculentum* sin.: *Solanum lycopersicum*, *Lycopersicon lycopersicum*) belong to the Solanaceae family and are one of the most widespread vegetables in the world [15]. Commercial greenhouses provide optimal growth conditions for these plants and are widely used to produce high-quality fruits and vegetables [17]. Moderate temperature and high humidity favor plant growth, but at the same time contribute to the spread of pests and diseases. In the greenhouse, fungal diseases develop, such as the blight (*Phytophthora infestans*) and gray mold (*Botrytis cinerea*) [18]. Insects and mites are also omnipresent in greenhouses and insecticides are widely used in the protection of tomatoes [19]. For this reason, for the control of pests, tomatoes grown in the greenhouse are sprinkled with different types of fungicides and insecticides [16, 17].

In this chapter, tomatoes grown outdoors were differentiated from those grown in greenhouses, using differentiation markers: the content of pesticides, micro-macro elements and isotopic. For this purpose, the concentrations of the 30 pesticides studied were determined applying the QuEChERS extraction method and detection and identification by GC-ECD and GC-MS. The content of micro and macro elements, essential elements, toxic metals, rare-earth elements and isotopic content was determined for tomatoes grown outdoors (7 samples) and for tomatoes grown in greenhouses (14 samples) and available on the Transylvanian market.

#### 6.1. Determination of pesticide residues in tomatoes grown in greenhouse and outdoors

Figure 6-1 shows a chromatogram of a fortified tomato sample at a level of 40 µg/ml of the extract from the two standard pesticide mixtures used during the study. Out of the 30 pesticides followed 20 were detected, identified and quantified. Only two samples showed no traces of pesticide residues and one sample showed only one pesticide residue (chlorpyrifos methyl). A total of 8 samples showed combinations of 2 and 3 pesticides (4 samples with 2 pesticides and 4 samples with 3 pesticides, out of the four samples, two are from tomatoes grown in the greenhouse and 2 grown outdoors). Ten of the thirty pesticides that were studied were not detected in any of the analyzed samples. Out of the 20 pesticide residues found, 17 are

insecticides, two herbicides and one fungicide. Detected levels of residues ranged from <LOQ to 5.74 µg/kg (except for two samples of tomatoes grown in the greenhouse where 60.07 µg/kg *lambda*-cyhalothrin and 63.15 µg/kg chlorpyrifos methyl respectively were detected, concentrations that are over the MRL set for tomatoes.



**Figure 6-1.** The GC-ECD chromatogram of the fortified tomato sample at 40 µg/kg of each compound a). Standard mixture of pesticides (AOAC) and b). Standard mixture of pesticides (Appendix IX)

Although pesticide residues detected in almost all the samples were below MRL, the detection of *lambda*-cyhalothrin and chlorpyrifos methyl above MRL in two of the samples and also the simultaneous presence of two and three pesticide residues may suggest that the principles of good agricultural practice are not respected [20].

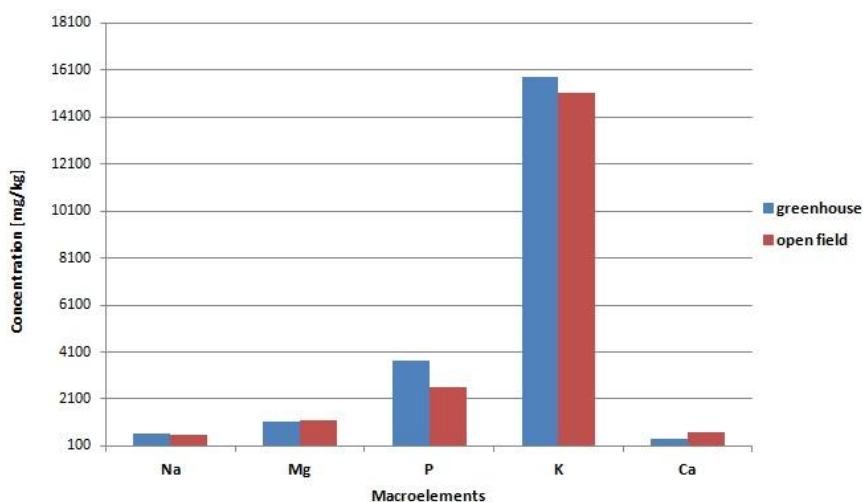
The R<sub>21</sub> tomato sample has the largest number of detected pesticide residues (9 pesticides). Endrin,  $\delta$ -BHC, chlorpyrifos methyl, and endosulfan I and II were the most common pesticides detected in the samples. The presence of more than one pesticide in one sample was more frequent in tomatoes grown in greenhouse than in tomatoes grown outdoors.

These results are a direct consequence of practices applied in pest management on each system. Under the outdoor growing conditions, the accumulation of pesticides on fruits can be reduced by the direct effect of precipitation and solar radiation. On the other hand, despite the

higher yields that can be achieved, growing conditions in greenhouses favor the development of outbreaks of arthropod pests and plant disease, which leads to the excessive use of pesticides. Pest control practices, continuous fruit baking, time spans between spraying and harvesting in both methods (greenhouse/outdoors) are not respected by producers. Our results do not indicate a clear difference in pesticide residues between tomato production systems, both systems presenting pesticide levels above the detection limit. The percentage of pesticide detected in each sample was higher for the greenhouse samples. Only  $\alpha$ -BHC and dieldrin were detected in the outdoors tomatoes, but in amounts below MRL.

## 6.2. Determination of macroelements in tomatoes grown in greenhouse and outdoors

The experimental study conducted on the determination of macroelements (Na, Ca, K, Mg, P) of the studied tomato samples showed a wide range of concentrations. The total content of macroelements present in the tomato samples studied can be arranged in the following order: K > P > Mg > Ca > Na. Higher concentrations were obtained for P and K. Comparing the macroelement content in the samples of greenhouse tomatoes versus outdoors tomatoes it was found they have close average values, 4036.55 mg/kg macroelements for outdoors grown tomatoes and 4483.903 mg/kg for those grown in the greenhouse (Figure 6-3). A bigger difference was observed for Ca, the mean concentration being 687.219 mg/kg for tomatoes grown in the outdoors versus 433.088 mg/kg for those grown in the greenhouse, this being justified by the differences in soil structure.

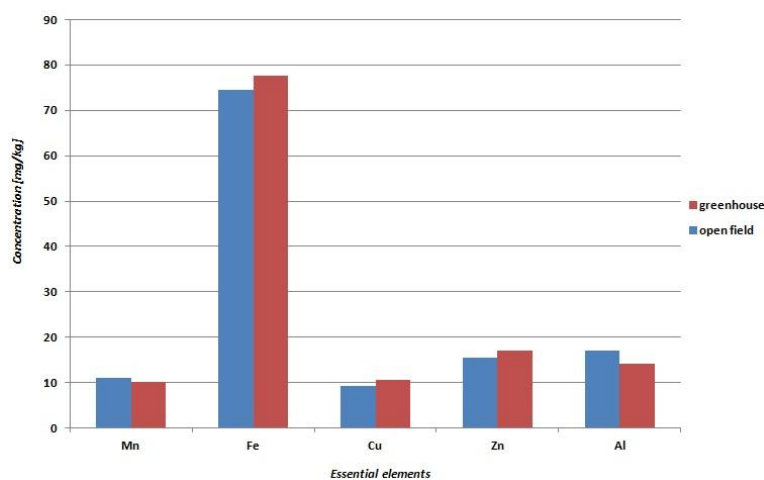


**Figure 6-3.** Diagram of macroelement content in comparative greenhouse/outdoor tomato samples

### 6.3. Determination of essential elements and microelements in tomatoes grown in greenhouse and outdoors

Mn was detected in all the studied tomatoes with an average of 10.524 mg/kg. Concentrations of Fe and Cu ranged between 24.736-211.539 mg / kg for Fe and 5.492-28.337 mg/kg for Cu. Zn concentrations ranged between 10.644-31.459 mg/kg. Ni concentrations had an average of 2.737 mg/kg. Al had values between 1.41-34.307 mg/kg . The St concentration had a range between <0.0005 - 0.683 mg/kg. The mean values of the microelement concentrations: B, Ti, V, Co, Ni, Rb, Sr, Ba, Sn, Li were 11,425 mg/kg for B, 1,310 mg/kg for Ti, 0.092 mg/kg for Co, 2.736 mg/kg for Ni, 16.645 mg/kg for Rb, 3.752 mg/kg for Sr, 0.108 mg/kg for Sn, 1.165 mg/kg for Ba, 0.085 mg/kg for Li.

By comparing the element concentrations of the outdoors versus greenhouse samples, as seen in Figure 6-4, tomatoes grown outdoors have lower values of Cu, Zn, Fe and Mn, Al than those grown in the greenhouse. High concentrations of Fe could be explained by the use of metal tools ( made of alloys high in Fe ).



**Figure 6-4.** Comparative study of the content of essential elements in samples of greenhouse/outdoor tomatoes

### 6.4. Determination of toxic metals in tomatoes grown in greenhouse and outdoors

The levels of Pb in the analyzed samples had a range between <0.0005-0.28 mg/kg, with the samples of tomatoes grown outdoors having the lowest values. Some of the samples both from greenhouse and outdoors showed values above the legal limit of 0.1 mg/kg. Cd was present in all the tomato samples studied with concentrations in the range of 0.028-0.26 mg/kg. The tomatoes grown outdoors had an average of 0.079 mg/kg of Cd and the tomatoes grown in greenhouse had an average of 0.096 mg/kg of Cd, values exceeding the legal limit of 0.05 mg/kg

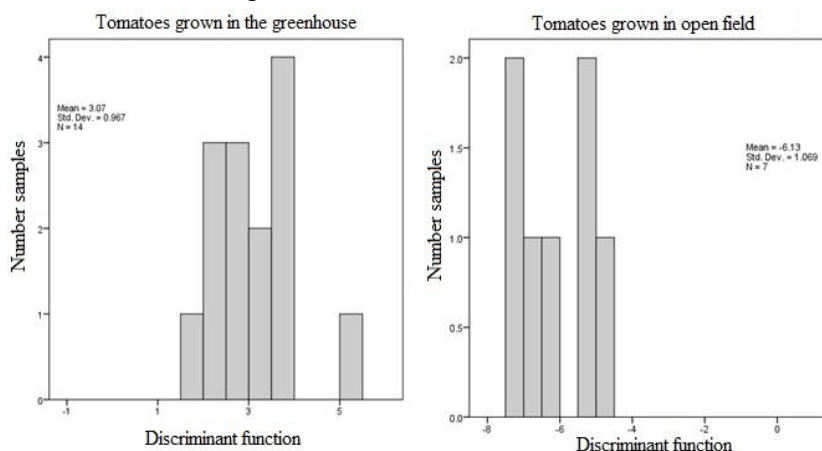
[21], [22]. As concentrations were in the range of 0.0005-0.109 mg/kg. The Cr content in the samples of the tomatoes grown outdoors is higher than of those grown in the greenhouse. There is little difference between the Hg content of greenhouse tomatoes and outdoors tomatoes.

### 6.5. Determination of critical minerals and rare-earth elements in tomatoes grown in greenhouses and outdoors

The concentration of all critical minerals was in the range of <0.0005-0.132 mg/kg, with the exception of Gd which had a higher value in a sample of greenhouse tomato. Rare-earth metal content was between <0.0005 and 0.833 mg/kg , W having the highest value in a sample of outdoor tomato.

### 6.6. Differentiation of samples of tomatoes grown outdoors versus tomatoes grown in greenhouses using chemometry

In the statistical processing of the experimental data obtained the elemental composition, and the isotopic and pesticidal content were used as independent variables. Linear analysis of discrimination was applied to the matrix formed, the two predefined groups being represented by the outdoor and the greenhouse tomatoes (the dependent variable). Applying this method, a 100% initial classification was obtained (Figure 6-5), with the same percentage for cross-validation. Due to the fact that two categories (groups) were compared, a statistically significant discriminating function was obtained which is capable of explaining 100% of the variability of the experimental data. This function has as main parameters the following elements: Al, Sc, Se, Dy, Pb,  $\delta^{18}\text{O}$ , 4,4'-DDT. These elements proved to be the best predictors to match an unknown sample to the outdoor tomatoes or greenhouse tomatoes.



**Figure 6-5.** Initial separation of the two tomato groups after application of the discriminant analysis

The main purpose of this PhD thesis was to perform a quality control of some vegetables and fruits commercialized on the Romanian market using complementary methods of analysis. The parameters of interest were: pesticide residue content, isotopic ratios of  $^2\text{H}/^1\text{H}$ ,  $^{18}\text{O}/^{16}\text{O}$  (to obtain information on geographic origin) and  $^{13}\text{C}/^{12}\text{C}$  (to have information on the botanical origin of vegetables and fruits), and the content of micro and macroelements. For this purpose the following analysis techniques were used: gas chromatography with flame ionization detector and electron capture detector, gas chromatography coupled with mass spectrometry, mass spectrometry for isotopic ratios and inductively coupled plasma mass spectrometry.

A total of 159 samples collected from supermarkets and farmers markets in Cluj-Napoca, Romania have been investigated. 50 samples of vegetables (carrot, potato, parsley, parsnip, green beans, zucchini, eggplant, cabbage, salad, cucumber, cauliflower, sweet pepper and kapia pepper) were investigated for pesticide residue content, 88 samples of berries and 21 samples of tomatoes were investigated for the isotopic, micro and macroelement content and the pesticide residue.

In order to determine the levels of pesticide residues in complex matrices such as vegetables and fruits, the QuEChERS extraction method was used. The novelty of the study was to optimize this method in order to obtain much cleaner extracts and to remove unwanted compounds from the matrix that could interfere with compounds of interest (usually present in very small amounts) during the analysis. The proposed method was used to monitor 30 organochlorine pesticides (commonly used in spraying vegetables and fruits) and subject to validation protocol to establish the method's performance and its applicability in the routine analysis of vegetables and fruits. The results obtained showed good selectivity, linearity, precision, sensitivity and reproducibility compared to literature data emerging in recent years, demonstrating that the method is suitable for multi-residue analysis of pesticides. Also experimental conditions used for pesticide detection were modified so that the analysis time of approximate 30 minutes was very reasonable.

The results of the investigation of 50 vegetable samples have provided important information on the pesticide contamination of five commonly consumed vegetables, and have

highlighted the need to control the use and application of plant protection products, in particular persistent pesticides such as  $\alpha$ -BHC, heptachlor epoxide, dieldrin and endrin. Tolyfluanide and chlorothalonil were the compounds most frequently detected. The largest amount of tolyfluanide residue was determined in a potato sample. Out of the 30 organochlorine pesticides monitored, 21 have been detected with 18 of them being insecticides, 2 fungicides and one herbicide. The presence of pesticide residues in vegetables and fruits could be caused by their illegal use or their timetable. Potatoes were the contaminated with the largest number of pesticides. Pesticide residue concentrations in the investigated samples were generally low, however, their concentration in some vegetables exceeded the maximum permitted levels.

Another novelty of this work was the combination of several complementary analytical methods in terms of food quality and safety, with particular reference to berries from Transylvania region. It presents the first isotopic fingerprinting of berries and the initiation of the first database containing all the determined parameters. This study is new at national level.

In order to differentiate between wild and cultivated fruit, the isotopic composition of carbon, oxygen, and hydrogen was determined by IRMS, and ICP-MS technique was used for complementary elemental content measurements.

Based on the obtained results there were significant statistical differences in the isotopic and micro-macroelement content of berries grown in different areas of Transylvania. Isotopic values of cultivated berries are higher than those of wild berries. This is explained by the difference in altitude, wild berries growing at higher altitudes than those cultivated. Precipitation at high altitudes has a lower isotopic composition compared to low altitudes. The results are consistent with the theory of isotope separations. Correlations between isotopic and elemental determinations were made using chemometric methods. Pesticide residue levels were determined only in cultivated berries with two pesticides detected ( $\alpha$ -BHC and chlorpyrifos methyl).

With the purpose to authenticate against geographic origin, 17 samples of strawberries produced in 7 different European countries were tested. For this purpose the stable isotope ratios were determined, the so called "bio-elements" ( $^2\text{H}/^1\text{H}$ ,  $^{18}\text{O}/^{16}\text{O}$ ,  $^{13}\text{C}/^{12}\text{C}$ ). The differences between the obtained isotopic ratios correlate perfectly with the climatic values of the cultivation region (the lowest values were recorded for a sample from Germany and the highest for a sample from Italy). From the results of mineral content in strawberries it can be seen that both in terms of quality and quantity, the elements strongly depend on the region the plant grows in.



Differentiation of strawberry samples in terms of mineral and metal content was determined based on the chemometric analysis (Pearson correlation and main component analysis). Of all the samples investigated only one from Greece contained pesticides. In order to identify the pesticide the coupled gas chromatograph with mass spectrometer was used. Through selective monitoring of  $m/z = 314$  ion a visible peak can be seen at min. 37.58. Based on the mass spectrum, the compound was identified as a common fungicide called iprodione commonly used in the treatment of strawberries.

Pesticide content, micro and macro element content and the isotopic ratios were used as differential markers in order to distinguish between tomatoes grown outdoors and tomatoes grown in greenhouses. The pesticide residue content ranged from below the limit of quantification to 5.74  $\mu\text{g}/\text{kg}$  with the exception of two samples of tomatoes grown in greenhouse that had 60.07  $\mu\text{g}/\text{kg}$  *lambda*-cyhalothrin and 63.15  $\mu\text{g}/\text{kg}$  chlorpyrifos methyl. Based on the data obtained, pesticides alone do not indicate a clear difference between the two tomato production systems, both systems presenting pesticides above the detection limit. The total content of macroelements present in the studied tomato samples can be arranged in the following order :  $\text{K} > \text{P} > \text{Mg} > \text{Ca} > \text{Na}$ . The measurements were also focused on the so called essential elements (Mn, Fe, Cu, Zn) and toxic metals (Pb, Cd, As, Cr and Hg), their concentrations being discussed as per investigated sample group. Oxygen isotopic ratios and rare-earth elements content have a high discriminant potential and can be used as markers in the differentiation of vegetable categories (organic/conventional and greenhouse/outdoor) .

The results of this study confirm the need to investigate the quality of vegetables and fruits and to create the premises for implementing a monitoring program and a high-performance laboratory at local level.

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