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FOOD SAFETY AND PESTICIDE RESIDUES IN PLANT-BASED FOOD: FOUR-YEAR MONITORING RESULTS

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SUMMARY: Food safety is crucial for protecting consumer health, as pesticides used in agriculture to safeguard crops may leave residues in food products. These residues can pose health risks, necessitating regulatory monitoring, such as Maximum Residue Levels (MRLs) for pesticide residues. In the European Union, these levels are stipulated by Regulation (EC) No 396/2005. The aim of this study was to analyze the amount of pesticides in food products from the Split-Dalmatia County, Croatia, from 2019 to 2023, using analytical methods LC-MS/MS and GC-MS/MS. The samples included fruits, vegetables, olive oil, and jams, with a focus on detecting and quantifying pesticide residues. The findings indicate that all food samples analyzed using LC-MS/MS and GC-MS/MS methods had pesticide levels below the detection limit, with the exception of a few isolated cases where pesticides such as malathion, permethrin, and deltamethrin were detected above this threshold but still within the permissible limits. The only exception was strawberry jam, in which captafol was found at concentrations exceeding the maximum residue level (MRL). The tested samples encompassed a variety of agricultural products, and the GC-MS/MS analysis revealed that out of 22 food items, 15 samples (68.2%) contained pesticide levels below the detection limit, while 6 samples (27.3%) showed pesticide residues above the detection limit but within the permissible limit. Only one sample (4.5%) had pesticide levels exceeding the maximum allowable limit. Similarly, the LC-MS/MS analysis found that 21 samples (95.2%) had pesticide levels below the detection limit, with only one sample (4.5%) exceeding this threshold. These results suggest that food safety regulations were generally well adhered to, with only a few isolated instances of detected pesticide residues requiring further monitoring. The results show that in the majority of the analyzed samples, pesticide levels were below the detection limit or within permitted values, indicating overall effective control of pesticides in food production. However, isolated cases in samples of kale, lemon, nectarine, and strawberry jam, where certain pesticides were detected above the detection limit or allowed concentrations, suggest a possible localized issue, deficiencies in monitoring, or potential sources of contamination during storage and transportation. It is recommended that further analyses and investigations be conducted to confirm the consistency of these findings and identify the causes of these irregularities, with the aim of improving the monitoring system.

Key words: GC-MS/MS, LC-MS/MS, pesticides, food safety

INTRODUCTION

Food safety is defined as a state with minimal or no risk of hazards or threats. (Zubak, 2015).

Ensuring the safety of food products and protecting consumer interests has become increasingly important among the general public, non-governmental organizations, professional associati-

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ons, international trade partners, and trade organizations (*Regulation (EC) No 178/2002*).

An essential aspect of food safety assessment is the monitoring of pesticide residues, which has become more precise and accurate thanks to ongoing technological advancements (*Sabljak et al., 2013*). Pesticides are substances used to suppress, eradicate, and prevent the occurrence of harmful organisms (*European Parliament, 2004*). They are widely used in conventional agricultural practices on a global level due to their critical role in reducing crop losses—without pesticides, losses in fruit production could reach 78%, grain production 32%, and vegetable production 54%. (*Tudi et al., 2021*). Although pesticides contribute significantly to crop protection, their use raises concerns over the potential presence of residues in raw and processed food products (*Regulation (EC) No 396/2005*). These residues result from pesticide applications in agriculture and the use of biocidal agents in veterinary medicine (*Food Quality and Phytosanitary Policy Administration, 2015*). Exposure to pesticides, whether acute or chronic, can negatively impact human health, largely due to the active ingredients in these chemicals (*Miletić et al., 2018*). Safety limits are established using metrics such as the acute reference dose (ARfD) and acceptable daily intake (ADI), which indicate the maximum amounts of pesticides that can be consumed over a short period or a lifetime without posing health risks (*Nasreddine and Parent-Massin, 2002*).

According to the Food Act, it is prohibited to place food on the market that is not safe for consumption (*Food Act, 2004, 2023*). Due to the potential for pesticide accumulation in the environment and on treated crops, pesticide residue monitoring is rigorously conducted (*Food Quality and Phytosanitary Policy Administration, 2015*). In addition, the European Union has established legally defined Maximum Residue Levels (MRLs) for pesticide residues in food and feed of plant and animal origin (*Regulation (EC) No 396/2005*). MRLs are determined under standard conditions that adhere to the principles of good agricultural practice (*Mešić et al., 2018*) and are set for each permitted pesticide substance based on the specific crop on which it is allowed to be used (*Lu, 1988*).

The aim of this study is to analyze pesticide levels in food products using reference methods that ensure reliable results. Samples were collected from Split-Dalmatia County, Croatia, between 2019 and 2023. The study investigates whether pesticide levels exceed the Maximum Residue Levels (MRLs), thereby providing insights into the overall safety of the food products in the area.

This research is important because it provides reliable data on pesticide levels, directly contributing to consumer protection and ensuring food quality. The precise analysis of pesticide residues allows for the identification of potential health risks and aids in establishing effective monitoring and regulatory measures.

Moreover, the connection to the local context is underscored by the fact that samples were collected in Split-Dalmatia County - a region significant for its agriculture and food industry. The results enable local authorities and farmers to identify specific issues in pesticide application, improve agricultural practices, and take appropriate measures to minimize potential sources of contamination. In this way, the research contributes to strengthening local food safety, economic development, and public health.

MATERIAL AND METHODS

Pesticides were determined following the HRN EN ISO 12 393-1,2,3:2013 standard. This European guideline provides basic instructions for the identification and quantification of pesticide residues in plant-based food products. Each method specified in the guideline is capable of detecting and measuring a specific range of organohalogen, organophosphorus, and/or organonitrogen pesticides that may be present as residues in plant foods. The guideline includes three methods, labeled as M, N, and P, which have undergone interlaboratory testing or are widely accepted across Europe. In this work is used only method M. Method M involves extraction with acetone, liquid partitioning with dichloromethane or light petroleum, and, if necessary, cleanup using Florisil (*HRN EN ISO 12 393-1,2,3:2013*).

In this study, 22 samples from Split-Dalmatia County were analyzed, comprising seven virgin

olive oil samples, five fresh vegetable samples (e.g., cucumber, kale, sweet potato, spinach, and fresh pepper), eight fresh fruit samples (excluding dried fig; e.g., almond, lemon, grapefruit, orange, peach, mandarin, and strawberry), and two strawberry jam samples—all sourced from market circulation and selected randomly. The analysis was conducted at the Teaching Institute of Public Health in Primorje-Gorski Kotar County, as the Public Health Institute of Split-Dalmatia County has not yet implemented this test.

A total of 250 analytes were determined in each sample using two techniques: LC-MS/MS and GC-MS/MS. For LC-MS/MS, the HPLC system used was the Sciex ExionLC (Massachusetts, United States) and the MS/MS system was the AB Sciex QTRAP 4500 (Massachusetts, United States). For GC-MS/MS, the GC system used was the Agilent 7890B GC System (California, United States) and the MS/MS system was the Agilent-7000 GC/MS Triple Quad (California, United States).

Analytical Methods

Sample preparation for LC-MS/MS and GC-MS/MS was performed to identify potentially harmful substances that may remain in or appear in food (Na et al., 2022).

Extraction and Re-extraction

Extraction is a separation process relying on the transfer of dissolved components between the initial mixture and a selected solvent that does not mix with the original solution (Croatian Encyclopedia, 2024).

Before weighing, the samples are ground and placed into numbered containers. Then, 5 g of the sample is carefully weighed and transferred to Erlenmeyer flasks, followed by the addition of 25 ml of ethyl acetate (PE) and 25 ml of dichloromethane (DCM). For moist samples, sodium sulfate (Na_2SO_4) is added prior to the solvents to remove excess water (HRN EN ISO 12 393-1,2,3:2013). Ethyl acetate (EtOAc) has a rich history as an extraction solvent. This method has been used since 1989 for monitoring pesticide residues and is applied to a wide range of pesticides, offering numerous advantages. It is particularly suitable

for the extraction of foods with a high sugar content, as sugar has limited solubility in ethyl acetate (Schürmann et al., 2024). Other studies have also employed dichloromethane as an extraction solvent for pesticide determination, demonstrating improved recoveries and reduced matrix effects when analyzing a wide range of target analytes (Debler and Gandrass, 2024). The solvents were chosen because the standard defines them with those specific characteristics. Furthermore, their prescribed usage confirms that they are appropriate for that type of pesticide.

Once prepared, the samples are placed on a shaker and allowed to process for 1 to 1.5 hours. After 1 hour, the samples are removed from the shaker and filtered through filter paper using a funnel into round-bottom flasks. All samples are then dried to complete evaporation at 30°C (HRN EN ISO 12 393-1,2,3:2013).

Defatting

After evaporation, virgin olive oil samples retain residual fat. This fat must be removed before column purification (Matus, 2016, Hrvatska enciklopedija, 2024). Reducing the co-extraction of lipids allows for larger injection volumes. It also decreases matrix interference. This enhancement improves the sensitivity of analyses. It benefits both target compounds and other molecules not predefined as analytes (Xie et al., 2024). For lipid removal, the solvent ANC:PE was used due to its efficiency in removing lipids. 50 ml of ACN:PE (acetonitrile:petroleum ether) in a 2:1 ratio is added to a flat-bottom flask in three portions. The mixture is transferred to a separating funnel. The funnel is gently shaken and vented. After shaking, the layers are allowed to separate. The bottom layer is drained into another flat-bottom flask and evaporated to dryness. The upper layer is discarded. (HRN EN ISO 12 393-1,2,3:2013).

Purification

The goal of purification is to separate compounds co-extracted with analytes and, if necessary or desired, to isolate analytes into different fractions (Kožul and Herceg-Romanić, 2009). Purification aimed at removing pigments, lipids, amino acids, and peptides is carried out using various

procedures employing organic solvents (*Siddhanta et al., 2001*). The presence of these particles can compromise the accuracy of the results (*ALWSCI, 2024*). The purification method varies depending on the extract, but the most common and effective approach involves adsorption chromatography with sorbents such as silica gel, aluminum oxide, Florisil, or activated carbon (*Focant et al., 2004*).

Column preparation begins by filling the columns with florisil and pre-soaking them in petroleum ether, ensuring optimal efficiency in sample cleanup. Column rinsing is carried out in a container placed underneath the column (usually round with a flat bottom) in three stages: in the first stage, 50 mL of petroleum ether is added; in the second stage, a combination of 35 mL of petroleum ether and 15 mL of dichloromethane is used; and in the third stage, 50 mL of dichloromethane is added.

After the residue in the flask evaporates, it is diluted with 25 mL of petroleum ether, divided into three separate portions. The sample is then passed through the prepared columns, and afterwards, it is evaporated to complete dryness using a Rotavapor (*HRN EN ISO 12 393-1,2,3:2013*).

GPC (Gel Permeation Chromatography)

Gel permeation chromatography, also known as size exclusion chromatography (*Rastija and Medić-Šarić, 2009*), is regarded as an extremely valuable and versatile purification technique. It is widely applied in pesticide residue analysis, particularly for fat removal, and for separating smaller molecules, such as food toxins, from high-molecular-weight compounds. For multi-residue pesticide analysis across a broad spectrum, alternative fat removal methods are often less suitable due to the low recovery rates of many pesticides. This method effectively separates small molecules from high-molecular-weight compounds, including lipids such as waxes, triglycerides, and phospholipids, as well as pigments (e.g., chlorophylls and carotenoids) and plant resins. (*Hans G. J. Mol, navesti samo prezime autora, 2007*). Additionally, the amount of extracted fat depends on the properties of the targeted analyte, which influences the choice of solvent (*Xu et al., 2021*).

GPC is a liquid chromatography technique that separates samples based on the hydrodynamic volume of individual components (*Direktiva Komisije 98/73/EZ, 2018*). The method purifies samples by removing impurities and fats, thereby collecting a clean extract containing pesticides from the analyzed sample (*Puntarić et al., 2012*). Separation occurs when the sample passes through a column filled with porous, swollen gel—a process also applied to the fractionation of monomeric and polymeric polyphenolic compounds (*Guadalupe et al., 2006*). GPC also enables the evaluation of the average mass and degree of polymerization of substances formed during sedimentation (*Saucier et al., 1997*). Molecules that are too large to pass through the pores of the column take the shortest path and elute first, while smaller molecules that can enter the pores are retained longer and elute later, resulting in chromatographic peaks appearing at later times. In addition to separating substances, this technique is used to determine the molecular weight of a substance. By measuring the retention times of standards, a calibration curve—showing the relationship between the molecular weight of the standards and their retention time—is constructed, from which the molecular weight of the sample can be determined (*Miller, 2009*).

LC-MS/MS and GC-MS/MS

Liquid chromatography–mass spectrometry (LC–MS) represents an analytical technique in chemistry that combines the physical separation capability of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Systems that integrate these two methods are widely used in chemical analysis because their individual functions complement and enhance each other. While liquid chromatography enables the separation of mixtures containing multiple components, mass spectrometry provides spectral data that can help in identifying or confirming the identity of the separated compounds (*de Hoffmann and Stroobant, 2007*).

The quantification limit was 0.005 mg/kg, with recoveries ranging from 73% to 128% (depending on the pesticide). Pesticides that were determined are shown in table 1.

Table 1. Pesticides that were determined using the LC-MS/MS method**Tablica 1. Pesticidi koji su se određivali LC-MS/MS metodom**

No	PESTICIDE	No	PESTICIDE
1.	3-hydroxycarbofuran	101.	Imazalil
2.	Acephate	102.	Imidacloprid
3.	Acetamipirid	103.	Ipconazole
4.	Acibenzolar-S-methyl	104.	Iprovalicarb
5.	Alanycarb	105.	Isoprocab
6.	Aldicarb	106.	Isoproturon
7.	Aldicarb sulfone	107.	Isoxaflutole
8.	Aldicarb sulfoxide	108.	Kresoxim-methyl
9.	Ametryn	109.	Linuron
10.	Aminocarb	110.	Lufenuron
11.	Amitraz	111.	Mandipropamid
12.	Azoxystrobin	112.	Mefenacet
13.	Benalaxyl	113.	Mepanipyrim
14.	Bendiocarb	114.	Mepronil
15.	Benfuracarb	115.	Mesotrione
16.	Benzoximate	116.	Metaflumizone
17.	Bitertanol	117.	Metalaxyl
18.	Bifenazate	118.	Metconazole
19.	Boscalid	119.	Methabenzthiazuron
20.	Bromuconazole-trans	120.	Methamidophos
21.	Bromuconazole-trans	121.	Methiocarb
22.	Buprimate	122.	Methomyl
23.	Buprofezin	123.	Methoprotryne
24.	Butafenacil	124.	Metobromuron
25.	Butoxycarboxim	125.	Metoxyfenozide
26.	Carbaryl	126.	Metribuzin
27.	Carbendazim	127.	Mevinphos E,Z
28.	Carbetamide	128.	Mexacarbate
29.	Carbofuran	129.	Monochrotophos
30.	Carboxin	130.	Monolinuron
31.	Carfentazone-ethyl	131.	Myclobutanil
32.	Chlorantraniliprole	132.	Neburon

33.	Chlorfluazuron	133.	Nitenpyram
34.	Chlorotoluron	134.	Novaluron
35.	Chloroxuron	135.	Noviflumuron
36.	Clethodim	136.	Nuarimol
37.	Clofentezine	137.	Omethoate
38.	Clothianidin	138.	Oxadixyl
39.	Cyazofamid	139.	Oxamyl
40.	Cycluron	140.	Paclobotrazol
41.	Cymoxanil	141.	Penconazole
42.	Cyproconazole	142.	Pencycuron
43.	Cyprodinil	143.	Phenmedipham
44.	Desmedipham	144.	Picoxystrobin
45.	Diclobutrazol	145.	Piperonyl butoxide
46.	Dicofol	146.	Pirimicarb
47.	Dicrotophos	147.	Prochloraz
48.	Diethofencarb	148.	Promecarb
49.	Difenoconazole	149.	Prometon
50.	Diflubenzuron	150.	Prometryne
51.	Dimethoate	151.	Propamocarb
52.	Dimethomorph	152.	Propargite
53.	Dimiconazole	153.	Propham
54.	Dimoxystrobin	154.	Propiconazole
55.	Dinotefuran	155.	Propoxur
56.	Dioxicarb	156.	Pymeton
57.	Diuron	157.	Pymetrozine
58.	Emamectin-benzoate	158.	Pyracarbolid
59.	Epoxiconazole	159.	Pyraclostrobin
60.	Etaconazole	160.	Pyridaben
61.	Ethiofenacarb	161.	Pyrimethanil
62.	Ethiprole	162.	Pyriproxyfen
63.	Ethirimol	163.	Quinoxifen
64.	Ethofumesate	164.	Rotenone
65.	Clethodim Z	165.	Secbumeton
66.	Etoxazole	166.	Siduron
67.	Famoxadone	167.	Simetryn
68.	Fenamidone	168.	Spinetoram

69.	Fenarimol	169.	Spinosad
70.	Fenazaquin	170.	Spirodiclofen
71.	Fenbuconazole	171.	Spiromesifen
72.	Fenhexamid	172.	Spirotetramat
73.	Fenobucarb	173.	Spiroxamine
74.	Fenoxycarb	174.	Sulfentrazone
75.	Fenpropopimorph	175.	Tebuconazole
76.	Fenpyroximate	176.	Tebufenozide
77.	Fenuron	177.	Tebufenpyrad
78.	Fipronil	178.	Tebuthiuron
79.	Flonicamid	179.	Teflubenzuron
80.	Fluazinam	180.	Temphos
81.	Flubendimide	181.	Terbumeton
82.	Fludioxonil	182.	Terbutryn
83.	Flufenacet	183.	Tetraconazole
84.	Flufenoxuron	184.	Tiabendazole
85.	Fluometuron	185.	Thiacloprid
86.	Fluoxastrobin	186.	Thiamethoxam
87.	Fluquinconazole	187.	Thidiazuron
88.	Flusilazole	188.	Thiobencarb
89.	Flutolanil	189.	Thlofanox
90.	Forchlorfenuron	190.	Thiophanate-methyl
91.	Formetanate HCL	191.	Triadimefon
92.	Fuberidazole	192.	Triadimenol
93.	Furalaxyl	193.	Trichlorfon
94.	Furathiocarb	194.	Tricyclazole
95.	Flutriafol	195.	Trifloxystrobin
96.	Halofenozide	196.	Triflumizole
97.	Hexaconazole	197.	Triflumuron
98.	Hexaflumuron	198.	Triticonazole
99.	Hexythiazox	199.	Vamidothion
100.	Hydramethylnon	200.	Zoxamide

Gas chromatography (GC) is a technique used to separate volatile components in a mixture. The process begins by heating a liquid sample until it vaporizes, after which the vapor is transported by a gas, such as helium or hydrogen. This gas, acting as the carrier or mobile phase, carries the sample through a narrow glass or metal column coated with a chemical substance that forms the stationary phase. As the vaporized substances travel through the column, they interact with its surface, causing the compounds to slow down according to their chemical properties. Consequently, different compounds pass through the column at varying speeds, resulting in their separation. Once separated, the individual compounds are transferred to a mass spectrometer for further analysis (*Agilent Technologies, 2017*).

The quantification limit was 0.005 mg/kg, with recoveries ranging from 71% to 121% (depending on the pesticide). Pesticides that were determined are shown in table 2.

Table 2. Pesticides that were determined using the GC-MS/MS method

Tablica 2. Pesticidi koji su se određivali GC-MS/MS metodom

No	PESTICIDE	No	PESTICIDE
1.	Alachlor	58.	Fenchlorfos (ronnel)
2.	Aldrin	59.	Fensulfothion
3.	Ametryn	60.	Fention
4.	Atraton	61.	Fenvalerate
5.	Atrazin	62.	Folpet
6.	Azinfos-metil	63.	Heptachlor
7.	BHC alpha isomer (alfa-HCH)	64.	Heptachlor epoxid
8.	BHC beta isomer (beta - HCH)	65.	Hexachlorobenzene
9.	BHC delta isomer (delta -HCH)	66.	Imazalil
10.	BHC gama isomer	67.	Iprodion

11.	(gama-HCH),lindan Bromacil	68.	Isodrin
12.	Butachlor	69.	Coumaphos
13.	Butylate	70.	Merphos
14.	Captafol	71.	Methoxychlor
15.	Captan	72.	Metolachlor
16.	Carbaryl	73.	Metribuzin
17.	Carbofuran	74.	Mevinphos
18.	Cis – Chlordane	75.	MGK 264
19.	trans – Chlordane	76.	Molinate
20.	Chlorfenapyr	77.	Monocrotophos
21.	Chlorfenvinphos	78.	Napropamide (Devrinol)
22.	Chlorpropham	79.	o,o,o-triethylphospho- horoithioate
23.	Chlorpyrifos	80.	Octamethylprop- hosphoramid
24.	Chlorpyrifos-methylg	81.	Parathion methyl
25.	Crotoxyphos	82.	Parathion ethyl
26.	Cycloate	83.	Paraoxon methyl
27.	Cyfluthrin I	84.	Pebulate
28.	Cyhalothrin I(lambda)	85.	Pendimethalin
29.	Cypermethrin II	86.	Pentachlorobenzene
30.	DDD,p,p	87.	Pentachlorophenol
31.	DDE,p,p	88.	Permethrin-cis
32.	DDT,p,p	89.	Permethrin-trans
33.	DEF(Tribufos)	90.	Phorate
34.	Deltamethrin	91.	Phosalone
35.	Demeton-O	92.	Posphamidone (E+Z)
36.	Malation	93.	Procymidone
37.	Diazinon	94.	Prometon
38.	Dichlorvos	95.	Prometryn
39.	Dicloran	96.	Propazine
40.	Dieldrin	97.	Propyzamide
41.	Dimethoate	98.	Prothiofos (Tokuthion)
42.	Dioxathion	99.	Simazine
43.	Diphenamid	100.	Simetrin
44.	Disulfoton	101.	Sulfotep
45.	Disulfoton sulfone	102.	Sulprofos(Bolstar)

46.	Endosulfan alpha	103.	Tefluthrin,cis
47.	Endosulfan beta	104.	Terbacil
48.	Endosulfan sulphate	105.	Terbufos
49.	Endrin	106.	Terbutryn
50.	Endrin aldehyde	107.	Thioazin
51.	Endrin ketone	108.	Tetrachlorvinphos (Stirophos)
52.	EPN	109.	Tetraethylpyrophos- phatc
53.	EPTC	110.	Tetradifon
54.	Esfenvalerate	111.	Tolyfluanid
55.	Ethion	112.	Trichloronate
56.	Ethoprophos	113.	Vinclozolin
57.	Famphur	114.	Vernolate

RESULTS

22 samples from the Split-Dalmatia County were analyzed, comprising seven virgin olive oil samples (31.8%), five fresh vegetable samples (22.7%) (cucumber, kale, sweet potato, spinach, fresh pepper), eight fresh fruit samples (except dried fig) (36.4%) (almond, lemon, grapefruit, orange, peach, dried fig, mandarin, strawberry), and two strawberry jam samples (9.1%) (Figure 1); (Authors based on research).

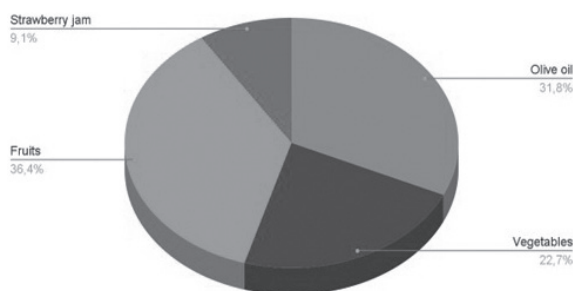


Figure 1. Samples Analyzed

Slika 1. Analizirani uzorci

To evaluate the effectiveness of pesticide residue analysis, LC-MS/MS and GC-MS/MS methods were employed and tested for sensitivity, linearity, selectivity, precision, and accuracy to confirm their reliability (Vrsaljko, 2022).

Table 3. LC-MS/MS and GC-MS/MS analysis of different types of olive oil**Tablica 3. LC-MS/MS i GC-MS/MS analiza različitih vrsta maslinovog ulja**

Sample	Number of tested pesticides	Method 1	Concentration mg/kg	Number of tested pesticides	Method 2	Concentration mg/kg
Olive oil 1	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	111 tested pesticides	<i>GC-MS-MS</i>	<0,005
Olive oil 2	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	113 tested pesticides	<i>GC-MS-MS</i>	<0,005
Olive oil 3	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	114 tested pesticides	<i>GC-MS-MS</i>	<0,005
Olive oil 4	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	112 tested pesticides	<i>GC-MS-MS</i>	<0,005
Olive oil 5	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	113 tested pesticides	<i>GC-MS-MS</i>	<0,005
Olive oil 6	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	113 tested pesticides	<i>GC-MS-MS</i>	<0,005
Olive oil 7	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	112 tested pesticides	<i>GC-MS-MS</i>	<0,005

Table 4. LC-MS/MS and GC-MS/MS analysis of Class II cucumber, fresh kale (Savoy cabbage), potato, spinach, and fresh pepper**Tablica 4. LC-MS/MS i GC-MS/MS analiza krastavca II. klase, svježeg kelja (verzota), krumpira, špinata i svježeg paprike**

Sample	Number of tested pesticides	Method 1	Concentration mg/kg	Number of tested pesticides	Method 2	Concentration mg/kg
Class II cucumber	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	111 tested pesticides	<i>GC-MS-MS</i>	<0,005
Fresh kale (Savoy cabbage)	199 tested pesticides	<i>LC-MS-MS</i>	<0,005	111 tested pesticides	<i>GC-MS-MS</i>	<0,005
Potato	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	114 tested pesticides	<i>GC-MS-MS</i>	<0,005
Spinach	197 tested pesticides	<i>LC-MS-MS</i>	<0,005	113 tested pesticides	<i>GC-MS-MS</i>	<0,005
Fresh pepper	197 tested pesticides	<i>LC-MS-MS</i>	<0,005	111 tested pesticides	<i>GC-MS-MS</i>	<0,005

All samples of various types of olive oil were analyzed using the LC-MS/MS method for 200 different pesticides. The tested pesticides in all samples were below the level of detection. The GC-MS/MS method analyzed between 111 and 114 different pesticides, depending on the type of olive oil, and in no sample did the pesticide levels exceed the detection limit (Table 3).

In the Class II cucumber sample, analyzed using the LC-MS/MS method for 200 pesticides and the GC-MS/MS method for 111 pesticides, pesticide levels did not exceed the detection li-

mit. The LC-MS/MS analysis of 199 pesticides in fresh kale (Savoy cabbage) found none above the detection limit. The LC-MS/MS analysis of 199 pesticides in fresh kale (Savoy cabbage) found none above the detection limit. Using the LC-MS/MS method, 200 pesticides were analyzed in potatoes, 197 in spinach, and 197 in fresh bell pepper, with all pesticide levels in these samples found to be below the detection limit. The GC-MS/MS method analyzed 114 pesticides in potatoes, 113 in spinach, and 111 in fresh bell pepper, and in all cases, the pesticide levels were also below the detection limit (Table 4).

Table 5. Pesticides found in analysis of fresh kale (Savoy cabbage)**Tablica 5. Pesticidi pronađeni analizom svježeg kelja (verzota)**

<i>Fresh kale (Savoy cabbage) GC-MS-MS</i>	Concentration mg/kg	MDK (mg/kg)
Demeton-O	0,0073	0,01
Pendimethalin	0,0106	0,05

The GC-MS/MS analysis of 111 pesticides found in fresh kale are two pesticides, demeton-O and pendimethalin, above the detection limit, as shown in Table 5.

In dried figs, 200 pesticides were examined using the LC-MS/MS method, with all remaining below the detection limit. Additionally,

Table 6. LC-MS/MS and GC-MS/MS analysis of dried figs, lemon, nectarine, orange, almond kernels, peach, grapefruit, strawberry jam 1, strawberries and strawberry jam 2**Tablica 6. LC-MS/MS i GC-MS/MS analiza suhih smokvi, limuna, nektarine, naranče, jezgre badema, breskve, grejpa, džema od jagoda 1, jagoda i džema od jagoda 2**

Sample	Number of tested pesticides	Method 1	Concentration mg/kg	Number of tested pesticides	Method 2	Concentration mg/kg
Dried figs	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	111 tested pesticides	<i>GC-MS-MS</i>	<0,005
Lemon	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	111 tested pesticides	<i>GC-MS-MS</i>	<0,005
Nectarine	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	111 tested pesticides	<i>GC-MS-MS</i>	<0,005
Orange	198 tested pesticides	<i>LC-MS-MS</i>	<0,005	115 tested pesticides	<i>GC-MS-MS</i>	<0,005
Almond kernels	213 tested pesticides	<i>LC-MS-MS</i>	<0,005	94 tested pesticides	<i>GC-MS-MS</i>	<0,005
Peach	197 tested pesticides	<i>LC-MS-MS</i>	<0,005	111 tested pesticides	<i>GC-MS-MS</i>	<0,005
Grapefruit	197 tested pesticides	<i>LC-MS-MS</i>	<0,005	113 tested pesticides	<i>GC-MS-MS</i>	<0,005
Strawberry jam 1	200 tested pesticides	<i>LC-MS-MS</i>	<0,005	110 tested pesticides	<i>GC-MS-MS</i>	<0,005
Strawberries				111 tested pesticides	<i>GC-MS-MS</i>	<0,005
Strawberry jam 2				111 tested pesticides	<i>GC-MS-MS</i>	<0,005

111 pesticides were analyzed using the GC-MS/MS method.

Lemon samples underwent LC-MS/MS analysis for 200 pesticides, none of which exceeded the detection limit. Similarly, nectarines were tested for 200 pesticides using the same method, with no detections above the limit.

The LC-MS/MS method was also applied to oranges, almond kernels, peaches, and grapefruits, analyzing 198, 213, and 197 pesticides, respectively, with none surpassing the detection limit. Likewise, GC-MS/MS analysis found no exceedances among 115 pesticides in oranges, 94 in almond kernels, 111 in peaches, and 113 in grapefruits.

Finally, an analysis of 200 pesticides in a type of strawberry jam using LC-MS/MS confirmed that none exceeded the detection limit, as shown in Table 6.

Table 7. LC-MS/MS and GC-MS/MS analysis of lemon**Tablica 7. LC-MS/MS i GC-MS/MS analiza limuna**

<i>Dried figs</i> <i>GC-MS-MS</i>	Concentration mg/kg	MDK (mg/kg)
Permethrin-cis	0,024	0,05
Permethrin-trans	0,061	0,05
<i>Lemon</i> <i>GC-MS-MS</i>	Concentration mg/kg	MDK (mg/kg)
Malation	0,678	2,0
<i>Nectarine</i> <i>GC-MS-MS</i>	Concentration mg/kg	MDK (mg/kg)
Deltamethrin	0,042	0,04
<i>Strawberry jam 1</i> <i>GC-MS-MS</i>	Concentration mg/kg	MDK (mg/kg)
Captan	0,0628	1,5
Captafol	0,158	0,02
<i>Strawberries</i> <i>GC-MS-MS</i>	Concentration mg/kg	MDK (mg/kg)
Captafol	0,019	0,02
<i>Strawberry jam 2</i> <i>GC-MS-MS</i>	Concentration mg/kg	MDK (mg/kg)
Captafol	0,009	0,02

The GC-MS/MS method detected 111 pesticides in dried figs, with all remaining below the detection limit except for permethrin-cis and permethrin-trans.

In lemon samples, GC-MS/MS analysis showed that 111 pesticides were below the detection limit, while malathion was present in concentrations exceeding the limit.

Similarly, 111 pesticides analyzed using the GC-MS/MS method were below the detection limit, except for deltamethrin, which was detected in quantities above the limit.

In another analysis using GC-MS/MS, 110 pesticides were found below the detection limit, while captan exceeded the detection limit and captafol surpassed the maximum residue level (MRL). Likewise, in a different type of strawberry jam, 111 pesticides analyzed by GC-MS/MS were below the detection limit, except for captafol, which was present in quantities above the limit. A similar result was observed in strawberries, where captafol was the only pesticide exceeding the detection limit.

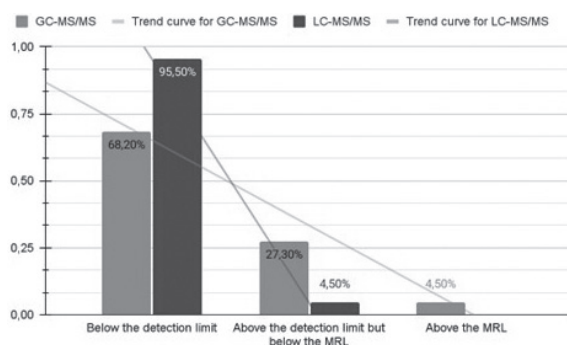


Figure 2. Overall results by food item analyzed using GC-MS/MS and LC-MS/MS

Slika 2. Ukupni rezultati po namirnici rađeni GC-MS/MS-om i LC-MS/MS-om

The GC-MS/MS analysis showed that out of 22 food items, 15 (68.2%) were below the detection limit, 6 (27.3%) samples were above the detection limit but below the MRL, and only 1 (4.5%) exceeded the MRL. For the LC-MS/MS method, only 1 (4.5%) sample was above the detection limit, while the remaining 21 (95.5%) were below the detection limit (Figure 2); (Authors based on research).

DISCUSSION

The growth in the use of mass spectrometry (MS) for analysis was further accelerated during the mid-to-late 1990s, leading to a transition from gas chromatography (GC) as the primary mass spectrometry technology to liquid chromatography (LC). LC enabled significantly simpler workflows and much faster analysis processing times (Grebe and Singh, 2011). GC-MS/MS became the principal technique for analyzing contaminants and residues in food due to its high sensitivity and selectivity and is widely used for the analysis of low-polarity, volatile, and thermally stable compounds (Xu et al., 2021). The application of GC-MS in pesticide residue analysis is summarized in analytical manuals for pesticides (Thier and Zeumer, 1992, van Zoonen, 1998), applications by instrument manufacturers (Agilent Technologies, 1999), or scientific studies (Cairns et al., 1993, Fillion et al., 2000, Wong et al., 2003). Today, liquid chromatography-mass spectrometry (LC-MS) technology has become widespread in clinical reference and testing labo-

ratories globally, gradually penetrating large and medium-sized hospitals and regional clinical laboratories (Grebe and Singh, 2011). Over the past decade, the LC-MS/MS method has played a key role in routine food safety monitoring, providing analytical parameters that meet regulatory quality requirements (Kovač et al., 2018).

Both methods were used to test for the presence of 200 pesticides in various food samples, including olive oil, vegetables, fruits, and strawberry jam. Results showed that most pesticides were below the detection limit, though some samples contained pesticides exceeding permissible levels.

Olive Oil: LC-MS/MS analysis of 200 pesticides detected no pesticide residues above the allowable limit. GC-MS/MS analysis, covering 111–114 pesticides depending on the type of olive oil, also found no pesticides exceeding the detection limit.

Vegetables: Similar results were observed in vegetable samples such as cucumbers, kale, potatoes, spinach, and pepper. Most pesticides were below the detection limit, with only two pesticides (demeton-O and pendimethalin) detected above the detection limit in kale samples.

Dried Figs and Lemons: LC-MS/MS analysis found no pesticide residues above the detection limit. However, GC-MS/MS detected malathion in lemons in amounts exceeding the detection limit.

Fruits: In nectarine, orange, almond kernels, peach, and grapefruit, LC-MS/MS analysis detected no pesticides above the detection limit. However, deltamethrin was found in nectarines above the detection limit.

Strawberry Jam: LC-MS/MS analysis showed no pesticides above the detection limit. GC-MS/MS detected captan in one type of jam and captafol in both types, in quantities exceeding the detection limit and maximum allowable concentrations.

Approximately 200 types of pests are known to directly or indirectly attack strawberries, prompting producers worldwide to use pesticides as an effective protective measure. A study in China found that 40% of strawberries contained residues of 10 or more pesticides. Since strawberries are

often consumed fresh and unwashed, many studies have raised public concern about pesticide residues in strawberries, which can pose health risks (Shao et al., 2021). In Hungary, a long-term study confirmed the mutagenic effects of two pesticides, captafol and malathion, on human cells under prolonged low-dose exposure (Martinek et al., 2024). The World Health Organization (WHO) classifies pesticides based on their toxicity into two categories: Class I, which includes extremely hazardous substances, and Class II, which includes slightly toxic pesticides. These chemicals are recognized as poisonous, dangerous, and harmful to human health. At low concentrations, they can act as inhibitors of sex hormones, leading to disruptions in sexual development, abnormal sex ratios, and altered mating behaviors in animals. Additionally, they negatively affect hormonal processes, including thyroid hormone production, which plays a crucial role in bone development. Exposure to pesticides can cause a range of acute symptoms, such as headaches, vomiting, skin rashes, respiratory issues, and convulsions. Long-term exposure to these substances may increase the risk of cancer. Residues of organochlorine pesticides can also damage the DNA of unborn babies, disrupt endocrine system function, and cause nerve and brain cell damage (Oyeyiola et al., 2017).

We are exposed daily to various chemicals found in food, the environment, and consumer products. However, risk assessments for these chemicals are still typically conducted individually, meaning that the risk analysis of dietary exposure to each chemical is carried out separately from the potential influence of simultaneous exposures to other chemicals. According to Regulation (EC) 396/2005, it is necessary to develop a methodology that takes into account cumulative and synergistic effects when establishing Maximum Residue Levels (MRLs) for pesticide active substances (Jensen et al. 2022). An increasing body of evidence indicates that combined exposure to multiple chemicals can lead to harmful effects, even when the levels of individual chemicals are below the threshold for harm (Kortenkamp and Faust, 2018). Over the past decades, government institutions and scientific bodies have been working intensively to develop multi-tiered models for assessing combined exposure to numerous chemicals for risk analysis purposes (Jensen et al. 2022).

Extra virgin olive oil (EVO) is recognized as a premium product of the Italian food industry and is one of the most valuable products in terms of both economic and nutritional value in Mediterranean countries. The health benefits of incorporating EVO into the diet have long been well-documented.

According to the research findings, the levels of pesticide residues in EVO do not pose a threat to consumer health. However, the presence of even small amounts of pesticides could jeopardize the commercial value of this product.

It should also be considered that EVO is a staple ingredient in the Mediterranean diet and there is a high likelihood that it is consumed alongside other contaminated foods. As a result, adverse effects on human health could occur due to the combined action of different contaminants, even when their levels are below the legally permitted limits. Therefore, continuous monitoring of contaminant levels in food is essential for properly assessing and managing the risks associated with chemicals in food products (*Erminia Schiano et al. 2024*).

The results demonstrate the high sensitivity and efficiency of both LC-MS/MS and GC-MS/MS in detecting pesticides in various food types. While most samples contained pesticide levels below the detection limit, some samples, such as kale, lemons, strawberries and nectarines, contained pesticide residues within the permitted concentrations. However, pesticide concentrations exceeding maximum allowable levels were found in strawberry jam samples, rendering them unsuitable for market distribution. To prevent such occurrences, the adoption of Good Agricultural Practices (GAP) is essential, focusing on cultivation, harvesting, storage, food processing, chemical application, worker hygiene, equipment maintenance, and traceability. The Rapid Alert System for Food and Feed (RASFF) is another critical tool in ensuring consumer safety, performing risk assessments, and adhering to EU-approved guidelines. The rise in active ingredient use in strawberry farming can be attributed to factors like pest and disease control, market demands, crop quality improvements, environmental adaptation, sustainable practices, and regulatory compliance. These findings highlight the necessity of increasing

pesticide analyses and evaluating cumulative dietary exposure to develop effective risk management measures and establish revised maximum residue levels (MRLs).

CONCLUSION

The results indicate that LC-MS/MS and GC-MS/MS methods are highly effective in detecting pesticides in various types of food. Although in most cases the pesticide concentration was below the detection level, in products such as kale, lemons, strawberries, and nectarines, pesticide concentrations were still recorded, albeit below the maximum allowable concentration. However, values exceeding the maximum allowable concentration were found in strawberry jam samples, which should not be available on the market, as the presence of even one pesticide above the allowable concentration renders the product unfit for consumption.

To prevent such cases, the adoption of Good Agricultural Practices (GAP) is necessary to reduce consumer risks associated with food production. These practices include activities such as cultivation, harvesting, storage, food processing, application of chemical agents, worker and equipment hygiene, and traceability.

Additionally, one of the tools in the process of protecting consumers and ensuring the availability of safe food on the market is the Rapid Alert System for Food and Feed (RASFF). This system conducts consumer risk assessments and employs guidelines and tools approved at the EU level. These tools play a critical role in ensuring consumer safety.

The increased use of active ingredients in strawberry farming can be attributed to a combination of factors, including the need for effective pest and disease control, meeting market demands, improving crop quality, adapting to environmental conditions, adopting sustainable practices, and complying with regulatory requirements.

Exposure to pesticides severely undermines public health due to its acute and long-term harmful effects, necessitating the strengthening of

institutions that monitor their use (PARA/ANVISA). Additionally, it is necessary to review the legislative framework for the registration of new pesticides and related compounds. To achieve this, it is crucial to conduct additional national research that will serve as the foundation for policies aimed at prevention and the promotion of health.

Future studies should focus on the cumulative exposure of consumers to pesticide residues from multiple sources and foods. Risk assessments are generally conducted individually for each pesticide, but with increasing evidence of combined exposure risks, there is a pressing need to develop methodologies that account for the synergistic and cumulative effects of different chemicals. Research should investigate how low concentrations of multiple chemicals may lead to harmful effects, even when individual pesticides fall below the maximum residue limits (MRLs). The development of multi-tiered models that simulate combined exposure to pesticides could provide a more accurate understanding of their potential health impacts.

As technology evolves, it is crucial to explore newer methods of pesticide detection that offer higher sensitivity and precision. While LC-MS/MS and GC-MS/MS have been effective in detecting pesticide residues, newer technologies such as high-resolution mass spectrometry (HRMS) or ambient ionization techniques could provide enhanced capabilities for detecting pesticides at even lower concentrations, minimizing false negatives and expanding the range of detectable contaminants. Research into the development of portable, field-deployable devices for real-time pesticide residue detection could also significantly improve food safety monitoring.

Connecting recommendations with international standards, such as the Codex Alimentarius, significantly emphasizes the importance of aligning pesticide regulations globally. The Codex Alimentarius is a collection of international guidelines and standards for food that plays a key role in protecting consumer health and ensuring fair trade.

Codex guidelines, including the Codex Standard for Pesticide Residues (Codex STAN 193-1995), serve as a framework for harmonizing national legislation and encourage countries to adopt their own regulations in accordance with international norms.

Linking recommendations with Codex guidelines can contribute to the alignment of maximum residue levels (MRL), which reduces trade uncertainty and enhances food safety. It also promotes the development of more efficient systems for monitoring pesticide residues in food, ensuring that consumers are not exposed to dangerous levels of chemicals. Alignment with international standards facilitates global cooperation and food trade by reducing barriers and ensuring uniform regulation.

Furthermore, the implementation of Codex guidelines promotes sustainable agricultural practices, which helps reduce environmental contamination and preserve biodiversity. This increases transparency and boosts consumer confidence in food safety, as they know that products meet international standards. Additionally, support for Codex guidelines enables the strengthening of capacities for pesticide analysis and control through education and technical assistance.

Integrating these international guidelines into national policies allows for a coordinated approach to food safety challenges, thereby contributing to global food security and the sustainability of food systems.

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DECLARATION OF INTERESTS

"Authors declare no conflict of interest"

LITERATURE

Agilent Technologies, Agilent GC/MS DRS Pesticide Screener, Flyer, 2017, Available on: <https://www.agilent.com/cs/library/flyers/public/5990-5310EN.pdf>, Accessed: 20.10.2024.

Agilent Technologies, RTL Pesticide MS Library, Product No. G1049A, 1999, Available on: http://www.chem.agilent.com/ecommerce/product/product_catalog_3.aspx?groupid=&sid=none&catid=&subcatid=&pid=38, Accessed: 20.1.2024.

ALWSCI Technologies, Solvent Filtration – Sample Purification: A Detailed Guide, 2024, Available on: <https://hr.alwsci.com/news/solvent-filtration-sample-purification-an-i-82561313.html>, Accessed on: 19.3.2025.

Cairns, T., Chiu, K. S., Navarro, D., Siegmund, E.: *Multiresidue pesticide analysis by ion-trap mass-spectrometry*, Rapid Commun Mass Spectrom, 7, 1993, 971–988.

Commission Directive 98/73/EC of 18 September 1998 adapting to technical progress for the 24th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Available at: <https://eur-lex.europa.eu/legal-content/HR/TXT/?uri=CELEX:31998L0073>, Accessed: 23.3.2024.

Croatian Encyclopedia, Extraction, Miroslav Krleža Lexicographical Institute, Available on: <https://www.enciklopedija.hr/natuknica.aspx?id=17468>, Accessed: 20.3.2024.

Croatian Encyclopedia, Solvents, Miroslav Krleža Lexicographical Institute, Available on: <https://www.enciklopedija.hr/clanak/otapala>, Accessed: 20.3.2024.

Croatian Normative Document. Foods of plant origin – Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS -- Part 1: General considerations (EN 12393-1:2013), Available at: <https://repozitorij.hzn.hr/norm/HRN+EN+12393-1%3A2013>, Accessed: 19.9.2024.

Croatian Normative Document. Foods of plant origin - Multiresidue methods for the determina-

tion of pesticide residues by GC or LC-MS/MS -- Part 2: Methods for extraction and clean-up (EN 12393-2:2013), Available at: <https://repozitorij.hzn.hr/norm/HRN+EN+12393-2%3A2013>, Accessed: 19.9.2024.

Croatian Normative Document. Foods of plant origin - Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS -- Part 3: Determination and confirmatory tests (EN 12393-3:2013), Available at: <https://repozitorij.hzn.hr/norm/HRN+EN+12393-3%3A2013>, Accessed: 19.9.2024.

de Hoffmann, E., Stroobant, V.: *Mass Spectrometry: Principles and Applications*, Wiley, West Sussex, England, 2002.

Debler, F., & Gandrass, J.: Development of an analytical method for the determination of more than 300 pesticides and metabolites in the particulate and gaseous phase of ambient air. *Analytical and bioanalytical chemistry*, 416, 2024, 12, 3059–3071. Available on: <https://doi.org/10.1007/s00216-024-05254-4>, Accessed: 23.3.2025.

Erminia Schiano, M., Sodano, F., Cassiano, C., Magli, E., Seccia, S., Grazia Rimoli, M., & Albri-zio, S.: Monitoring of seven pesticide residues by LC-MS/MS in extra virgin olive oil samples and risk assessment for consumers. *Food chemistry*, 442, 2024. Available on: <https://doi.org/10.1016/j.foodchem.2024.138498>, Accessed: 23.3.2025.

European Parliament, Information Articles on the European Union, Chemicals and Pesticides, 2004, Available at: <https://www.europarl.europa.eu/factsheets/hr/sheet/78/kemikalije-i-pesticidi>, Accessed on: 19.3.2024.

Fillion, J., Sauve, F., Selwyn, J.: Multiresidue method for the determination of residues of 251 pesticides in fruits and vegetables by gas chromatography/mass spectrometry and liquid chromatography with fluorescence detection, *J AOAC Int*, 83, 2000, 698–713.

Focant, J-F., Pirard, C., De Pauw, E.: Automated sample preparation-fractionation for the measurement of dioxins and related compounds in biological matrices: a review, *Talanta*, 63, 2004, 1101-13.

Food Act, N.N. 018/2023, Available on: https://narodne-novine.nn.hr/clanci/sluzbeni/full/2023_02_18_302.html, Accessed: 18.11.2024.

Food Act, Official Gazette of BiH, br. 50/04, Available on: https://www.msb.gov.ba/dokumenti/7Zakon_o_hrani.pdf, Accessed: 18.11.2024.

Food Quality and Phytosanitary Policy Administration, Annual Report on the Implementation of the National Monitoring Program for Pesticide Residues in and on Food for the Period of 2014, Ministry of Agriculture, Zagreb, 2015.

Grebe, S. K., Singh, R. J.: LC-MS/MS in the Clinical Laboratory - Where to From Here?, *Clin Biochem Rev.*, 32, 1, 2011, 5-31.

Guadalupe, Z., Soldevilla, A., Sáenz-Navajas, M. P., & Ayestarán, B.: Analysis of polymeric phenolics in red wines using different techniques combined with gel permeation chromatography fractionation, *Journal of chromatography A*, 2006, 1112, 1-2, 112-120, Available at: <https://doi.org/10.1016/j.chroma.2005.11.100>, Accessed: 22.9.2024.

Prezime zarez prvo slovo imena točka dvotočka Hans G.J. Mol: Food Toxicants Analysis, Elsevier Science, Wageningen, 2007.

Jensen, B. H., Petersen, A., Petersen, P. B., Christensen, T., Fagt, S., Trolle, E., Poulsen, M. E., & Hinge Andersen, J.: Cumulative dietary risk assessment of pesticides in food for the Danish population for the period 2012-2017. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 168, 2022. Available on: <https://doi.org/10.1016/j.fct.2022.113359>, Accessed: 19.3.2025.

Kortenkamp, A., Faust, M.: Regulate to reduce chemical mixture risk, *Science*, 361, 2018., 224-226, Available on: 10.1126/science.aat9219, Accessed: 19.3.2025.

Kovač, M., Šubarić, D., Bulaić, M., Kovač, T., Šarkanj, B.: Jučer maskirani, a danas modificirani – što je sljedeće s mikotoksinima?, *Arh Hig Rada Toksikol.*, 69(3), 2018, 214-214, Available at: <https://doi.org/10.2478/aiht-2018-69-3108>, Accessed: 19.10.2024.

Kožul, D., Herceg-Romanić, S.: Analiza polikloriranih dibenzo-p-dioksina i polikloriranih dibenzofurana u tlu i sedimentu., *Arh Hig Rada Toksikol.*, 60, 2, 2009, 243-257, Available at: <https://doi.org/10.2478/10004-1254-60-2009-1922>, Accessed: 19.10.2024.

Lu, F. C.: Acceptable daily intake: Inception, evolution, and application, *Regulatory Toxicology and Pharmacology*, 8, 1988, 1, 45-60.

Martinek, R., Lózsa, R., Póti, Á., Németh, E., Várady, G., Szabó, P., & Szüts, D.: Comprehensive investigation of the mutagenic potential of six pesticides classified by IARC as probably carcinogenic to humans, *Chemosphere*, 362, 2024, 142700. Available on: <https://doi.org/10.1016/j.chemosphere.2024.142700>, Accessed on: 20.10.2024.

Matus, I.: *Organska i anorganska nevodena otapala*, Završni rad, Sveučilište u Zagrebu, Fakultet šumarstva i drvne tehnologije, Zagreb, 2016, Available on: <https://urn.nsk.hr/urn:nbn:hr:108:579247>, Accessed: 23.3.2024.

Matus, I.: *Organska i anorganska nevodena otapala*, Završni rad, Sveučilište u Zagrebu, Fakultet šumarstva i drvne tehnologije, Zagreb, 2016, Available on: <https://urn.nsk.hr/urn:nbn:hr:108:579247>, Accessed: 23.3.2024.

Mešić, A., Juran, I., Pajač Živković, I.: Važnost doze pesticida u dostizanju ciljeva moderne poljoprivrede, osobito zdravstvenu ispravnost hrane, *Glasiло biljne zaštite*, 18, 5, 2018, 427-433. Available on: <https://hrcak.srce.hr/236991>, Accessed: 23.3.2024.

Miletić, M., Murati, T., Puntarić, A., Bilandžić, N., Kmetić, I.: Utjecaj pripremnih tehnika i termičkih postupaka procesiranja na sadržaj ostataka pesticida u namirnicama, *Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam*, 13, 3-4, 2018, 78-85. Available on: <https://doi.org/10.31895/hcptbn.13.3-4.12>, Accessed: 23.4.2024.

Miller, J. M.: Chromatography, *Encyclopedia of Applied Physics*, 2009.

Na, T. W., Seo, H. J., Jang, S. N., Kim, H., Yun, H., Kim, H., Ahn, J., Cho, H., Hong, S. H., Kim, H. J., & Lee, S. H.: Multi-residue analytical

method for detecting pesticides, veterinary drugs, and mycotoxins in feed using liquid- and gas chromatography coupled with mass spectrometry, *Journal of chromatography*, 1676, 2022, 463257. Available at: <https://doi.org/10.1016/j.chroma.2022.463257>, Accessed: 21.10.2024.

Nasreddine, L., Parent-Massin, D.: Food contamination by metals and pesticides in the European Union. Should we worry?, *Toxicology Letters*, 127, 2002, 29-41.

Oyeyiola, A. O., Fatunsin, O. T., Akanbi, L. M., Fadahunsi, D. E., & Moshood, M. O.: Human Health Risk of Organochlorine Pesticides in Foods Grown in Nigeria. *Journal of health & pollution*, 7(15),2017, 63–70. <https://doi.org/10.5696/2156-9614-7.15.63>

Puntarić, D., Muškulin, M., Bošnjir, J. et al.: *Zdravstvena ekologija*, Medicinska naklada, Zagreb, 2012.

Rastija V, Medić-Šarić M.: Kromatografske metode analize polifenola u vinima. *Kemija u industriji*, 58(3), 2009,121-128. Available on: <https://hrcak.srce.hr/32964>, Accessed: 14.03.2025.

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety: Official Journal of the European Union. Available at: <https://eur-lex.europa.eu/legal-content/HR/TXT/?uri=celex:32002R0178>, Accessed: 19.3.2024.

Regulation (EC) No. 396/2005 of the European parliament and the Council; on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC (2005): Official Journal of the European Union. Available at: <https://eur-lex.europa.eu/legal-content/HR/TXT/?uri=CELEX:32005R0396>, Accessed: 19.3.2024.

Sabljak, I., Brlek, V., Grubelić, M. et al.: *Ostaci pesticida u poljoprivrednim proizvodima tijekom 2013. godine*, Euroinspekt Croatiakontrola d.o.o, Stručni rad, 2013.

Saucier, C., Bourgeois, G., Vitry, C., Roux, D., Glories, Y.: Characterization of (+)-Catechin–Acetaldehyde Polymers: A Model for Colloidal State of Wine Polyphenols, *J. Agric. Food Chem.* 1997, 45, 4, 1045–1049.

Schürmann, A., Crüzer, C., Duss, V., Kämpf, R., Preiswerk, T., & Huebschmann, H. J.: Automated micro-solid-phase extraction clean-up and gas chromatography-tandem mass spectrometry analysis of pesticides in foods extracted with ethyl acetate. *Analytical and bioanalytical chemistry*, 416(3), 2024, 689–700. Available on: <https://doi.org/10.1007/s00216-023-05027-5>, Accessed: 19.3.2025.

Shao, W. C., Zang, Y. Y., Ma, H. Y., Ling, Y. E., & Kai, Z. P.: Concentrations and Related Health Risk Assessment of Pesticides, Phthalates, and Heavy Metals in Strawberries from Shanghai, China, *Journal of food protection*, 84, 12, 2021, 2116–2122. Available on: <https://doi.org/10.4315/JFP-21-165>, Accessed: 20.10.2024.

Siddhanta, A. K, Goswami, A. M., Ramavat, B. K., Mody, K. H., Mairh, O. P. : Water soluble polysaccharides of marine algal species of Ulva (Ulvales, Chlorophyta) of Indian waters. *Indian J Mar Sci.* 30, 2001, 166–172.

Thier, H. P., Zeumer, H.: *Manual of pesticide residue analysis*, II., Weinheim: Wiley VCH., 1992, 26–28.

Tudi M., Daniel Ruan H., Wang L., Lyu J., Sadler R., Connell D., Chu C., Phung DT.: Agriculture Development, Pesticide Application and Its Impact on the Environment. *Int J Environ Res Public Health.* 18(3), 2021. Available on: <https://www.mdpi.com/1660-4601/18/3/1112>, Accessed: 19.3.3024.

van Zoonen, P.: *Analytical methods for residues of pesticides in foodstuffs*, 6th edition. Part I. Annex B., The Hague: General Inspectorate for Health Protection, 1998, 1–8.

Vrsaljko, A.: Pesticidi i mikotoksini u jezgri bajama u ekološkim uvjetima Ravnih Kotara – organski uzgoj, *Pomologia Croatica*, 26, 1-4, 2022, 125-140. Available on: <https://doi.org/10.33128/pc.26.1-4.6>, Accessed: 20.10.2024.

Wong, J. W., Webster, M. G., Halverson, C. A., Hengel, M. J., Ngim, K. K., Ebeler, S. E.: Multi-

residue pesticide analysis in wines by solid-phase extraction and capillary gas chromatography-mass spectrometric detection with selective ion monitoring, *J Agric Food Chem*, 51, 2003, 1148–1161.

Xie, H., Sdougkou, K., Bonnefille, B., Papazian, S., Bergdahl, I. A., Rantakokko, P., & Martin, J. W.: Chemical Exposomics in Human Plasma by Lipid Removal and Large-Volume Injection Gas Chromatography-High-Resolution Mass Spectrometry. *Environmental science & technology*, 58(40), 2024, 17592–17605. Available on: <https://doi.org/10.1021/acs.est.4c05942>, Accessed: 19.3.2025.

Xu, M. L., Gao, Y., Wang, X., Han, X. X., & Zhao, B.: Comprehensive Strategy for Sample Preparation for the Analysis of Food Contaminants and Residues by GC- MS/MS: A Review of Recent Research Trends, *Foods*, 10, 2021, 2473. Available at: <https://doi.org/10.3390/foods10102473>, Accessed on: 23.3.2024.

Zubak, J.: Ljudska sigurnost: sigurnost hrane i glad kao dimenzije međunarodne sigurnosti, *Polemos*, XVIII, 36, 2015, 163-179. Available on: <https://hrcak.srce.hr/156742>, Accessed: 18.11.2024.

SIGURNOST HRANE I OSTACI PESTICIDA U HRANI BILJNOG PODRIJETLA: REZULTATI ČETVEROGODIŠNJEG MONITORINGA

SAŽETAK: Sigurnost hrane ključna je za zaštitu zdravlja potrošača, budući da pesticidi koji se koriste u poljoprivredi za zaštitu usjeva mogu ostaviti ostatke u prehrambenim proizvodima. Ti ostaci mogu predstavljati zdravstvene rizike, što zahtijeva regulatorni nadzor, poput utvrđivanja maksimalnih razina ostataka (MRL) pesticida. U Europskoj uniji, te su razine propisane Uredbom (EZ) br. 396/2005. Cilj ovog istraživanja bio je analizirati količinu pesticida u prehrambenim proizvodima iz Splitsko-dalmatinske županije, Hrvatska, u razdoblju od 2019. do 2023. godine, koristeći analitičke metode LC-MS/MS i GC-MS/MS. Uzorci su uključivali voće, povrće, maslinovo ulje i džemove, s naglaskom na detekciju i kvantifikaciju ostataka pesticida. Rezultati pokazuju da su svi analizirani uzorci pomoću LC-MS/MS i GC-MS/MS metoda imali razine pesticida ispod granice detekcije, osim u nekoliko izoliranih slučajeva gdje su pesticidi poput malationa, permetrina i deltametrina otkriveni iznad tog praga, ali i dalje unutar dopuštenih granica. Jedini izuzetak bio je džem od jagoda, u kojem je captafol pronađen u koncentracijama koje prelaze maksimalnu razinu ostataka (MRL). Testirani uzorci obuhvatili su razne poljoprivredne proizvode, a GC-MS/MS analiza otkrila je da je od 22 prehrambena proizvoda, 15 uzoraka (68,2%) imalo razine pesticida ispod granice detekcije, dok je u 6 uzoraka (27,3%) utvrđena prisutnost ostataka pesticida iznad granice detekcije, ali unutar dopuštenih granica. Samo jedan uzorak (4,5%) imao je razine pesticida koje su prelazile maksimalno dopuštenu granicu. Slično tome, LC-MS/MS analiza pokazala je da je 21 uzorak (95,2%) imao razine pesticida ispod granice detekcije, dok je samo jedan uzorak (4,5%) premašio taj prag. Ovi rezultati sugeriraju da su propisi o sigurnosti hrane uglavnom dobro poštovani, uz nekoliko izoliranih slučajeva detektiranih ostataka pesticida koji zahtijevaju daljnji nadzor. Rezultati pokazuju da je u većini analiziranih uzoraka razina pesticida bila ispod granice detekcije ili unutar dopuštenih vrijednosti, što ukazuje na učinkovit nadzor pesticida u proizvodnji hrane. Međutim, izolirani slučajevi u uzorcima kelja, limuna, nektarine i džema od jagoda, gdje su određeni pesticidi otkriveni iznad granice detekcije ili dopuštenih koncentracija, upućuju na moguć lokalizirani problem, nedostatke u nadzoru ili potencijalne izvore kontaminacije tijekom skladištenja i transporta. Preporučuje se daljnja analiza i istraživanje kako bi se potvrdila dosljednost ovih nalaza i utvrdili uzroci nepravilnosti, s ciljem poboljšanja sustava nadzora.

Ključne riječi: GC-MS/MS, LC-MS/MS, pesticidi, sigurnost hrane

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