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Abstract of the Ph.D. Thesis

CHROMATOGRAPHIC METHODS USED IN FOOD QUALITY CONTROL

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Aims and objectives of the research

The use of food additives in the food industry is considered a necessity in the current context. However, some substances in this category have been proven to be toxic, causing various side effects. These can range from immediate symptoms, such as nausea and dizziness, to severe long-term conditions, including cancer or liver diseases, due to their gradual accumulation in the body.

Although fruits and vegetables are perceived as a healthy option and are recommended by nutritionists for every meal, it is important to consider that pesticides are increasingly used in cultivation. These chemicals accumulate in the produce and eventually reach the human body. The excessive use of pesticides in recent years has led to substantial accumulation in food, soil, air, and water, ultimately affecting human health. This poses a significant risk, making it crucial to monitor the quantities of pesticides used.

This thesis aims to explore the development of new chromatographic methods for the determination of food additives in vegetable-based products and the detection of pesticide residues accumulated in various vegetables.

The purpose of these studies is to raise awareness about the risks of excessive use of food additives and pesticides, and especially the danger of exposing the body to them.

Considering the national situation in recent years regarding the use of food additives and pesticides in agriculture, this paper's main objective is to develop chromatographic methods applicable to food quality control for the determination of both food additives and pesticide residues. The focus is on vegetables sourced from agro-food markets, retail stores, and those labeled as BIO and ECO, as well as vegetable-based products.

1. Food additives

Food additives are chemical substances intentionally added to food products to enhance their characteristics, such as taste, appearance, texture, shelf life, or stability. The classification of food additives includes several categories, such as preservatives, colorants, emulsifiers, sweeteners, and flavor enhancers, each playing a specific role in maintaining food quality. The purpose of food additives is to improve or preserve certain characteristics of foods demanded by consumers, whether physical, chemical, biological, or sensory (Velazquez, 2019). Many low-calorie snacks and convenient ready-to-eat foods would not be possible without the use of food additives.

Food additives have proven useful in maintaining the food distribution network and have contributed to consumer convenience by providing safe, healthy, and visually appealing ready-to-eat products, helping to keep pace with rapid urbanization and the growing demand for "fast" food (Mwale, 2023).

Although many additives are considered safe for consumption, controversies persist regarding their impact on human health. Certain additives, especially when used excessively or in combination with other substances, may have negative effects, such as allergic reactions, digestive disorders, or even long-term risks, including chronic diseases.

1.1.Flavor enhancers

Flavor enhancers are used to impart taste or aroma to foods and are found in a wide range of products, from confectionery and soft drinks to cereals, cakes, and yogurts, typically in relatively small amounts. A flavor additive can be a single chemical compound or a mixture of compounds, either synthetic or natural, added to food to enhance its natural flavor, introduce a new flavor, or replace those lost during food processing.

The most well-known additive in this category is monosodium glutamate (MSG) (Figure 1), listed under the code E621 and considered a safe additive by EFSA or FDA when consumed in moderate doses. Chemically, it is the sodium salt of glutamic acid, a non-essential amino acid that naturally occurs in various foods (cheeses, fish, certain vegetables).

Monosodium glutamate (MSG) is frequently used to enhance the natural flavor of certain foods, particularly in soups, broths, canned and frozen vegetables, spice mixes, meat, poultry, seafood, and various sauces. Currently, MSG is commercially produced through bacterial fermentation of starch and molasses, followed by a reaction with ammonium salts. EFSA has currently established a safe acceptable daily intake for glutamates at 30 mg/kg/day.

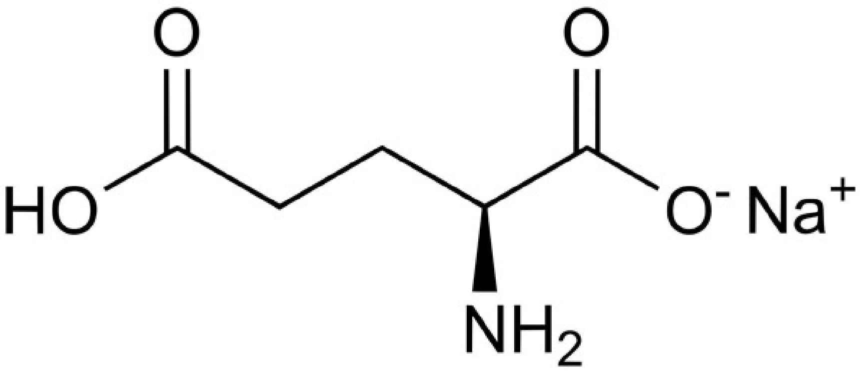

Figure 1. Chemical structure of monosodium glutamate (MSG)

Over time, the consumption of MSG has been the subject of controversies regarding its health effects. In the past, it was associated with the so-called "Chinese Restaurant Syndrome," characterized by symptoms such as headaches, dizziness, nausea, and palpitations. However, scientific studies have failed to confirm a direct link between MSG and these reactions in healthy individuals.

Reactions to monosodium glutamate (MSG) can be cumulative, meaning that occasional consumption, such as once a week, is unlikely to cause negative effects. However, repeated intake of the same product over a longer period—such as daily consumption of fast food, snacks, or soy sauce—may increase the likelihood of adverse effects. This prolonged exposure can amplify certain risks associated with MSG, including discomfort or sensitivity, nausea, and even obesity.

2. Pesticides

Pesticides are used in agriculture to protect crops from pests, diseases, and weeds, thus contributing to increased production and food safety. However, excessive or improper use can have negative effects on the environment, human health, and biodiversity. The ideal pesticide would destroy the target pest without causing harmful effects on humans, non-target plants and animals, or the environment.

Pesticides are classified based on their chemical structure, origin, and target organism. They can either be synthetic inorganic pesticides (produced synthetically) or biological pesticides (bio-pesticides). Essentially, synthetic inorganic pesticides are of synthetic origin and work by directly destroying pests. Pesticides can have negative effects on health and the environment. Higher levels of pesticides have been detected in fruits and vegetables, which are essential foods in the population's diet. Dietary pesticide intake (especially the ingested amount) varies according to age groups, which can lead to differing levels of pesticide exposure. Due to negligence in the correct use of pesticides in agriculture—aimed at increasing production quantities and making products more visually appealing—or their excessive application, more and more hazardous residues are accumulating in food.

Synthetic chemical pesticides are considered one of the greatest threats to food safety and quality at the European level. For this reason, strict measures have been adopted regarding the use of synthetic pesticides, with some already banned by the European Union. Restricting synthetic pesticides is a measure taken to reduce their negative impact on the environment and human health.

Biopesticides are compounds or agents that occur naturally and are derived from animals, plants, certain minerals, or microorganisms such as bacteria, viruses, fungi, protozoa, microalgae, and nematodes. Over time, it has been reported that biopesticides effectively control certain pests and plant pathogens while simultaneously reducing adverse effects on crops, the environment, and human health (Cai, Dimopoulos, 2025). Natural pesticides undoubtedly seem healthier, but the limitation lies in the amount of a specific substance ingested. For instance, a copper derivative is used as a fungicide in organic farming, but if ingested in inappropriate amounts, it can be toxic. Rotenone is known for its high toxicity to fish.

In this context, "organic" agriculture was designed and introduced as a sustainable production system that excludes the use of synthetic pesticides and chemical fertilizers, promoting ecological practices to protect soil, water, and biodiversity. Organic farming uses biopesticides or naturally occurring chemical pesticides that have evolved in plants. It focuses on crop rotation, the use of compost and natural fertilizers, as well as biological pest control. Products obtained through organic farming are considered healthier because they do not contain chemical residues and adhere to strict certification standards.

The principles of organic farming are based on the responsible use of natural resources through practices such as crop rotation, composting, the use of organic fertilizers, and biological pest control. These methods help maintain soil fertility, reduce water pollution, and protect local ecosystems.

Personal contributions

3. Matrix-matching calibration as a new approach for quantitation of monosodium glutamate in food seasoning powders using high performance thin layer chromatography (Rad et al., 2025)

3.1. Introduction

Monosodium glutamate (MSG) is the sodium salt of the L – glutamic acid, a widespread amino acid naturally found in two forms: bonded with others amino acids in proteins and peptides and free as glutamate in different foodstuffs such as meat, seafood, vegetables and fruits. When glutamic acid is bonded it is tasteless, but in the free form it enhances the natural flavor of certain foods, being responsible for the fifth taste called "umami" taste.

The potential harmful effects of MSG consumption on human health, as well as the widespread use of this flavor enhancer, have required the development of rapid and robust methods for its determination in various food samples in order to ensure food safety, food quality and authenticity.

The aim of this research was the developing of a simple, sensitive and specific HPTLC method to quantify the MSG in food seasoning powders (FSPs), without pre-treatment of sample or using advanced equipment.

3.2. Experimental part

For this study, a homemade vegetable mix was used, consisting of carrots, root parsley, parsnips, celery, red and yellow bell peppers, red and white onions, and fresh parsley. In addition to this mix, 6 different types of commercially available bases for dishes were also used:

- Delikat
- Maggi Secretul gustului
- Rollton – 12 vegetables
- Vigora
- Mirodenia (with the label" no added MSG")
- Vegeta Naturella (with the label" no added MSG")

The stock solution of MSG was obtained by dissolving 0.01 g accurately weighed in 20 mL distilled water and sonicated the mixture for 15 min and filtered through a filter paper. Aliquot of 1 g of each seasoning powder sample accurately weighed was mixed with 50 mL water and then was subjected to same operation as stock solution.

The homemade seasoning powder was obtained from carrots, red and yellow peppers, white and red onions roots, parsley leaves and roots, celery roots and parsnip roots. The vegetables were washed, weighed, sliced, and then they were dried in the oven to constant weight, grinded and mixed to obtain the homemade seasoning powder. Aliquot of 1 g of this powder and 1 g of each dried vegetable was mixed with 20 mL water and the solutions were sonicated for 15 min and then filtered. The obtained filtrates were kept in the refrigerator until analysis.

The chromatographic analysis was performed on HPTLC silica gel 60 F$_{254}$ glass plates. Chromatographic elution was done at room temperature, in a TLC twin through chamber (Camag) pre-saturated for 30 min with mobile phase consisting of a mixture of 1- propanol - acetic acid - water 6:2:1 (v/v/v). After development, the dried plate was immersed for 3 s in 1 % ninhydrin solution prepared in acetone and heated at 60°C for 5 min.

Statistics for calibration

The following calibration methods were analyzed in this study:

- linear equation – $y = ax + b$
- quadratic equation – $y = ax2 + bx + c$
- logarithmic equation – $y = a\ln x + b$
- power equation – $y = axb$

The following criteria were used for evaluation of calibration equations:

- determination coefficient: $R^2 = 1+SSR/SST$ (1)

where *SSR* - *sum squared regression* - is the sum of the residuals squared, and *SST* - *total sum of squares* - is the sum of the distance the data is away from the mean all squared.

- standard error in the estimate errors:

$$s = \frac{\sqrt{\sum(y_i - \hat{y}_i)^2}}{n-1} \qquad (2)$$

where *yi* is the dependent variable, ˆ*yi* is the predicted value for the calibration equation and *n* is the number of data points

- prediction Sum of Squares:

$$PRESS = \sum_{i=1}^{n} (y_i - \hat{y}_{i(i)})^2 \tag{3}$$

where yi is the dependent variable and $\hat{}yi(i)$ is the value of the omitted response value predicted based on the regression equation using n-1 observations.

The proposed HPTLC method will be validated in terms of selectivity, limit of quantification (LOQ) and limit of detection (LOD). The following formulas were used to calculate the two limits>:

$$LOD = (3{,}3 \times sd)/a \tag{4}$$

$$LOQ = (10 \times sd)/a \tag{5}$$

where sd is standard deviation and a is slope.

3.3. Results and discussions

For the quantitative determination of MSG in vegetables, the standard calibration curve was obtained by HPTLC analysis of the MSG solutions of different concentrations. Also, since it was found that vegetables also contain native MSG, the effects of the matrix for the MSG-matrix combination were evaluated based on the matrix-matched calibration curve in order to determine the MSG as accurately as possible in real samples of seasoning powders.

Following the chromatographic analysis, different calibration methods, linear, quadratic and non-linear regression models, were tested in both cases. Selecting an appropriate calibration model, respectively the order of the calibration curve is an important part of the quantitative analysis and method validation, calibration equations being the subject of many studies because there is no general model for all cases. The evaluation of linear, quadratic and non-linear equations to be used in chromatography was presented in the literature and has demonstrated that quadratic and non-linear equations determine a better fitting agreement than linear equations due to slightly curved calibration curves (Rodrıguez et al., 1993; Huber, 1997; Chen and Chen, 2022).

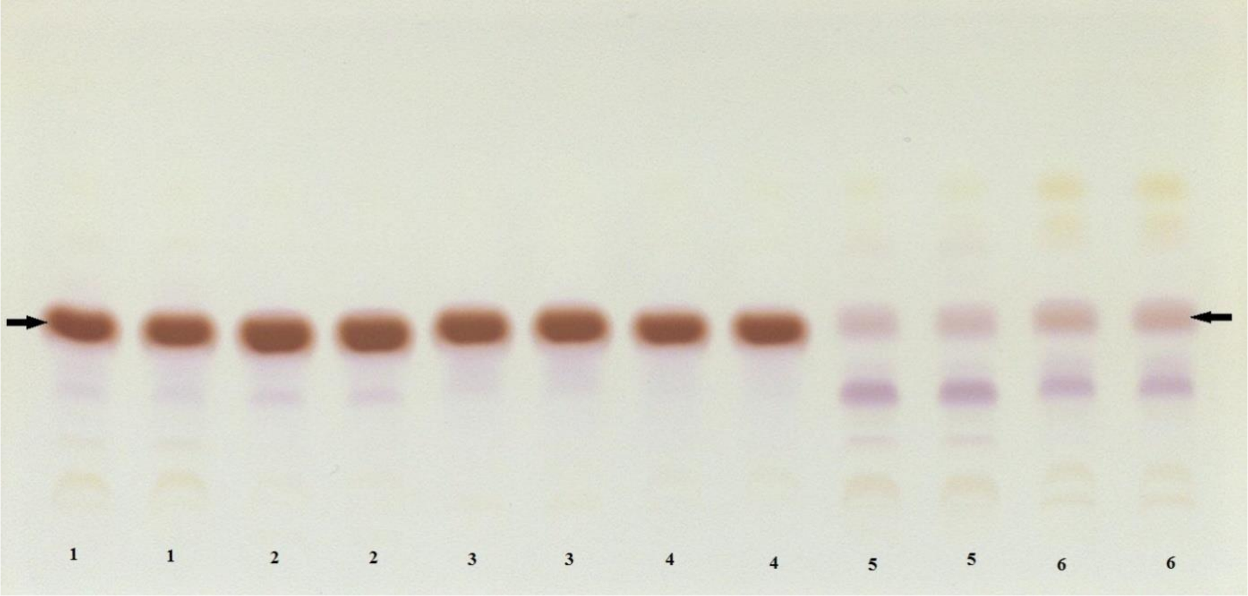

Figure 1. The HPTLC separation of commercial FSPs obtained on silica gel 60 F254 with the mixture of 1-propanol - acetic acid - water 6:2:1 (v/v/v) as mobile phase, derivatization with nynhidrin and detection in visible light.1- Rollton, 2- Delikat, 3-Maggi, 4-Vigora, 5-Mirodenia, 6-Vegeta Naturella

For the evaluation of calibration equations, the determination coefficient (R^2) and standard error in the estimate errors (s) is used as criteria of fitting-agreement and Prediction Sum of Squares (*PRESS*) is used for comparison of the prediction ability for tested equations. Even if R^2 is often used as a criterion for evaluating calibration equations, it cannot be the only criterion because it has been proven that there is no acceptable value for R^2 and, moreover, this criterion can be flawed, its numerical value being easily manipulated. In most cases, the values of R^2 used as an acceptable criterion are higher than 0.99. The s is a useful criterion because it measures the accuracy of the predictions made by a regression model and has the same unit as the response for detection techniques. It is known that the smaller the value of s, the better the regression line describes the values of a data set. The *PRESS* evaluates the predictive capacity of a model, being a method of validating it. This criterion can be used to compare regression models because, for different calibration equations, the smaller the *PRESS* value, the better the predictive ability.

Table 1. The equation of calibration curves and evaluating parameters.

Type of calibration	**Equation of the curve**	R^2	*s*	***PRESS***
Standard	**$y = -111{,}1 \pm 8{,}2x^2 + 1262 \pm 33x + 404{,}6 \pm 11{,}4$**	0,9984	52,82	35980
	$y = 660{,}1 \pm 11{,}2x + 936{,}7 \pm 27{,}7$	0,9558	276,7	501609
	$y = 1328 \pm 14\ln x + 1736 \pm 8$	0,9883	142,4	304215
	$y = 1556 \pm 4x^{0{,}6172+-0{,}0024}$	0,9930	160,4	61880
Matrice	**$y = -68.4 \pm 4.1x^2 + 864.9 \pm 18.1x + 1300 \pm 2$**	0,9998	18,13	4066
	$y = 522{,}7 \pm 2{,}2x + 1517 \pm 16$	0,9741	209,9	321902
	$y = 1131 \pm 10\ln x + 2062 \pm 2$	0,9931	68,9	79523
	$y = 2119 \pm 6x^{0{,}3879+-0{,}0051}$	0,9992	29,66	7358

As can be seen from the values obtained, if only R^2 were taken into account, there wood be more equations that should be taken into account. Thus, in the case of standard calibration, the quadratic equation ($R^2 = 0.9984$) and the power equation ($R^2 = 0.9930$) would equally well describe the fitting of the data. Looking at the other parameters in the case of standard calibration, it is observed that both *s* and *PRESS* have the lowest values in the case of the quadratic equation, so it can be concluded that this equation offers the best fit and prediction.

Instead, in the case of matrix-match calibration, although 3 equations can describe the data fitting with the same accuracy, namely the quadratic equation ($R^2 = 0.9998$), logarithmic equation ($R^2 = 0.9931$) and power equation ($R^2 = 0.9992$), the best fitting agreement and prediction is also given by the quadratic equation. As a result, the second-degree polynomial equation (the bolded equations from the Table 1) will be used for the both types of calibration curves (Figure 3 and 4), based on which the MSG will be determined.

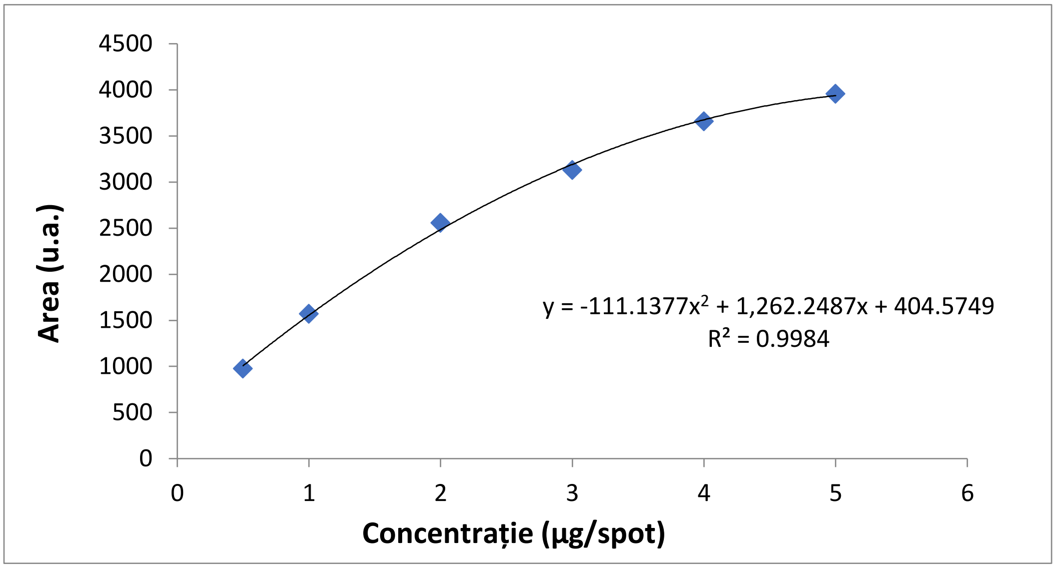

Figure 3. Standard calibration curve used for determination of MSG from vegetables

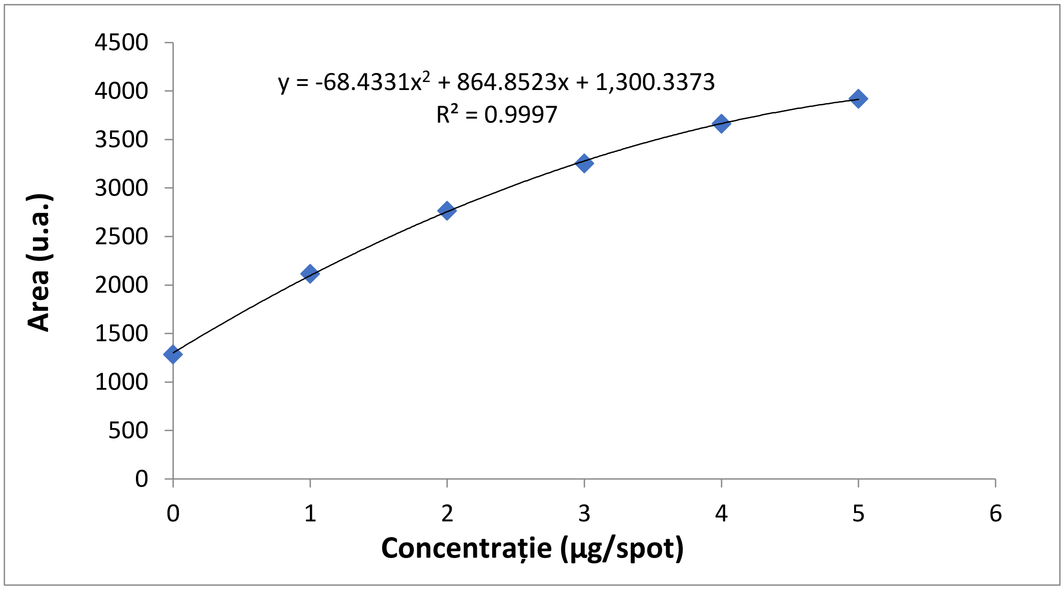

Figure 4. Matrix-matched curve used for determination of MSG from FSPs

The following results were obtained captured in **Table 2 and Table 3.**

Table 2. The amount of MSG in dry vegetables (LOD = 136,3 μg/g, LOQ = 449,8 μg/g)

Vegetable	**MSG (mg/g)**
Carrot	5,749 ± 0.372
Parsnip	2,597 ± 0.096
Celery	4,093 ± 0.173
Parsley root	1,331 ± 0.056
Parsley leaves	2,665 ± 0.148
Red pepper	0,914 ± 0.060
Yellow pepper	5,017 ± 0.211
White onion	2,510 ± 0.149
Red onion	0,623 ± 0.120
Homemade seasoning powder	3,174 ± 0.032

In order to mimic the prevailing conditions where the MSG must be determined and also the possible interactions between the MSG and other compounds present in the matrix that could be alter the observed response, the matrix-matched calibration curve was made (matrix effect, ME, %).

If the %ME values are between 0 % and 20 % the matrix effects are considered low, values between 20 % and 50 % denote medium effects, and values ≥ 50 % indicate strong effects (Koloka et al., 2023). In our case, the obtained value of %ME is – 30 % indicating medium effects of matrix determined by interferences between added MSG and native MSG from matrix. Also, the negative value obtained for %ME revealed negative matrix effects, demonstrating that matrix components suppress their signals (Dong et al., 2012). The result demonstrates that the use of the matrix-matched calibration curve will allow the accuracy problems of MSG quantification in FSPs to be overcome in a single step.

The MSG contents are different from one sample to another, sample 2 containing the highest amount of MSG. The obtained results for samples 1–4 are in agreement with those reported by Krishna et al. (2010), but native MSG is not taken into account in this research. The fact that the MSG content of samples 1–4 differs from that of samples 5 and 6 can be explained by the fact that they could contain added MSG. The low MSG content of samples 5 and 6 could be explained by the fact that even if they do not contain added MSG, according to the manufacturers' specifications, their vegetable content could be relatively low, having in their composition a high amount of sodium chloride.

Table 3. The amount of MSG in the commercial FSPs (LOD = 66.83 μg/g, LOQ = 220.5 μg/g)

Food seasoning powder	**MSG (mg/g)**
Rollton	18,246 ± 0.207
Delikat	25,013 ± 0.324
Maggi Secretul gustului	21,946 ± 0.288
Vigora	18,911 ± 0.048
Mirodenia	0,124 ± 0.012
Vegeta Naturella	0,461 ± 0.017

MSG content of samples 1–4 differs from that of samples 5 and 6 can be explained by the fact that they could contain added MSG. The low MSG content of samples 5 and 6 could be explained by the fact that even if they do not contain added MSG, according to the manufacturers' specifications, their vegetable content could be relatively low, having in their composition a high amount of sodium chloride. It can be seen that samples 1–4 contain added MSG, the concentration of the addition being several times higher compared to the MSG content that could exist naturally in the vegetables from which they were prepared. Even if the quantities of MSG determined do not exceed the maximum recommended limits, they must be taken into account in the total daily intake of MSG, considering the various sources of MSG that exist in the diet.

3.4. Conclusions

In this study a simple cost-effective HPTLC method for MSG determination in vegetables and commercial FSP was developed. Comparing with the existing procedures, the developed method consists in fewer simple steps of sample preparation, no derivatization or purification steps being needed. The presented method can be a useful tool for the determination of MSG concentration in routine analysis of different seasoning food products, for their quality control.

Although fitting to nonlinear and quadratic equations can be challenging, the results indicate that the quadratic equation fits better than the linear equation to the data considered here.

Also, matrix calibration is more effective for determining MSG in FPS because MSG can exist natively in the vegetables from which FPS is obtained.

Regular consumption of MSG for a long period of time can lead to some diseases, which should lead to more awareness of the dangerous effects of MSG, and natural alternatives to MSG should be promoted.

4. Validated Methods for the Identification and Quantification of Pesticide Residues in Vegetables and Vegetable-Based Food Products

4.1. LC-MS/MS analysis and validation of pesticides residue in vegetables asserted to be organic

4.1.1. Introduction

The excessive use of pesticides, fertilizers, and other production inputs has led to increased environmental pollution, affecting water, air, and soil, while endangering agricultural ecosystems (Peng et al., 2025). Several studies indicate a growing consumer interest in environmental issues and health-related risks, alongside a greater openness to purchasing "clean-label" foods, as well as "organic" fruits and vegetables (Morone et al., 2021).

In this study, the pesticide content in various vegetable samples was analyzed using a multi-residue analysis method (MRM) with a liquid chromatograph coupled to a triple quadrupole mass spectrometer (LC–QQQ–MS). The mass spectrometer operated in ESI – positive and negative product ion mode.

Over a two-year period (2023–2024), 160 samples were collected during the seasons when specific vegetable varieties were available. From greenhouses and solariums, lettuce, spinach, dill, parsley, and cucumbers were cultivated between January and April, while onions and peppers were grown from September to November. Throughout the summer (May–October), vegetables were cultivated directly in open fields. Among these samples, 40% were found to contain pesticide residues.

4.1.2. Experimental part

The LC method was validated for peppers in terms of linearity, accuracy, limit of detection (LOD), and limit of quantification (LOQ), achieving good linearity with correlation coefficients exceeding 0.98.

The chromatographic methods were the ones used in the previous study. The analysis was performed with MRM, using a HPLC liquid chromatograph *Agilent 1260 Infinity Prime LC* and a mass spectrometer *Agilent Hunter 6495,* with ion source temperature of 200°C. The mobile phases used were A: ultra-pure water + 0.1% formic acid and B: acetonitrile + 0.1% formic acid, with a flow rate of 0.45 mL/min, ionization gas was nitrogen, the injection volume 2 µL and the run time 29 minutes.

4.1.3. Results and discussions

Farmers use a diverse range of pesticides, both synthetic and organic, and therefore the simultaneous determination of pesticide residues in vegetables requires a better understanding of current pesticide use patterns and safety concerns. One hundred and sixty vegetable samples cropped from Transylvania (central-west part of Romania) harvested in 2023 and 2024 were analyzed for pesticides residues as part of the method evaluation. The vegetables for this study were selected taking into account the types most commonly consumed for detoxification purposes to increase quality of life. Around 40% of the samples analyzed were contaminated with pesticides.

Analyzing the results (**Table 11**), the presence of pesticides is observed in all the samples analyzed, in some cases even exceeding the MRLs.

Table 11. The results obtained on determination of pesticide residues in tested vegetables:

Vegetables	**Number of residues**	**Date of sampling**	**Pesticide**	**Conc. (mg/kg)**	**MRL (mg/kg)**
peppers	2	04.06.2023	acetamiprid	0,022	0,3
			tiametoxam	0,021	0,7
peppers	1	01.07.2023	flonicamid	0,049	0,3
peppers	2	23.07.2024	difenoconazole	0,011	0,9
			flonicamid	0,021	0,3
peppers	2	30.07.2024	flonicamid	0,017	0,3

			metalaxil	0,048	0,5
peppers	2	05.11.2023	boscalid	0,033	3
			imidacloprid	0,047	0,9
peppers	2	05.11.2023	boscalid	0,029	3
			imidacloprid	0,035	0,9
peppers	3	11.11.2024	acetamiprid	0,017	0,3
			flonicamid	0,072	0,3
			imidacloprid	0,100	0,9
peppers	2	18.11.2024	acetamiprid	0,048	0,3
			flonicamid	0,080	0,3
cucumbers	3	20.03.2023	acetamiprid	0,237	0,3
			propamocarb	0,126	5
			azoxystrobin	0,052	1
cucumbers	1	26.03.2024	propamocarb	0,200	5
cucumbers	2	26.03.2024	propamocarb	0,125	5
			dimetomorf	0,161	0,5
cucumbers	3	03.04.2023	propamocarb	0,353	5
			fludioxonil	0,012	0,5
			ciprodinil	0,011	0,4
cucumbers	2	16.04.2024	boscalid	0,014	4
			imidacloprid	0,151	0,5
cucumbers	2	13.05.2023	metalaxil	0,025	0,01
			acetamiprid	0,029	0,6
cucumbers	1	21.05.2024	metalaxil	0,012	0,5
cucumbers	1	08.11.2023	flonicamid	0,022	0,5
cucumbers	2	12.11.2024	propamocarb	0,014	5

			flonicamid	0,017	0,5
green onion	2	18.03.2023	azoxystrobin	0,253	10
			difenoconazole	0,112	9
green onion	3	16.04.2024	fluopicolid	0,050	10
			difenoconazole	0,023	9
			azoxystrobin	0,071	10
green onion	3	02.04.2023	azoxystrobin	0,034	10
			difenoconazole	0,038	9
			famoxadone	0,046	0,01
green onion	2	02.04.2024	tiametoxam	0,078	0,01
			linuron	0,033	0,01
dill	2	16.04.2023	azoxystrobin	0,253	70
			difenoconazol	0,462	10
dill	2	23.04.2024	pendimetalin	0,034	0,6
			difenoconazole	0,316	10
dill	3	07.05.2023	azoxystrobin	0,049	70
			tebuconazol	3,748	2
			difenoconazole	0,056	10
dill	2	21.05.2024	azoxystrobin	0,088	70
			difenoconazol	0,069	10
carrots	2	23.04.2023	boscalid	0,013	2
			tebuconazol	0,019	0,6
carrots	3	02.04.2024	mandipropamid	0,045	0,01
			boscalid	0,148	2
			fenhexamid	0,038	0,01

carrots	2	30.07.2023	azoxystrobin	0,017	1
			difenoconazole	0,012	0,4
carrots	1	28.10.2024	fludioxonil	0,040	1
parsnip	2	15.05.2024	boscalid	0,016	2
			tebuconazol	0,015	0,4
parsnip	2	15.10.2024	azoxystrobin	0,030	1
			difenoconazole	0,014	0,4
parsley root	3	20.03.2023	azoxystrobin	0,074	70
			tebuconazol	0,164	2
			piraclostrobin	0,111	2
parsley root	6	02.04.2024	propamocarb	0,383	30
			difenoconazole	0,116	10
			fluopicolide	0,821	9
			flonicamid	0,105	6
			piraclostrobin	0,089	2
			epoxiconazol	0,241	0,02
parsley root	5	07.05.2023	penconazole	0,087	0,02
			azoxystrobin	0,290	70
			piraclostrobin	0,037	2
			pendimetalin	0,024	2
			difenoconazole	0,478	10
parsley root	3	08.05.2024	pendimetalin	0,019	2
			difenoconazole	0,393	10
			azoxistrobin	0,270	70
parsley root	3	23.09.2023	acetamiprid	0,080	3

			difenoconazole	0,672	10
			azoxystrobin	0,348	70
parsley root	5	15.10.2024	azoxystrobin	0,274	70
			dimethomorph	0,338	10
			tebuconazole	0,227	2
			difenoconazole	0,365	10
			piraclostrobin	0,084	2
lettuce	3	29.01.2023	mandipropamid	0,140	25
			boscalid	29,9	50
			difenoconazol	0,173	4
lettuce	6	20.03.2023	carbendazim	0,686	0,1
			spinosad	0,047	4
			azoxystrobin	0,133	10
			fenhexamid	11,4	50
			piraclostrobin	1,3	2
			tiophanat-methyl	0,967	0,1
lettuce	4	12.03.2024	boscalid	0,015	50
			acetamiprid	0,42	1,5
			azoxystrobin	4,24	10
			difenoconazole	0,476	4

This demonstrates that, although they were declared as organic products, the analyzed vegetables were obtained by treating the crops with various pesticides. Most pesticides are found in one sample of lettuce (10) and in one sample of parsley leaves (6), but there are also samples of vegetables in which only one pesticide residue is found. The cases in which MRLs are exceeded are probably due to failure to comply with the break time specified by the manufacturer of each pesticide or the use of excessively high doses, and also to insufficient information of vegetable producers regarding the influence of the microclimate in greenhouses and solariums on the degradation of the pesticides used. It is important to note that in a lettuce sample, there is in addition to other pesticides an organic pesticide, spinosad that is the sum of spinosyn A and spinosyn D (**Figure 6, 7**). Although the concentration of pesticides found in a sample is below the MRLs, the number of pesticides in the sample should cause concern, such as the case of the lettuce sample harvested on 20.03.2023.

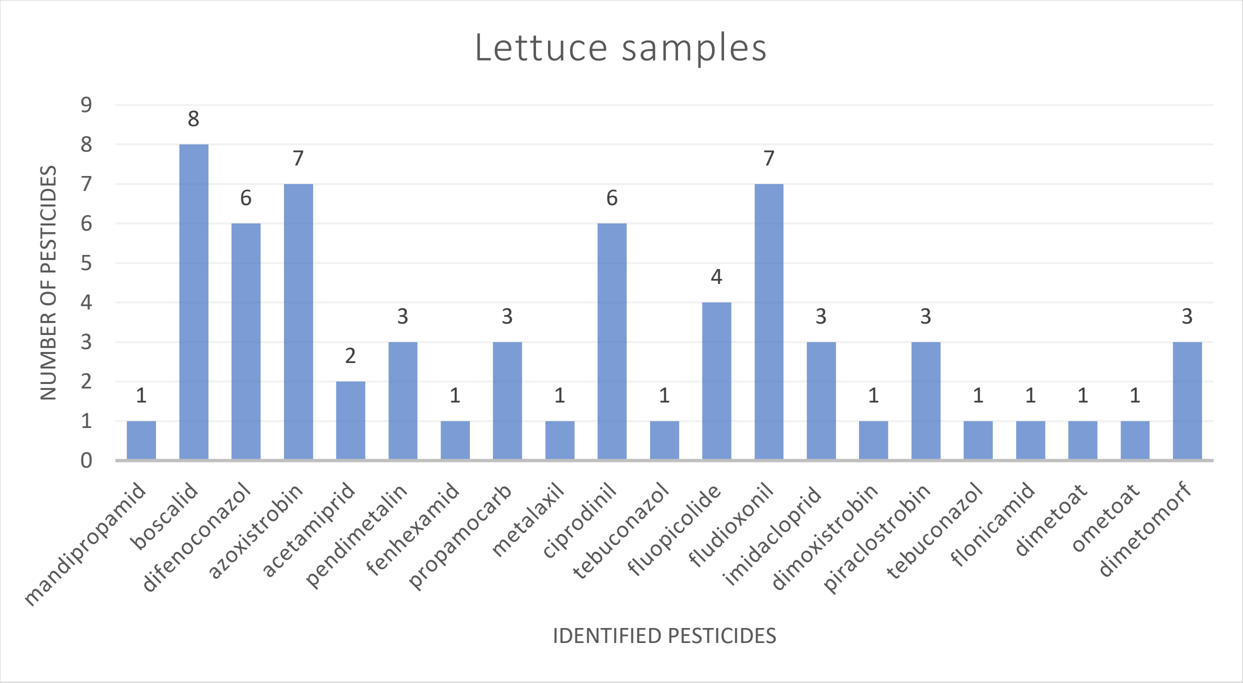

Figure 6. Identified pesticides residues in the lettuce samples

It can be seen that the most commonly used pesticides are fluopicolide, azoxystrobin, boscalid and difenoconazole (**Figure 8**). The first three are fungicides, with a wide range of use, and can be applied to several varieties of vegetables and fruits. Flonicamide and acetamiprid belong to neonicotinoids, insecticides that are mainly used on leafy vegetables. They are also classified as highly toxic to birds, bees, and aquatic life, as is spinosad, a bio-insecticide. Several insecticides belonging to neonicotinoids have been banned by the European Union from 2018 onwards. As can be seen from Table 11, some samples even contain pesticides withdrawn from the market, e.g. carbendazim, thiophanate-methyl, imidacloprid, thiamethoxam, linuron.

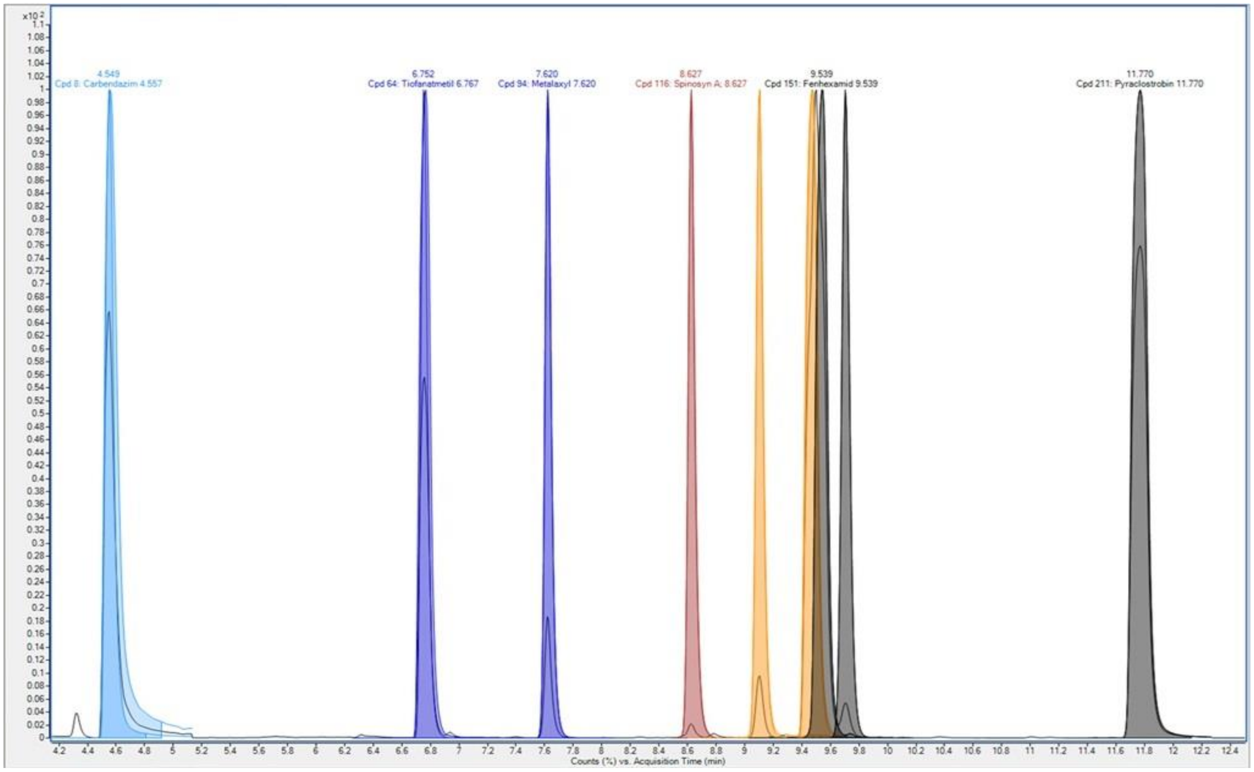

Figure 7. Chromatogram of the lettuce sample cropped on 20.03.2023

The explanation would be that farmers had large stocks of products with the respective active substances, knew their effectiveness, and used them, without consulting the regulations in force.

Over a two-year period, a significant reduction in the number of pesticides containing samples is observed. Thus, of the total samples collected in 2023, pesticides are found in 47% of them, while in the case of those collected in 2024, pesticides are found in 34% of the samples. This trend suggests that the restrictions and regulations imposed on pesticide use in agriculture have had a positive impact, contributing to the reduction of excessive pesticide application in the cultivation of fruits and vegetables.

However, this could also mean that farmers switched from synthetic pesticides to organic ones, which do not have clear regulations for the AILs and may not be detected during analysis. Organic pesticides, despite being derived from natural sources, can still pose significant environmental and health risks. Some organic pesticides, such as rotenone and spinosad, have been linked to toxicity in non-target organisms, including beneficial insects, aquatic life, and even humans.

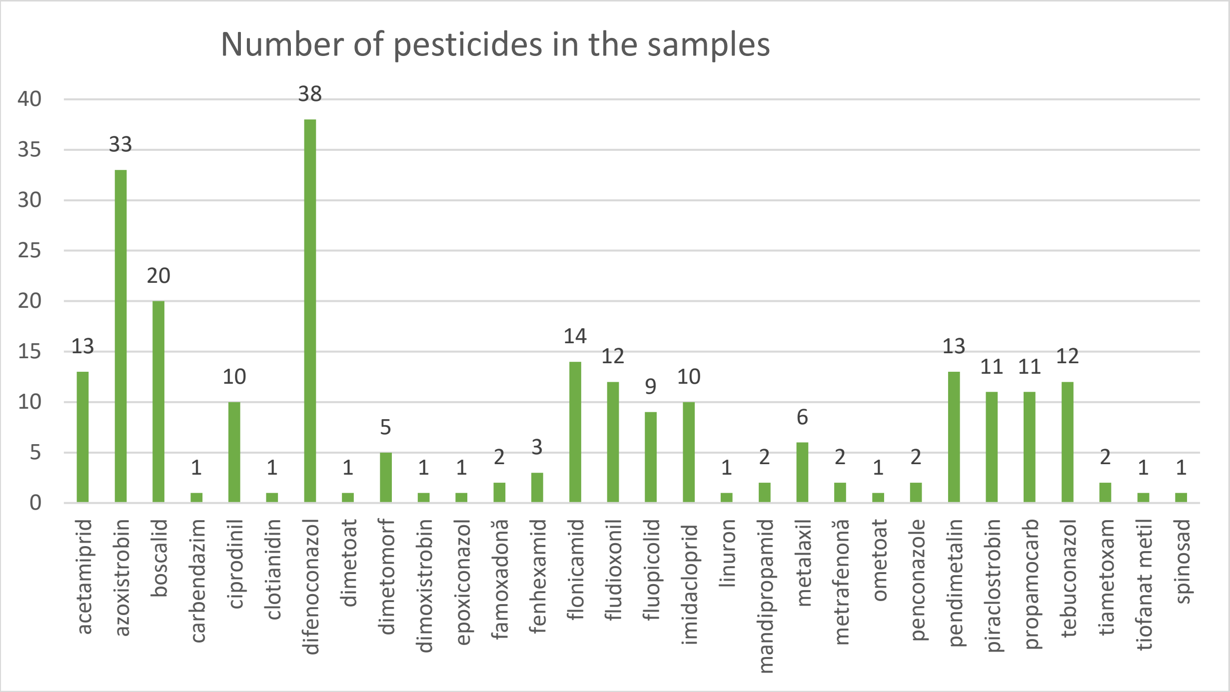

Figure 8. Number of pesticide residues in analyzed samples.

Additionally, excessive use can lead to bioaccumulation and environmental persistence, potentially disrupting ecosystems. While they are often considered a safer alternative to synthetic pesticides, their application must still be carefully regulated to minimize unintended harmful effects.

4.1.4. Conclusions

Consumers are becoming increasingly conscious of environmental and health degradation and more open to purchasing green, eco-labeled products. This trend presses the farmers to switch from synthetic pesticides to organic pesticides, which translates into decreasing in the number of pesticide residues found in the analyzed samples. The term “organic” does not mean “pesticide-free” and does not mean nontoxic, as organic pesticides can be harmful as or more than synthetic pesticides. The level of pesticide residues in an organically labeled vegetable can be just as harmful as in a non-organically labeled product.

Even though, eating vegetables is advised by global dietary guidelines as a way to prevent disease, maintaining a strict organic vegetables-based diet does not necessarily ensure a better quality of life or improved health. The word “ecological” does not exclude the use of pesticides and the level of pesticide residues in vegetables grown in greenhouses or solariums, even if purchased from local producers, can be harmful.

4.2. Determination of 2,4-dichlorophenoxyacetic acid (2,4-d) from tomatoes by LC-MS/MS analysis (Costea et al., 2022)

4.2.1. Introduction

2,4-dichlorophenoxyacetic acid (2,4-D) is the active ingredient in several formulation of herbicides recommended for the control of broadleaf weeds. Other uses include the control of aquatic weeds, some woody vegetation, and site preparation and conifer release in forests (Menn, 1978). 2,4-D was used as herbicide in the herbicide Agent Orange, a 1:1mixture of 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Agent Orange was a herbicide widely used during the Vietnam war, and was often contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), which result from the manufacture of 2,4,5-T, and this contaminant has high potential to be carcinogenic, teratogenic, and fetotoxic (Lilienfeld, Gallo, 1989).

Tomatoes are considered one of the most sensitive crops regarding 2,4-D and its derivatives. As a growth stimulator, it is applied in sublethal doses ranging between 0.42 - 13.44 g s.a /ha directly on the plants, in different stages of growth, from the beginning of flowering. For tomatoes, the tolerance to 2,4-D increases a lot with the age of the plant.

Due to the appearance on the Romanian market of some tomatoes of a abnormally shape (appearance of a tassel), with signs of phytotoxicity due to exposure to overdoses of the herbicide 2,4-D used as a growth regulator, the growth stimulation products based on 2,4-D were withdrawn from market.

4.2.2. Experimental part

For this LC-MS analysis, an AGILENT liquid chromatograph equipped with a quaternary pump model 1200, autosampler and a mass spectrometer triple quadrupole AGILENT 6410A, ionization source type Multi mode ionization (MMI), with electrospray ionization (ESI) in the negative mode**.**

Injection volume was 5µL and flow rate of mobile phase was 0.35 ml /min. Mobile phase A was water and phase B was acetonitrile with 0.1% formic acid.

The sample extraction method is described in **Figure 14**.

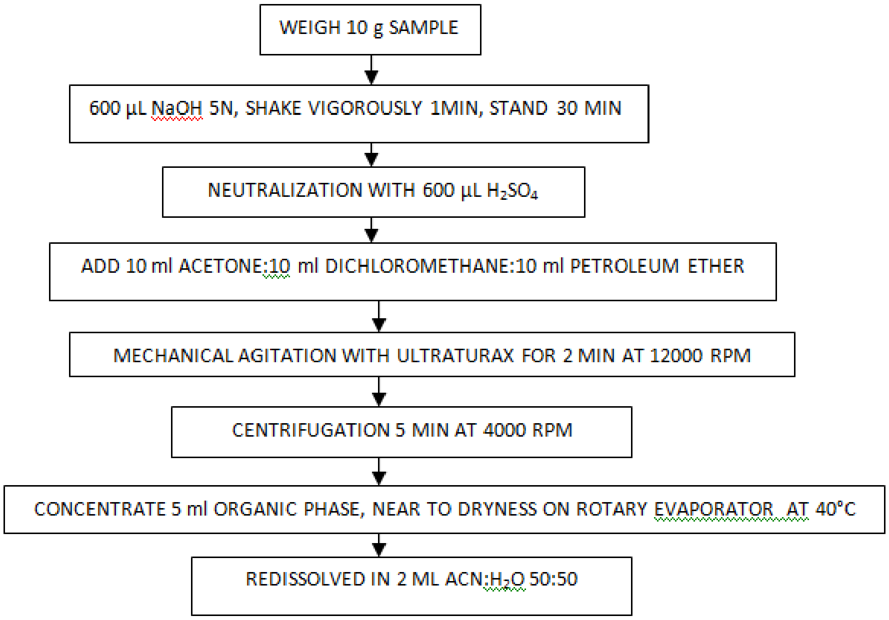

Figure 14. Extraction procedure of 2,4-D from tomatoes

4.2.3. Results and discussions

Due to its polar nature 2,4-D is difficult to analyze; it is partially linked to the matrix compounds. A good increase in extractability can be achieved by alkaline hydrolysis (e.g. NaOH, K2CO3), but even in this situation the recovery rates are up to 65%. A European method (Alkaline hydrolysis preceding QuEChERS for breaking up conjugates (prior to adding acetonitrile)) has been developed for the determination of phenoxyacid pesticides in flour, including 2,4-D, which uses alkaline hydrolysis in the extraction method, whose part of the analysis method has many elements in common with the one proposed in the present study, the significant difference being in the method of extraction (Anastassiades M., 2007, EURL – SRM).

Also, the analysis method by LC-MS/MS QQQ was a challenge because in matrices of plant origin and animal origin 2,4-D expressed as 2,4-D contain a sum of 2,4-D, its salts, its esters and its conjugates.

In order to establish the most efficient method of extracting 2,4-Dfrom tomatoes, we compared the QuEChERS method and the Miniluke method, with an alkaline hydrolysis previously applied for both. Both qualitative (chromatographic) and quantitative results were against the QuEChERS method. In **Figures 12** and **13** were presented the differences between the shape, the amplitude and the areas of the chromatographic peaks obtained by the two extraction methods (HA QuEChERS - alkaline hydrolysis and HA MiniLuke- alkaline hydrolysis), the LC-MS analysis method being the same.

The LOQ (0.025 mg/kg) was much lower than the MRL's established by the EU legislations for tomatoes (0.05 mg/kg). Retention time, regression coefficients (R^2), LOQ, recoveries, coefficient of variation RSD, matrix effects and MRL for 2,4-D in tomatoes are presented in **Table 12.**

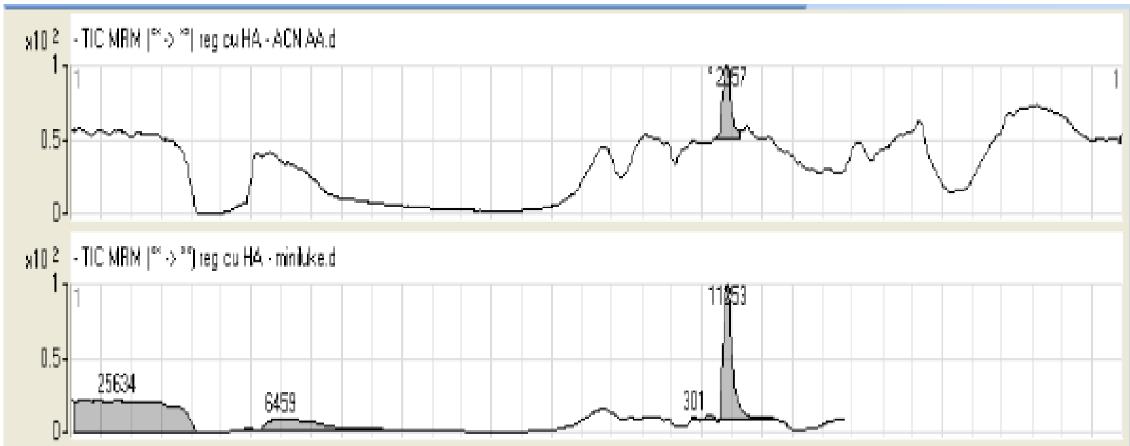

Figure 12. TIC chromatograms for QuEChERS HA - ACN AA and HA Miniluke extraction method

Table 12. Retention time, R^2, LOQ, recoveries, coefficient of variation, RSD (n=5), matrix effects and MRL for tomatoes

RT (min)	R^2	LOQ	Spiking level 0.05 mg/kg			Spiking level 0.15 mg/kg			MRL (mg/kg)
			Recovery (%)	RSD (%)	Matrix effect	Recovery (%)	RSD (%)	Matrix effect	
4,311	0,99068	0,025	110,5	5,60	- 4,5	95,8	3,29	- 5,5	0,05

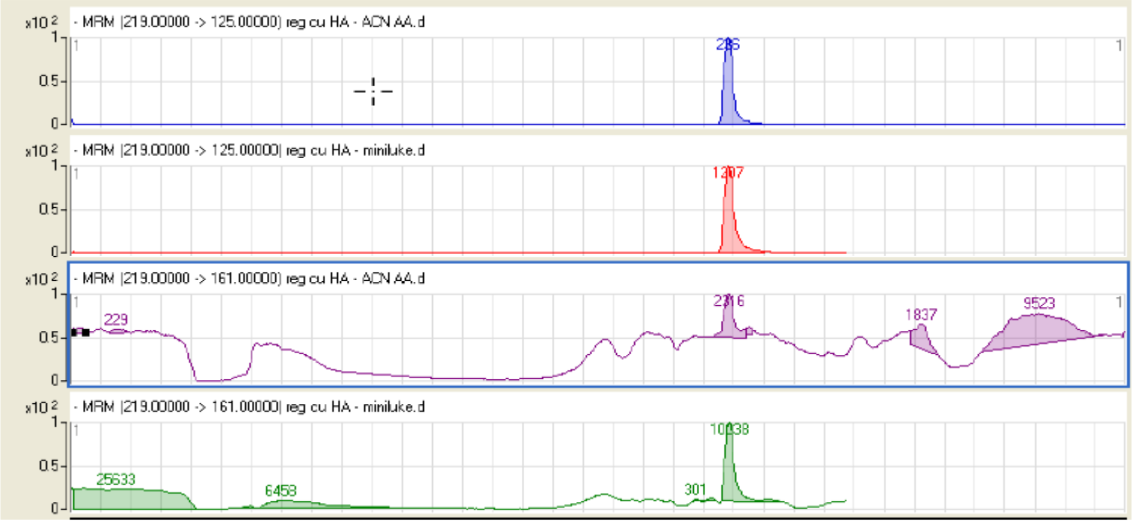

Figure 13. Peak areas for transitions 219→125 and 219→161 with HA Quechers (ACN AA) and HA Miniluke extraction method

4.2.4. Conclusions

The analysis method was validated on the matrix of tomatoes (matrix with high water content) and strawberries (matrix with high acidity content), and its efficiency was proven by participating in interlaboratory European tests organized by the European Reference Laboratory for the Analysis of Pesticide Residues from Almeria, Spain where very good Z scores were obtained.

The methods of analysis and extraction of 2,4-D proved effective for matrices with high water content (tomatoes) and those with high acidity (strawberries), but for samples with high starch content (cereals), the extraction method must be modified and adapted, by introducing a certain amount of cold water before the solvent extraction process. The amount of water varies depending on the type of sample (type of cereals) and its granulation after grinding.

4.3. LC- MS/MS analysis of pesticides residue in dried vegetables

4.3.1. Introduction

Crops treated with pesticides contain an unpredictable amount of these chemicals; therefore, finding alternatives for food decontamination becomes imperative. The pesticide levels can be reduced by subjecting plant-based products to various technological processes. A technological process consists of a set of activities that modify the chemical and physical properties of a plant product, transforming it into a safe food for consumption. Reducing pesticide residue concentrations in plant-based products can be achieved through methods such as heat treatment, exposing fruits and vegetables to different temperatures (Hrynko et al., 2023).

Washing with water or soaking in salt solutions and certain chemical substances, such as chlorine, chlorine dioxide, hydrogen peroxide, ozone, acetic acid, and detergents, has proven to be highly effective in reducing pesticide levels. Preparatory steps, such as peeling, trimming edges, and removing pits, help eliminate residues from external surfaces. Various heat treatments, including pasteurization, blanching, boiling, steaming, frying, or drying, have also been found valuable in breaking down different pesticides, depending on the type of pesticide and the duration of the treatment (Bajwa, Sandhu, 2014).

Different drying methods have varying effects on pesticide residues. In raisin production, sun drying led to a fourfold increase in residue concentration, while oven drying, preceded by washing, resulted in a decrease.

In this study, pesticide residues were analyzed in vegetable samples subjected to heat treatment, specifically drying (dehydration). The vegetables used in this experiment included red, yellow, and green bell peppers, red and white onions, carrots, root celery, and root parsley. These were purchased from various supermarkets and local markets between May and August 2024 (**Table 13**).

Table 13. Vegetables samples

Vegetable	**Purchased from**
Carrot	Local supermarket, Romania
Red peppers, bio	Local Supermarket, Holland, labelled „BIO”
Red peppers	Local market, Romania
Yellow peppers, bio	Local Supermarket, Holland, labelled „BIO”
Yellow peppers	Local market, Romania
Green peppers, bio	Local Supermarket, Holland, labelled „BIO”
Green peppers	Local market, Romania
Parsley, root	Local market, Romania
Celery, root	Local Supermarket, Holland
White and red onion	Local market, Romania

4.3.2. Experimental part

The chromatographic methods were the ones used in Chapter 4. 1.. The analysis was performed with MRM, using a HPLC liquid chromatograph *Agilent 1260 Infinity Prime LC* and a mass spectrometer *Agilent Hunter 6495,* with ion source temperature of 200°C. The mobile phases used were A: ultra-pure water + 0.1% formic acid and B: acetonitrile + 0.1% formic acid, with a flow rate of 0.45 mL/min, ionization gas was nitrogen, the injection volume 2 µL and the run time 29 minutes.

4.3.3. Results and discussions

In Table 14 are the results for 7 most common pesticides, Following the analysis, it was found that for certain pesticides, dehydration led to an almost 100% reduction in concentration, bringing it below the LOQ.

Table 14. Identified pesticides from the dried vegetables.

Sample	**Pesticide**	**LOQ**	**Conc. (mg/kg)**	**MRL (mg/kg)**
Green pepper	acetamiprid	0,01	**0.200**	0,09
	azoxystrobin	0,01	**0.011**	3,0

	boscalid	0,01	< LOQ	3,0
	difenoconazole	0,01	**0.480**	0.9
	fenhexamid	0,01	**0.013**	3,0
	imidacloprid	0,01	**0.069**	0,9
	penconazole	0,01	< LOQ	0,2
Red pepper	acetamiprid	0,01	**0.095**	0,09
	azoxystrobin	0,01	**0.099**	3,0
	boscalid	0,01	**0.020**	3,0
	difenoconazole	0,01	**0.092**	0.9
	fenhexamid	0,01	< LOQ	3,0
	imidacloprid	0,01	**0.071**	0,9
	penconazole	0,01	**0.053**	0,2
Yellow pepper	acetamiprid	0,01	< LOQ	0,09
	azoxystrobin	0,01	< LOQ	3,0
	boscalid	0,01	< LOQ	3,0
	difenoconazole	0,01	< LOQ	0,9
	fenhexamid	0,01	< LOQ	3,0
	imidacloprid	0,01	< LOQ	0,9
	penconazole	0,01	< LOQ	0,2
Celery	acetamiprid	0,01	< LOQ	0,01
	azoxystrobin	0,01	< LOQ	1,0
	boscalid	0,01	< LOQ	2,0
	difenoconazole	0,01	< LOQ	2,0
	fenhexamid	0,01	< LOQ	0,01
	imidacloprid	0,01	< LOQ	0,01
	penconazole	0,01	< LOQ	0,01
Parsley	acetamiprid	0,01	< LOQ	0,01
	azoxystrobin	0,01	< LOQ	1,0
	boscalid	0,01	< LOQ	2,0
	difenoconazole	0,01	< LOQ	0,4

	fenhexamid	0,01	< LOQ	0,01
	imidacloprid	0,01	< LOQ	0,01
	penconazole	0,01	< LOQ	0,01
Onion	acetamiprid	0,01	< LOQ	0,02
	azoxiytrobin	0,01	< LOQ	10
	boscalid	0,01	**0.027**	5,0
	difenoconazole	0,01	< LOQ	0,5
	fenhexamid	0,01	< LOQ	3,0
	imidacloprid	0,01	< LOQ	0,01
	penconazol	0,01	< LOQ	0,01
Carrot	acetamiprid	0,01	< LOQ	0,01
	azoxystrobin	0,01	< LOQ	1,0
	boscalid	0,01	< LOQ	2,0
	difenoconazole	0,01	**0.011**	0,4
	fenhexamid	0,01	< LOQ	0,01
	imidacloprid	0,01	< LOQ	0,01
	penconazol	0,01	< LOQ	0,01
Green pepper, bio	acetamiprid	0,01	< LOQ	0,09
	azoxystrobin	0,01	< LOQ	3,0
	boscalid	0,01	< LOQ	3,0
	difenoconazol	0,01	< LOQ	0,9
	fenhexamid	0,01	< LOQ	3,0
	imidacloprid	0,01	< LOQ	0,9
	penconazole	0,01	< LOQ	0,2
Yellow pepper, bio	acetamiprid	0,01	< LOQ	0,09
	azoxystrobin	0,01	< LOQ	3,0
	boscalid	0,01	< LOQ	3,0
	difenoconazole	0,01	< LOQ	0,9
	fenhexamid	0,01	< LOQ	3,0
	imidacloprid	0,01	< LOQ	0,9

	penconazole	0,01	< LOQ	0,2
Red pepper, bio	acetamiprid	0,01	< LOQ	0,09
	azoxystrobin	0,01	< LOQ	3,0
	boscalid	0,01	< LOQ	3,0
	difenoconazole	0,01	< LOQ	0,9
	fenhexamid	0,01	< LOQ	3,0
	imidacloprid	0,01	< LOQ	0,9
	penconazole	0,01	< LOQ	0,2
Crude mix	acetamiprid	0,01	**0,604**	N.A
	azoxystrobin	0,01	**0,048**	N.A
	boscalid	0,01	**0,028**	N.A
	difenoconazole	0,01	**0,066**	N.A
	fenhexamid	0,01	< LOQ	N.A
	imidacloprid	0,01	< LOQ	N.A
	penconazole	0,01	< LOQ	N.A
Vegetable mix, dried one day	acetamiprid	0,01	< LOQ	N.A
	azoxystrobin	0,01	< LOQ	N.A
	boscalid	0,01	< LOQ	N.A
	difenoconazole	0,01	**0,035**	N.A
	fenhexamid	0,01	< LOQ	N.A
	imidacloprid	0,01	< LOQ	N.A
	penconazole	0,01	< LOQ	N.A
Vegetable mix, dried 5 days	acetamiprid	0,01	< LOQ	N.A
	azoxystrobin	0,01	< LOQ	N.A
	boscalid	0,01	< LOQ	N.A
	difenoconazole	0,01	**0,011**	N.A
	fenhexamid	0,01	**0,014**	N.A
	imidacloprid	0,01	< LOQ	N.A
	penconazole	0,01	< LOQ	N.A

It is noteworthy that during extractions, the organic vegetable samples exhibited a more intense coloration than the others (**Figure 18**). This could indicate that organic vegetables contain other compounds that facilitate the extraction of pigments and colorants into the solvent mixture used for pesticide extraction.

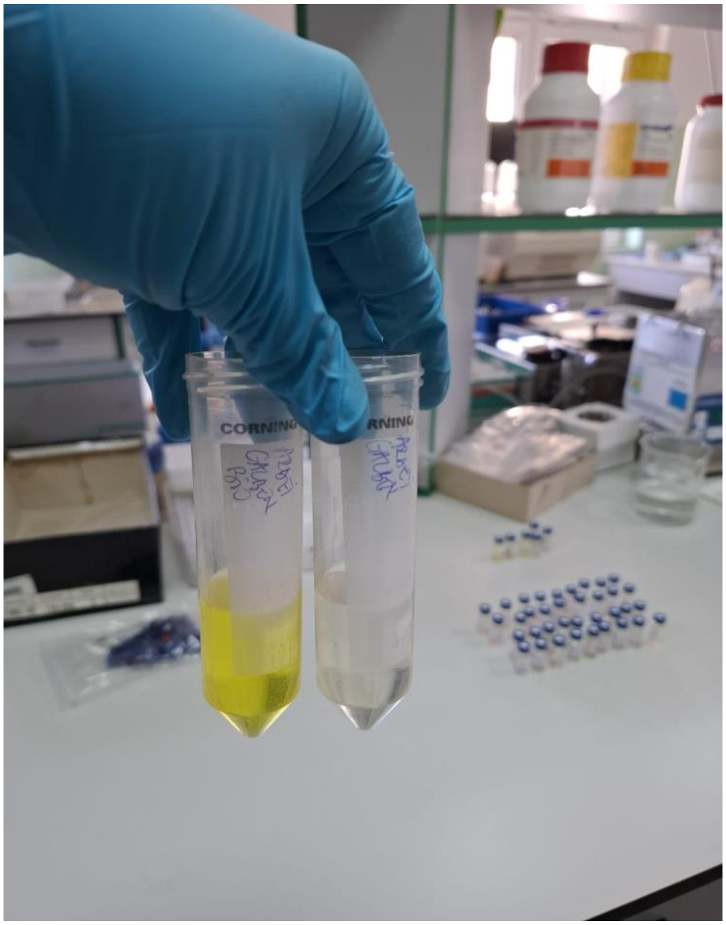

Figure 18. Yellow peppers samples, the one on the left with BIO label.

4.3.4. Conclusions

This study aimed to determine whether heat treatment, specifically drying (dehydration), reduces pesticide residues in vegetables. The results demonstrated that pesticide residues in the analyzed samples decreased by 80–90%, which is highly favorable. This is especially significant considering that the samples were not subjected to any prior physical treatments, such as washing, peeling, or seed removal.

Washing vegetables is a crucial step in food safety, helping to remove pesticide residues, pathogenic microorganisms, and surface impurities. Using water, saline solutions, or natural disinfectants can significantly reduce chemical and biological contaminants, enhancing the safety of vegetable consumption. Heat treatment further aids in eliminating pathogenic microorganisms and potential toxins, lowering the risk of foodborne illnesses. Depending on the method used, cooking vegetables can also reduce contaminant levels, such as pesticides, through evaporation, co-distillation, or thermal degradation.

Different drying methods, such as sun drying, oven drying, or industrial dehydration, affect both the nutritional value of vegetables and their long-term stability. The drying process can concentrate certain nutrients but may also lead to the loss of heat- and oxygen-sensitive vitamins, such as vitamin C and some B-complex vitamins.

Choosing an optimal drying method is essential for preserving the organoleptic and nutritional properties of vegetables, thereby maintaining their health benefits.

Additionally, applying proper washing, peeling, and cooking techniques is crucial. These methods can significantly reduce pesticide residues, minimizing potential health risks for consumers.

General conclusions

In this paper, the focus was on the quantitative determination of food additives in vegetable-based products and pesticide residues in vegetables sold in markets and supermarkets in Romania, which subsequently have repercussions on the health of organisms, humans, and the environment.

The study conducted on vegetable-based products used in culinary preparations highlights that they may contain significant amounts of monosodium glutamate (MSG), whose constant and long-term consumption can be associated with various health issues and chronic conditions. Furthermore, the research emphasizes that exposure to MSG may have immediate adverse effects on sensitive consumers, manifested through symptoms such as headaches, dizziness, or gastrointestinal discomfort. On the other hand, the study demonstrated that commercial vegetable bases can be replaced with a homemade mixture made exclusively from vegetables, containing only naturally occurring glutamic acid in reduced quantities. This alternative not only eliminates the risks associated with excessive MSG consumption but also contributes to a healthier diet without synthetic additives. In this context, better consumer education on the impact of added ingredients in processed products and the promotion of natural solutions to improve the nutritional quality of diets is essential.

For the determination of MSG, a simple and rapid HPTLC method was employed, which involves simplified steps in sample preparation. This method can be utilized in routine analyses to determine the concentration of MSG in various products, in this case, vegetables and vegetable-based food products.

For the determination of pesticide residues, a validated HPLC method coupled with a mass spectrometer was employed. This method successfully identified over 200 pesticides applied to fruits and vegetables. In the presented study, the most frequently used pesticides in vegetable cultivation were determined and monitored over a two-year period. Samples of vegetables grown in different seasons were analyzed, revealing numerous pesticides exceeding the MRL.

Although the comparative analysis of the data indicates a reduction in the number of samples containing pesticides in 2024 compared to 2023, this trend does not conclusively prove that pesticide use by farmers has decreased. One possible explanation could be the transition from synthetic pesticides to organic ones, for which the legislative framework is not yet well-defined, and maximum residue limits (MRL) are not imposed for all substances used. The lack of clear regulations regarding organic pesticides could lead to underreporting of their presence in food, potentially distorting the perception of the actual contamination levels.

Additionally, current testing methods are better suited for detecting synthetic pesticides, which could affect the results of the analyses and create the impression of an artificial reduction in pesticide use in agriculture. The shift to organic pesticides also presents challenges in establishing appropriate safety standards and assessing their impact on consumer health and the environment.

Therefore, updating legislation and expanding monitoring methods are necessary to accurately reflect the use of all pesticide types and ensure consumer protection.

The final part of the thesis involves the analysis of dried vegetables and the homemade vegetable mixture, conducted to determine the presence of pesticide residues. This analysis revealed a significant reduction in pesticide levels following the application of thermal treatments, such as drying. The experiment's results demonstrated that this process can substantially decrease pesticide levels, underscoring the importance of preliminary treatments applied to vegetable products prior to consumption.

This finding confirms that processing methods such as drying, boiling, or blanching can help reduce consumers' exposure to harmful chemicals. In this context, the implementation of appropriate pre-treatment techniques for vegetables and fruits becomes essential for food safety, reducing the risks associated with pesticide ingestion and promoting healthier eating habits. Moreover, these results support the need for further research into the impact of various processing methods on chemical contaminants in food products.

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3. Liana Maria Costea, **Raluca Rad**, Claudia Cimpoiu, Adela Megheșan, Determination of 2,4-dichlorophenoxyacetic acid (2,4-D) from tomatoes by LC-MS/MS analysis, *STUDIA UBB CHEMIA*, **2022**, 67, 249-259.

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1. **Raluca Rad**, Claudia Cimpoiu, Anamaria Hosu, Quantitative analysis of Monosodium Glutamate from food seasoning powders using high performance thin layer chromatography, Conferința Națională de Chimie, Călimanești – Căciulata, Romania, 04-07 Octombrie 2022.
2. **Raluca Rad**, Claudia Cimpoiu, Liana Costea, Adela Megheșan, Determination of the number and content of pesticides residues found in the vegetables harvested from Transylvanian area, 4th Young Researchers' International Conference on Chemistry and Chemical Engineering (YRICCCE IV), Debrecen, Hungary, 01-03 Iunie 2023.