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Evaluation of 146 pesticides in peels, mesocarp, and seeds of thirty-five cultivars of apples (*Malus domestica* Borkh.) grown under the same edaphoclimatic region

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ABSTRACT

In order to help optimize pesticide management and application strategies, we analyzed pesticide residues in thirty commercial and five regional apple cultivars from the same edaphoclimatic region in Portugal. Each sample was carefully divided into peel, pulp (mesocarp), and seeds. Analytical assessments were carried out utilizing a QuEChERS technique followed by LC-MS/MS, with 146 pesticide as targets. Regional cultivars tested confirmed for six different pesticide residues, ranging in concentrations from 0.01 to 1.46 mg/kg. However, there were nineteen compounds in the commercial samples, with concentrations ranging from 0.01 to 0.212 mg/kg. The comparison of 21 cultivars under controlled conditions improves the interpretation of residue data by establishing a clear correlation between the active substances sprayed and their presence in the fruit. Residues were predominantly concentrated in peel and seed fractions, while mesocarp tissues generally presented lower contamination levels, suggesting limited penetration of several compounds into the edible portion of the fruit. Differences in tissue distribution were associated with cultivar characteristics and physicochemical properties of the pesticides, particularly lipophilicity. The results obtained allow us to understand the distribution of pesticide residues in specific apple tissues and highlight the importance of residue monitoring systems to promote consumer safety and sustainable agricultural practices.

1. Introduction

The Mediterranean diet pyramid positions fruits at its foundation due to their health benefits and illness prevention properties. Fruits vary greatly, but because of the minerals, fiber, and bioactive compounds they contain, they are still vital components of a balanced diet (Harris

Abbreviations: ADI, Acceptable Daily Intake; D-SPE, dispersive solid-phase extraction; MeOH, Methanol; ACN, Acetonitrile; EtOH, Ethanol; EAc, Ethyl acetate EAc; EFSA, European Food Safety Authority; FA, Formic Acid; Anh, $MgSO_4$, Anhydrous magnesium sulfate; NaCl, Sodium chloride; HQ, Hazard Quotient; PSA, Primary Secondary Amine bonded silica; QuEChERS, Quick, Easy, Cheap, Effective, Rugged, and Safe; QTRAP, Quadropule Ion Trap; DNC, Dinitrocarbanilide or 1,3-bis4-nitrophenylurea; TPP, Triphenylphosphate; LOQ, Limit of quantification; LC-MS-MS, Liquid Chromatography with tandem mass spectrometry; MRL, Maximum residue limit; MIP, Molecularly imprinted polymer; PRMLs, Pre-harvest residue limits; SPE, Solid-phase extraction; RSDr, Repeatability; RSDw, Within-laboratory reproducibility.

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et al., 2022; Peng et al., 2022). Producing 951.9 million metric tons of fruit in 2023 demonstrated the importance of fruits for human health, agricultural output, international commerce and food safety (FAO, 2024). After bananas (139.3 million metric tons) and watermelons (104.9 million metric tons), apples (*Malus domestica* L.) were the third most produced fruit in the world, with 97.3 million metric tons (FAO, 2024). Apple production has increased dramatically over the past few decades, going from 58.1 million metric tons in 2003–97.3 million metric tons in 2023 (FAO, 2024), despite the fact that apples are one of the most extensively grown crops in the world. This production extends over 4.62 million hectares globally (FAO, 2024). Half of the world's apples were produced in China in 2023, 49.6 million metric tons, confirming Asia's dominance as the apple-producing region. (FAO, 2024). Among the other major producers, the FAO (2024) lists the US at 5.15 million metric tons, Turkey at 4.6 million, Poland at 3.89 million, and Italy at 2.88 million metric tons. The Food and Agriculture Organization of the United Nations reported that 13,940 ha of apple crops yielded 292,230 metric tons in 2023. About three quarters of this output is eaten raw, as fruit, while the other quarter is transformed into other foods like juice, purée, or applesauce (Andrés et al., 2016; Mignard et al., 2021).

Each variety is unique in its size, color, hardness, shape, texture, scent, juiciness, flavor (including sour, sweet, and bittersweet sensations), and nutritional content (Mignard et al., 2021;Tao et al., 2023).

Apples are recognized for their nutritional value and high content of bioactive compounds, particularly phenolic compounds. These compositional characteristics may also influence pesticide retention and tissue-specific distribution (Feng et al., 2021; Jakobek and Matić, 2024).

Lončarić et al. (2020) and Teixeira et al. (2023) reported that apple peel contains higher concentrations of bioactive compounds, particularly phenolic compounds, than the mesocarp (Lončarić et al., 2020; Teixeira et al., 2023; Campos-Mañas et al., 2019; Tirado et al., 2010; van den Berg et al., 2020).

Due to the intensive phytosanitary management required in apple orchardsapples are frequently associated with the occurrence of multiple pesticide residues. Factors such as the physicochemical characteristics of the pesticide, its systemicity and lipophilicity, the time of its application, the conditions under which it is applied, and the cultivar-specific properties all have a role in how residues behave in fruits. Specifically, for a lot of apple varieties, the distribution of residues among the peel, pulp, and seeds is still not well understood. Additional research is required to elucidate the mechanisms regulating tissue-specific pesticide dispersion in apples.

While pesticides are essential for agricultural production and food security, they also give rise to legitimate concerns about environmental contamination and pesticide residues in food. According to Campos-Mañas et al. (2019), the agro-food industry, a significant contributor to Europe's GDP, must strike a balance between efficient pest management and ensuring the safety of food (Campos-Mañas et al., 2019). The use of pesticides and the behavior of pests are both impacted by climate change, which makes it more difficult to maintain safe and sustainable food production systems (Tirado et al., 2010; van den Berg et al., 2020).

In order to safeguard the health of consumers, Regulation (EC) No. 396/2005 and its amendments established maximum residue limits (MRLs) for feeds and foods derived from both plants and animals.All EU member states are required by the European Food Safety Authority (EFSA) to establish annual monitoring programs to detect illicit pesticide residues in food. For the purpose of summarize the results of these official checks in all EU Member States, Norway, and Iceland, both of which are part of the European Economic Area, EFSA publishes reports annually (Marchese, 2024).

To successfully isolate pesticide analytes from food matrix, researchers have employed various extraction techniques (Barchanska et al., 2018; Anastassiades et al., 2003). The search for pesticides is laborious and time-consuming. The acronym "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, and Safe) describes a novel extraction method developed by Anastassiades et al. (2003). The process begins with acetonitrile extraction and continues with d-SPE for cleaning and partitioning. The initial target of this approach was food matrices with a high water content (>75%) and low fat content. Several adjustments allowed it to be used on dry and greasy dishes. Not only are QuEChERS-based methods efficient, but they also have a number of other advantages, such as being easy to use, recovering compounds with a wide range of polarities very well, having short analytical times, being very precise, and requiring less organic solvent (Tu and Yang, 2022).

In the Western region in central Portugal, which is famous for having the perfect climate for growing apples, this project aims to measure and map the levels of pesticide residues in commercial and regional apple types grown in the same edaphoclimatic zone. Beginning with SANTE Document No. SANTE/11312/2021v2 (SANTE, 2024) as its basis, the study establishes the LC-MS/MS analytical technique for measuring pesticide residues in apples. It then looks at how much residue is in different parts of the fruit (peel, pulp or mesocarp, and seeds) and compares the profiles of the residues in commercial and regional varieties, produced in accordance with the currently applicable Integrated Pest Management (IPM) standards.

Phytosanitary intensity, agronomic management, and cultivar selection may vary significantly among apple cultivation systems. Regional cultivars are frequently associated with traditional or locally adapted production systems, while commercial cultivars are typically associated with intensive production systems, often optimized for storage performance and productivity. Even in IPM systems, producers are facing difficult challenges, associated with the emergence of new pests or resistance phenomena resulting from the limited availability of chemical families currently available on the market. Despite this, traditional models, which are less focused on the market, are subject to less treatment pressure and residue occurrence patterns may be influenced by these distinctions.

During the growth season, all apple cultivars at the experimental station were treated with the identical phytosanitary solutions. However, it is unclear what phytosanitary measures were taken with a different group of apple cultivars that were also researched in the same area of Portugal. In the end, the goal of the study is to provide valuable information that can help with food safety management and sustainable apple production.

2. Materials and methods

2.1. Chemicals and consumables

After consulting with LG standards and Sigma-Aldrich Corporation (St. Louis, MO, USA), 146 pesticide reference standards were purchased. The following solvents were acquired from Merck (Darmstadt, Germany): n-hexane, ethyl acetate (EAc), formic acid (FA), acetonitrile (ACN), methanol (MeOH), toluene, acetone, and acetone. The water was treated using Millipore's Milli-Q Plus system, which operates in Molsheim, France. We acquired analytical-grade salts from Fluka in Seelze, Germany, including anhydrous magnesium sulfate (MgSO4) and sodium chloride (NaCl). Sigma-Aldrich of Madrid, Spain, obtained the sodium citrate dibasic sesquihydrate, while AppliChem of Darmstadt, Germany, procured the tri-sodium citrate 2-hydrate. Supelco (SupelcleanTM, Bellefonte, PA, USA) was contacted in order to get primary secondary amine (PSA) and bonded silica C18. Using Whatman Mini-UniPrep® syringeless filters (0.2 μm, Merck, Darmstadt, Germany), the last extract that was meant to be injected into the apparatus was filtered.

2.2. Experimental design

Apple samples were collected from a total of thirty-five cultivars in two harvesting periods: between July and November 2021 and between July and October 2022. Given that these fruits were not intended for consumption and that, for many cultivars, the optimal harvest period is

not yet known because they are still in preliminary testing prior to potential commercial introduction, the harvest dates were defined without consideration of pre-harvest intervals. Harvests were therefore grouped whenever possible to minimize travel to the various collection sites. All samples were obtained from the Vieira de Natividade Fruit Research Station (Alcobaça, Portugal), which belongs to the National Institute for Agrarian and Veterinary Research (INIAV, I.P., Portugal) and comprise material originating both from its own experimental fields and from experimental fields of collaborating producers. These samples are used to perform preliminary analyses within studies on adaptability, production viability, or preparation for commercial introduction, and were also used to investigate residues dynamics in the different components of the fruit in order to obtain baseline information that will contribute to defining appropriate treatment strategies and schedules for each one.

Apple cultivars were divided into two categories based on their agronomic origin and cultivation context: five regional and thirty commercial cultivars (including test or pre-commercial cultivars). Regional cultivars were traditional or locally adapted types connected with regional production systems and agricultural heritage, whereas commercial cultivars were those commonly cultivated for large-scale production and market distribution. This classification was deemed essential for pesticide residue interpretation since cultivar-specific features such as peel properties, wax composition, and fruit morphology can influence pesticide deposition, retention, penetration, and dissipation.

The regional cultivars included *Pardo Lindo*, *Repinau*, *Pêro de Borbela*, *Pêro Coimbra*, and *Noiva* and the commercial cultivars analyzed were *Granny Smith*, *Redfu*, *Challenger*, *Rubelite*, *Story*, *Fuji San*, *Fuji Spur*, *Inobi*, *Fuji Fubrax*, *Pixie*, *Bonita*, *Candine Espanha*, *Esmeralda*, *Candine França*, *Evelina*, *F7E3*, *UEB6581*, *Fengapi*, *Fujion*, *Fuji Aztec*, *Dalinette*, *Rubin Fuji*, *Gala*, *I3GS*, *Alpigala*, *I3D7*, *Galaval*, *Gemini*, *Gaia*, and *Bigbucks*.

All apple cultivars were subjected to phytosanitary treatments during the growing season, regardless of their origin, following the crop-protection calendar in accordance with integrated production guidelines.

While standardized phytosanitary schedules and monitored agronomic conditions were implemented for experimental station cultivars, slight differences in cultivar maturation and harvest timing may have influenced residue levels due to variable pesticide dissipation periods. Nevertheless, the systematic documentation of phenological stages, treatment dates, and cultivation conditions minimized major variability associated with residue behavior and enhanced the traceability and interpretation of residue occurrence.

Cultivars maintained at the Vieira de Natividade Fruit Research Station under standardized phytosanitary schedules and monitored agronomic practices were considered to be under controlled conditions, whereas cultivars obtained from collaborating producers were classified were considered to be under uncontrolled conditions due to potential variability in orchard management and environmental factors.

Details of the treatment schedule are provided in Tables 1 and 2.

2.3. Preparation of analytical standards

Individual pesticide stock solutions (~1000 μg/L) were prepared in appropriate solvents, chosen according to the solubility of each analyte, include acetonitrile (ACN), methanol (MeOH), toluene, and acetone.

An intermediate stock solution and a working solution were prepared by diluting the individual stock standard solutions appropriately. Two internal standards were used: triphenyl phosphate (TPP) for analytes

Table 2
Orchard characteristics and treatment conditions.

Orchard	Location	Cultivars included	Treatment conditions
Experimental Station (ENFVN)	P1	Granny Smith; Challenger; Story; Fuji Fubrax; Fuji Aztec	Same treatment schedule applied across the Station plots
Experimental Station (ENFVN)	P2	Redefu 20; Rubelite; Fuji Spur; Bonita; UEB6581	Same treatment schedule applied across the Station plots
Experimental Station (ENFVN)	P3	Gala	Same treatment schedule applied across the Station plots
Maiorga	–	F7E3; Fujion; I3GS; I3D7; Gemini; Gaia; Smeralda	Treatments applied by the Maiorga producer
Acipreste	–	Alpigala; Galaval; Bigbucks; Fengapi	May have received the same treatments as the Maiorga group (same producer)
Acipreste	–	Evelina	Treatments applied by Acipreste producer
Cela	–	Inobi; Pixie; Dalinette; Fuji San	Same treatment schedule applied by this producer
Cela	–	Candine (Spain); Candine (France)	Treatments applied by another producer

Table 1
Fungicide and insecticide applications in relation to apple phenological stages during the growing season.

Cultivars	Month	Date	Pesticide	Functional class	Phenological state
Regional cultivars and Experimental Station (ENFVN) P1, P2, P3	**April**	01/april	Pyriprozyfen and Hexythiazox	Pyriprozyfen: Insecticide (Insect growth regulator); Hexythiazox: Acaricide (mite growth inhibitor)	Phase of the appearance of the floral clusters [D3 (54)]
	June	02/june			Phase of the beginning of petal fall [G (67)]
	April	13/april	Cyprodinil	Fungicide (Anilinopyrimidine)	Phase in which the bunches are spread apart [E (58)
		20/april			Phase of early flowering [F (61)]
		26/april	Difenoconazole	Fungicide (Triazole, DMI – demethylation inhibitor)	Phase of early flowering [F (61)]
	May	05/may	Difenoconazole		Full flowering phase [F2 (65)]
	June	02/june	Difenoconazole		Phase of the beginning of petal fall [G (67)]
		21/june			Fruit enlargement phase [J (74)]
	May	05/may	Kresoxim-methyl	Fungicide (QoI – quinone outside inhibitor), by disrupting the fungal mitochondrial respiratory chain	Phase of full flowering [F2 (65)]
		20/may			Phase of early petal fall [G (67)]
	June	02/june			Phase of early petal fall [G (67)]
	June	09/june	Tebuconazole	Fungicide (Triazole)	Setting phase
			Indoxacarb	Insecticide (sodium channel blocker; DMI – demethylation inhibitor)	Setting phase
		30/june	Indoxacarb		Fruit enlargement phase [J (74)]
	July	13/july	Indoxacarb		Fruit enlargement phase [J (74)]
	August	03/ august			Fruit enlargement phase [J (74)]

Concentrations and application conditions according to DGADR (2011).

detected in positive ionization mode, and 4,4′-dinitrocarbanilide (DNC) for those detected in negative mode. The working solution was prepared at concentrations of 2.5 ng/μL and 1 ng/μL, depending on the analyte, by dilution of the corresponding stock solutions. All solutions were stored at −20 °C until use.

A standard mixture containing the target pesticides at a concentration of 250 μg/L was prepared. Working standard solutions (10–120 μg/L) were subsequently prepared and used exclusively for the preparation of the matrix-matched calibration standards.

For matrix-matched calibration, levels of 10, 20, 40, 60, 80, and 100 μg/L were prepared by mixing equal volumes (500 μL) of each working standard solution with 500 μL of a blank matrix extract.

2.4. Sample preparation strategy and extraction procedure

Each cultivar included in the study was represented by a selection of 10–12 fruits. Fruits were manually separated into three anatomical components: peel, seeds, and pulp (mesocarp). Each component was individually homogenized using a grinder homogenizer (Ultra-Turrax® T25, Janke & Kunkel IKA, Staufen, Germany). To make sure that all the samples were the same, samples that were typical of each cultivar were obtained from different parts of each plant.

Prior to analysis, samples were preserved at −20 °C in clean, inert containers to prevent contamination.

In order to remove pesticide residues from apple samples, the QuEChERS method was modified in accordance with the European Standard EN 15662 (2018) (SANTE, 2024). Document No. SANTE/11312/2021v2, which becomes effective as of 01/01/2024, details the methodology utilized for analysis and how they were interpreted (Pihlström et al., 2021).

A representative 10 g subsample of homogenized apple tissue was extracted with acetonitrile. Subsequently, a salt mixture containing magnesium sulfate, sodium chloride, and citrate buffering salts (4:1:1:0.5, w/w/w/w; pH 5.0–5.5) was added. The mixture was vigorously shaken and then centrifuged to facilitate phase separation. An aliquot of the organic phase was cleaned using dispersive solid-phase extraction (dSPE) with primary secondary amine (PSA, 150 mg) and magnesium sulfate (900 mg) to remove residual water and matrix components.

To 1 mL of the cleaned extract, 220 μL of acetonitrile was added. Then, 0.5 mL aliquots were spiked with internal standards (triphenyl phosphate, TPP, and 4,4′-dinitrocarbanilide, DNC) before being directly injected into the liquid chromatography-tandem mass spectrometry (LC-MS/MS) system for analysis.

2.5. Liquid chromatography tandem mass spectrometry analysis (LC-MS/MS)

Pesticide residues were detected and quantified using a QTRAP 5500 + MS/MS system (SCIEX, Foster City, CA, USA) equipped with a TurboV source and an electrospray ionization (ESI) probe, coupled to an UHPLC Nexera X2 system (Shimadzu, Kyoto, Japan). Chromatographic separation was performed on a Synergi Fujion-RP column (80 Å, 50 × 2 mm, 4 μm particle size; Phenomenex, Torrance, CA, USA). Sample injection volume was set at 10 μL, with a mobile phase flow rate of 1 mL/min. The mobile phases consisted of ultrapure water (phase A) and methanol (phase B), each containing 0.1% formic acid. Elution was carried out under a binary gradient mode, with a total runtime of 18 min.

Mass spectrometric data were acquired in multiple reaction monitoring (MRM) mode, with synchronized positive and negative ESI ionization (Table S1). Instrumental parameters were optimized as follows: ion spray voltage 4500 V, source temperature 600 °C, curtain gas (CUR) at 35 psi, and gas 1 and gas 2 at 40 and 60 psi, respectively. Curtain gas, gas 1 and gas 2 are nitrogen with a purity > 99.9%. Optimization of ion transitions (quantifier and qualifier ions) and collision energies for each analyte was performed via direct injection of standard solutions at approximately 1 μg/mL.

2.6. Methodology validation

The SANTE/11312/2021v2 document gives rules for method validation and quality control in regular pesticide residue analysis. We followed these rules for method validation. We looked at a number of important factors, including as specificity, linearity, accuracy (which was measured through recovery assays), and precision (which was measured through repeatability as RSDr and within-laboratory reproducibility as RSDwR). When specificity was less than 30%, matrix effects were less than 20%, recoveries were between 70% and 120%, and accuracy was less than 20%, the method was deemed acceptable.

2.6.1. Linearity and Limit of quantification (LOQ)

The analytical method was validated at the lowest analyte concentration that met both identification and quantification requirements adequately in order to define the limit of quantification (LOQ). Linearity and LOQ were assessed through matrix-matched calibration curves over a concentration range of 0.01–0.10 mg/kg. Working standard solutions expressed in μg/L were used only to prepare the matrix-matched calibration standards, whereas linearity was evaluated using matrix-matched concentrations expressed in mg/kg.

2.6.2. Accuracy

The accuracy of the method was determined through recovery assays. Recovery was determined by spiking a blank (white) matrix at four concentration levels (0.01, 0.02, 0.04, and 0.05 mg/kg), with six replicates per level. The recovery percentage was calculated as the ratio between the mean experimentally measured concentration and the spiked concentration.

The validated calibration range encompassed the anticipated residue concentrations in the analyzed samples. To guarantee quantification within the validated linear range of the method, appropriate dilution procedures were implemented prior to reanalysis whenever sample concentrations exceeded the upper calibration limit.

2.7. Statistical analysis

The statistical analysis of the data was performed with IBM® SPSS® Statistics, version 30.0.0.0. (Chicago, IL, USA), carrying out one-way analysis of variance (ANOVA), when the normality and homogeneity of variances were validated. The Tukey test was evaluated to examine the disparities among average values. Significance was defined at $p < 0.05$. Results were expressed as mean value ± the standard deviation (SD) of three replicates.

3. Results and discussion

3.1. Validation of the LC-MS/MS method

The method was successfully validated for determining 146 pesticide residues in apples. All target analytes met the acceptance criteria established by the SANTE/11312/2021v2 guidance document (Table 3).

Selectivity was assessed by analyzing blank apple samples (non-contaminated samples) to verify the absence of interfering chromatographic peaks at the retention times corresponding to the target pesticides, in accordance with the SANTE/11312/2021v2 criteria. LOQ was also evaluated, and the signal intensity of pesticides was at least five times higher than any signal detected in blank samples, corresponding to a signal ratio below 20%.

Calibration curves were established for pesticide quantification over concentration ranges of 0.01–0.1 mg/kg for the majority of analytes, except monocrotophos, which was calibrated from 0.01 to 0.08 mg/kg. Calibration standards were prepared both in organic solvent and in

Table 3
Maximum residue limits (MRL) of the target pesticides (MRL) according to European Legislation in force and results of the validation methodology reporting performance parameters, including limit of quantification (LOQ), linearity, repeatability, reproducibility, accuracy, and uncertainty, in accordance with EU guidelines for pesticide residue analysis.

Pesticides	MRL (mg/kg)	Linearity range (mg/kg)	Linearity R^2	LOQ (mg/kg)	Repeatability (CV %)	Reproducibility (CV %)	Accuracy (Recovery %)	Uncertainty (%)
Acetamiprid	0.4	0.01–0.1	0.998	0.01	4.82	4.38	88.99	11
Azoxystrobin	0.01	0.01–0.1	0.995	0.01	7.65	7.62	83.15	32
Bitertanol	0.01	0.01–0.1	0.986	0.01	17.2	15.7	107.4	29
Bixafen	0.01	0.01–0.1	0.994	0.01	5.05	5.46	95.98	14
Boscalid	2	0.01–0.1	0.998	0.01	4.81	6.00	85.46	16
Bupirimate	0.3	0.01–0.1	0.996	0.01	11.9	15.5	86.52	46
Buprofezin	0.01	0.01–0.1	0.998	0.01	5.36	4.73	100.7	34
Cadusafos	0.01	0.01–0.1	0.993	0.01	7.00	11.0	88.50	32
Carbaryl	0.01	0.01–0.1	0.993	0.01	8.03	11.5	96.81	37
Carbendazim	0.2	0.01–0.1	0.994	0.02	13.5	13.4	78.75	21
Carbofuran	0.001	0.01–0.1	0.994	0.01	5.61	8.53	95.08	28
Carbofuran−3-hydroxy		0.01–0.1	0.993	0.01	10.6	12.8	88.92	34
Carboxin	0.03	0.01–0.1	0.999	0.01	5.39	8.70	64.90	29
Chlorantraniliprole	0.4	0.01–0.1	0.995	0.01	9.12	10.8	96.10	41
Chlorfenvinphos	0.01	0.01–0.1	0.997	0.01	4.38	7.39	99.65	28
Chlorpyrifos	0.01	0.01–0.1	0.974	0.01	9.68	10.5	97.10	21
Chlorpyrifos-methyl	0.01	0.01–0.1	0.989	0.01	7.05	15.7	91.30	47
Clofentezine	0.5	0.01–0.1	0.998	0.01	5.04	9.70	89.36	44
Clothianidin	0.4	0.01–0.1	0.992	0.01	9.46	8.31	89.39	20
Coumaphos		0.01–0.1	0.994	0.01	7.85	14.3	92.80	47
Cymoxanil	0.01	0.01–0.1	0.998	0.01	8.66	9.87	88.40	23
Cyproconazole	0.1	0.01–0.1	0.997	0.01	4.08	4.49	87.60	12
Cyprodinil	2	0.01–0.1	0.997	0.01	5.05	7.71	86.90	24
Demeton-S-methylsulfone	0.01	0.01–0.1	0.980	0.01	15.6	19.4	92.20	38
Diazinon	0.01	0.01–0.1	0.990	0.01	5.61	18.9	93.30	45
Dichlorvos	0.01	0.01–0.1	0.992	0.01	5.76	12.9	88.07	46
Dicrotophos		0.01–0.1	0.993	0.01	8.89	10.9	91.70	28
Difethofencarb	0.01	0.01–0.1	0.984	0.01	6.17	8.53	93.58	34
Difenoconazole	0.8	0.01–0.1	0.993	0.01	6.65	15.0	90.54	47
Diflubenzuron	0.01	0.01–0.1	0.998	0.01	4.64	10.5	100.8	36
Dimethoate	0.01	0.01–0.1	0.997	0.01	5.67	7.65	91.55	17
Dimethomorph	0.01	0.01–0.1	0.996	0.01	4.75	8.31	88.03	27
Diniconazole	0.01	0.01–0.1	0.993	0.01	5.30	8.86	94.14	21
DMST		0.01–0.1	0.997	0.01	7.15	12.2	91.87	36
EPN		0.01–0.1	0.981	0.01	7.18	13.6	98.08	41
Epoxiconazole	0.01	0.01–0.1	0.997	0.01	5.07	9.48	92.87	31
Ethiofencarb		0.01–0.1	0.976	0.01	10.6	14.2	85.48	37
Ethion	0.01	0.01–0.1	0.978	0.01	12.8	12.7	97.29	23
Ethirimol	0.06	0.01–0.1	0.990	0.02	11.9	17.9	62.79	44
Ethoprophos	0.01	0.01–0.1	0.997	0.01	4.65	6.66	93.97	27
Etrimfos		0.01–0.1	0.980	0.02	14.6	10.9	75.74	36
Fenamidone	0.01	0.01–0.1	0.995	0.01	5.33	5.72	87.12	15
Fenamiphos sulfone		0.01–0.1	0.998	0.01	4.79	5.14	93.90	12
Fenamiphos sulfoxide		0.01–0.1	0.998	0.01	4.41	3.60	87.67	7
Fenamiphos	0.01	0.01–0.1	0.996	0.01	6.02	10.2	87.51	37
Fenarimol	0.1	0.01–0.1	0.997	0.01	4.08	5.00	92.00	14
Fenazaquin	0.15	0.01 – 0.1	0.992	0.01	7,14	20.0	80.80	42
Fenhexamid	0.01	0.01–0.1	0.997	0.01	11.5	10.2	82.86	21
Fenitrothion	0.01	0.01–0.1	0.996	0.01	7.16	9.22	94.25	26
Fenoxycarb	0.7	0.01–0.1	0.996	0.01	6.79	5.87	87.33	18
Fenpropathrin	0.01	0.01–0.1	0.997	0.01	9.54	10.2	92.43	33
Fenpropidin	0.01	0.01–0.1	0.996	0.01	6.69	6.18	97.27	12
Fenpropimorph	0.01	0.01–0.1	0.994	0.01	5.25	6.99	88.19	19
Fenpyroximate	0.3	0.01–0.1	0.983	0.01	14.8	18.6	74.72	35
Fenthion oxon sulfone		0.01–0.1	0.995	0.01	6.51	9.50	87.13	37
Fenthion oxon sulfoxide		0.01–0.1	0.997	0.01	5.27	5.35	86.43	15
Fenthion oxon		0.01–0.1	0.994	0.01	6.22	4.78	85.87	12
Fenthion sulfone		0.01–0.1	0.996	0.01	5.85	5.26	94.00	13
Fenthion sulfoxide		0.01–0.1	0.998	0.01	5.40	8.12	87.89	28
Fenthion	0.01	0.01–0.1	0.997	0.01	6.22	7.99	93.36	27
Flufenoxuron	0.01	0.01–0.1	0.997	0.01	7.78	13.6	87.70	43
Fluopyram	0.8	0.01–0.1	0.996	0.01	4.53	4.12	90.84	10
Fluquinconazole	0.01	0.01–0.1	0.996	0.01	5.25	4.87	87.55	7
Flusilazole	0.01	0.01–0.1	0.998	0.01	4.44	3.67	90.04	10
Flutriafol	0.4	0.01–0.1	0.994	0.01	5.88	6.82	90.16	15
Fonofos		0.01–0.1	0.992	0.01	8.39	14.0	83.40	38
Fosthiazate	0.02	0.01–0.1	0.998	0.01	5.03	5.83	92.47	20
Hexaconazole	0.01	0.01–0.1	0.988	0.01	4.86	10.2	93.89	27
Hexythiazox	0.4	0.01–0.1	0.987	0.01	8.22	16.1	106.0	37

(*continued on next page*)

Table 3 (*continued*)

Pesticides	MRL (mg/kg)	Linearity range (mg/kg)	Linearity R^2	LOQ (mg/kg)	Repeatability (CV %)	Reproducibility (CV %)	Accuracy (Recovery %)	Uncertainty (%)
Imazalil	0.01	0.01–0.1	0.996	0.01	9.08	10.1	84.52	19
Imidacloprid	0.01	0.01–0.1	0.990	0.01	13.2	10.6	90.50	17
Indoxacarb	0.01	0.01–0.1	0.996	0.01	8.56	9.75	92.58	30
Iprodione	0.01	0.01–0.1	0.997	0.01	7.03	8.84	86.46	29
Iprovalicarb	0.01	0.01–0.1	0.993	0.01	11.3	13.3	87.43	36
Isoprocarb		0.01–0.1	0.995	0.01	5.16	6.45	91.98	19
Isoprothiolane	0.01	0.01 – 0.1	0.995	0.01	7.45	10.7	92.20	30
Isoproturon	0.01	0.01–0.1	0.995	0.01	4.91	5.76	92.45	17
Kresoxim-methyl	0.2	0.01–0.1	0.994	0.01	14.3	14.2	96.56	35
Linuron	0.01	0.01–0.1	0.996	0.01	5.63	5.52	89.19	15
Lufenuron	1	0.01–0.1	0.997	0.01	7.17	7.37	87.43	22
Malaoxon	0.02	0.01–0.1	0.998	0.01	5.45	6.49	91.85	22
Malathion		0.01–0.1	0.978	0.01	11.9	12.9	99.02	37
Mepanipyrim	0.01	0.01–0.1	0.996	0.01	6.25	9.24	96.16	27
Metaflumizone	0.9	0.01–0.1	0.992	0.01	10.5	14.2	90.25	45
Metalaxyl	1	0.01–0.1	0.997	0.01	5.59	10.6	74.91	36
Metalaxyl-M		0.01–0.1	0.996	0.01	6.32	6.16	85.51	19
Metazachlor	0.02	0.01–0.1	0.995	0.01	6.74	5.76	99.34	14
Metconazole	0.02	0.01–0.1	0.994	0.02	5.90	8.58	89.81	24
Methiocarb sulfoxide	0.03	0.01–0.1	0.995	0.01	6.60	8.56	78.40	31
Methiocarb		0.01–0.1	0.993	0.01	7.25	11.1	86.84	38
Metobromuron	0.02	0.01–0.1	0.993	0.01	4.39	6.02	97.63	18
Metribuzin	0.1	0.01–0.1	0.996	0.01	5.14	5.15	85.20	13
Mevinphos	0.01	0.01–0.1	0.998	0.01	4.79	4.21	96.10	7
Monocrotophos	0.01	0.01–0.08	0.995	0.01	9.83	11.4	91.88	30
Myclobutanyl	0.6	0.01–0.1	0.977	0.01	4.78	14.9	97.66	34
Oxadixyl	0.01	0.01–0.1	0.995	0.01	6.58	11.6	84.27	31
Oxydemeton-methyl	0.01	0.01–0.1	0.978	0.01	16.7	15.99	83.56	28
Paclobutrazole	0.05	0.01–0.1	0.996	0.01	4.46	10.2	81.99	39
Paraoxon-ethyl		0.01–0.1	0.993	0.01	6.58	6.96	97.05	21
Paraoxon-methyl	0.01	0.01–0.1	0.994	0.01	6.40	5.67	86.79	11
Parathion	0.05	0.01–0.1	0.989	0.01	6.64	10.9	106.8	20
Penconazole	0.15	0.01–0.1	0.996	0.01	5.22	4.55	86.49	10
Pencycuron	0.02	0.01–0.1	0.994	0.01	6.61	5.88	92.66	10
Pendimethalin	0.05	0.01–0.1	0.981	0.01	7.98	11.2	97.31	26
Phenthoate		0.01–0.1	0.982	0.02	10.7	10.3	101.9	22
Phosalone	0.01	0.01–0.1	0.987	0.01	11.5	11.6	89.54	20
Phosphamidon	0.01	0.01–0.1	0.996	0.01	5.52	6.14	86.89	17
Phoxim	0.01	0.01–0.1	0.986	0.01	9.02	15.7	104.6	41
Pirimiphos-ethyl		0.01–0.1	0.984	0.04	9.90	9.06	89.66	19
Pirimiphos-methyl	0.01	0.01–0.1	0.990	0.01	7.26	18.2	92.09	36
Prochloraz	0.03	0.01–0.1	0.985	0.01	10.4	12.6	79.75	41
Profenofos	0.01	0.01–0.1	0.994	0.01	6.25	11.4	89.95	36
Propiconazole	0.01	0.01–0.1	0.996	0.01	4.41	4.36	94.21	9
Propoxur	0.005	0.01–0.1	0.994	0.01	10.1	12.3	89.3	37
Propyzamide	0.02	0.01–0.1	0.998	0.01	5.92	4.58	89.53	9
Prothioconazole-desthio	0.01	0.01–0.1	0.999	0.01	4.46	6.65	91.76	22
Pyraclostrobin	0.5	0.01–0.1	0.990	0.01	7.51	9.90	104.4	26
Pyrazophos	0.01	0.01–0.1	0.997	0.01	6.13	5.71	95.42	13
Pyridaben	0.9		0.976	0.02	7.15	20.6	92.71	17
Pyrimethanil	15	0.01–0.1	0.996	0.01	4.98	4.67	89.55	9
Pyriproxyfen	0.2	0.01–0.1	0.979	0.01	10.1	15.3	99.56	45
Quinoxyfen	0.05	0.01–0.1	0.983	0.02	9.17	11.6	92.97	31
Rotenone	0.01	0.01–0.1	0.995	0.01	6.04	7.35	92.98	25
Spinosad A	0.3	0.01–0.1	0.991	0.02	10.3	19.8	82.68	39
Spinosad D		0.01–0.1	0.996	0.01	8.06	13.3	88.50	45
Spiroxamine	0.01	0.01–0.1	0.997	0.01	5.08	6.71	87.57	22
Tebuconazole	0.3	0.01–0.1	0.996	0.01	4.52	8.99	88.96	33
Tebufenpyrad	0.3	0.01–0.1	0.992	0.01	9.8	12.0	88.41	34
Teflubenzuron	0.01	0.01–0.1	0.995	0.01	5.14	5.21	87.17	9
Terbuthylazine	0.01	0.01–0.1	0.991	0.01	5.64	7.45	88.56	20
Tetraconazole	0.3	0.01–0.1	0.992	0.01	7.69	7.70	80.85	18
Tetramethrin		0.01–0.1	0.990	0.01	10.3	18.1	82.85	48
Thiabendazole	4	0.01–0.1	0.990	0.02	12.0	17.6	69.90	40
Thiacloprid	0.3	0.01–0.1	0.998	0.01	5.35	3.8	89.27	8
Thiamethoxam	0.3	0.01–0.1	0.998	0.01	13.3	17.1	80.69	44
Thiodicarb	0.01	0.01–0.1	0.994	0.01	8.30	9.67	85.89	30
Tolclofos-methyl	0.01	0.01–0.1	0.978	0.01	6.82	14.1	102.7	38
Triadimefon	0.01	0.01–0.1	0.996	0.01	4.98	5.05	100.0	11
Triadimenol	0.01	0.01–0.1	0.996	0.01	5.58	8.51	90.86	27
Triazophos	0.01	0.01–0.1	0.996	0.01	5.96	7.78	93.24	20
Tricyclazole	0.01	0.01–0.1	0.997	0.01	5.56	5.23	79.48	12
Trifloxystrobin	0.7	0.01–0.1	0.994	0.01	5.81	7.31	94.45	21
Triflumuron	0.01	0.01–0.1	0.994	0.01	5.17	5.44	93.87	16

(*continued on next page*)

Table 3 (*continued*)

Pesticides	MRL (mg/kg)	Linearity range (mg/kg)	Linearity R^2	LOQ (mg/kg)	Repeatability (CV %)	Reproducibility (CV %)	Accuracy (Recovery %)	Uncertainty (%)
Zoxamide	0.02	0.01–0.1	0.998	0.01	5.51	7.91	89.80	25
Fludioxonil*	5	0.01–0.1	0.990	0.01	10.5	14.5	97.34	45
Methoxyfenozide*	0.01	0.01–0.1	0.998	0.01	8.16	8.38	94.36	20

* Ionization in negative mode.

matrix-matched extracts to compensate for matrix-induced signal suppression or enhancement. Linearity was evaluated using the coefficient of determination (R^2), with 146 analytes demonstrating R^2 values > 0.98 across most of the calibration range. However, within the concentration interval under consideration, about 7.5% of the analytes showed R^2 values lower than 0.98, indicating non-linearities that might be attributable to matrix effects or limits of the instruments.

Lower than 20% relative standard deviations under repeatability settings (RSDr). Six separate extractions (replicates) of the target matrix were conducted at four different fortification levels (0.01 mg/kg, 0.020 mg/kg, 0.04 mg/kg, and 0.050 mg/kg) to evaluate reproducibility. Three separate operators assessed reproducibility over the course of three days. Table 3 shows that all analytes had relative standard deviations under repeatability conditions (RSDr) less than 20%, which is the acceptability criterion set out by the SANTE/11312/2021v2 guidance document.

3.2. Pesticide residues in apple cultivars

Using a validated LC-MS/MS technology, thirty commercial apple cultivars and five regional ones were tested for the presence of various pesticide residues, in compliance with the requirements set out by the European Commission (SANTE/11312/2021v2) (Pihlström et al., 2021). The distribution patterns across distinct fruit components might be assessed by doing residue analysis separately in the peel, pulp (mesocarp), and seed tissues.

Reliability in residue monitoring is enhanced by the standardization of cultivation and management procedures, which reduces the impact of common confounding factors in heterogeneous production systems. The interpretation of residue data is enhanced when both commercial and regional cultivars are used under standardized settings. This makes it possible to trace the existence of the active substances in the fruit back to their administration.

The fact that all 29 cultivars were grown and harvested from the same experimental orchard in the same agronomic and edaphoclimatic circumstances is the study's strongest point. Tables 1 and 2 show that the research station consistently followed the crop protection calendar while treating samples (as a phytosanitary schedule). The field experiments that examined the adaptability of various cultivars to the edaphoclimatic conditions in Western Portugal yielded the results shown in Tables 4–6. Several locations in the same region served as test sites.

In addition, we can learn more about the mechanisms behind the chemicals' specific penetration and distribution by dissecting the fruit into its peel, pulp, and seed. Compliance with regulations and consumer exposure are both affected by these results.

3.2.1. Pesticide residues in regional apple cultivars

Pesticide residue patterncns varied among the region's five apple types, each with its own morphological and phenological traits. The results demonstrated that the behavior and dynamics differed across cultivars when comparing the quantities of residue in the seed, pulp, and peel. Pesticides were applied according to the standard crop protection schedule during crucial phenological stages like cluster separation, flowering, fruit setting, and fruit enlargement (Table 1). This consistent treatment approach allows for a robust evaluation of the residue profiles of the regional cultivars.

The overall amounts of pesticide residues in each regional cultivar by active ingredient, are shown in Fig. 1A. Although the relative contributions of indoxacarb, difenoconazole, and tebuconazole differed among cultivars, indoxacarb was always the most prevalent pesticide. It is worth mentioning that the cultivars *Pêro Coimbra* and *Pardo Lindo* had the highest total residues (more than 2 mg/kg), whereas *Noiva* had the lowest levels (1.2 mg/kg) overall.

Fig. 1B shows the consistent pattern that was found while analyzing the tissue-specific distribution of pesticide residues in all of the regional apple varieties. Moreover, Fig. 1 C shows this values by pesticide while Fig. 1D shows the contribution of each regional cultivar to the detected residues by compound (mg/kg sample).The peel consistently retained

Table 4
Comparative levels of pesticide residues in Regional Apple Cultivars after the same phytosanitary treatment.

Apple's Cultivar		Cyprodinil (LMR- 2 mg/kg)	Difenoconazole (LMR- 0.8 mg/kg)	Hexythiazox (LMR- 0.4 mg/kg)	Indoxacarb (LMR- 0.01 *mg/kg)	Kresoxim-methyl (LMR- 0.2 mg/kg)	Pyriproxyfen (LMR- 0.05**mg/kg)	Tebuconazole (LMR- 0.3 mg/kg)
Noiva	**S**	n.d	0.010 ± 0.0006^{a}	0.005 ± 0.001^{ab}	0.018 ± 0.0006^{b}	n.d	n.d	0.016 ± 0.0006^{cd}
	P	n.d	0.007 ± 0.0017^{a}	0.006 ± 0.0015^{b}	0.037 ± 0.0015^{d}	n.d	0.01 ± 0.0053^{a}	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Pero de borbela	**S**	n.d	n.d	n.d	0.039 ± 0.0006^{d}	n.d	n.d	0.014 ± 0.0001^{bc}
	P	n.d	n.d	n.d	0.064 ± 0.0006^{e}	n.d	n.d	0.010 ± 0.0001^{a}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Pero coimbra	**S**	n.d	0.029 ± 0.0017^{d}	n.d	0.03 ± 0.0012^{c}	n.d	n.d	0.043 ± 0.0015^{f}
	P	n.d	0.025 ± 0.0001^{c}	0.01 ± 0.001^{b}	0.066 ± 0.0006^{e}	n.d	n.d	0.047 ± 0.0006^{g}
	E	n.d	n.d	n.d	0.010 ± 0.0001^{a}	n.d	n.d	0.012 ± 0.0001^{ab}
Pardo lindo	**S**	n.d	0.010 ± 0.0001^{a}	n.d	0.026 ± 0.001^{c}	n.d	n.d	0.018 ± 0.000^{d}
	P	n.d	0.023 ± 0.0006^{c}	n.d	0.146 ± 0.005^{g}	n.d	0.01 ± 0.0006^{a}	0.014 ± 0.0006^{bc}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Repineau	**S**	n.d	0.017 ± 0.0006^{b}	0.006 ± 0.0017^{b}	0.039 ± 0.0035^{d}	0.013 ± 0.0006^{b}	n.d	0.030 ± 0.0001^{e}
	P	n.d	n.d	0.008 ± 0.0006^{b}	0.094 ± 0.002^{f}	0.006 ± 0.0015^{a}	0.012 ± 0.0006^{b}	0.010 ± 0.0001^{a}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d

S-seeds; P- peels; E-Pulp; n.d.- not detected; Letters indicate significative difference within values of the same column ($p < 0.05$). * Indoxacarb LMR in apple is 0.01 mg/kg, established by Regulation (EU) 2024/376. This study was conducted before that regulation came into effect; thus, the previous limit of 0.5 mg/kg (Reg. (EU) 2015/845) was applicable at the time. ** Pyriproxyfen LMR in apple is 0.05 mg/kg, established by Regulation (EU) Reg. (EU) 2023/1753. This study was conducted before that regulation came into effect; thus, the previous limit of 0.2 mg/kg (Reg. (EU) 2023/679) was applicable at the time.

Table 5
Comparative levels of pesticide residues in commercial apple cultivars grown under unknown phytosanitary treatments.

Cultivar		Boscalid	Carbendazim	Metribuzin	Pirimicarb-desmethyl	Pyraclostrobin	Spinosad D	Tebufenpyrad	Thiacloprid	Trifloxystrobin	Chlorantraniliprole	Kresoxim-methyl	Cyprodinil	Fenoxycarb	Pirimicarb	Hexythiazox	Pyriproxyfen	Tebuconazole	Difenoconazole	Indoxacarb
FUJI SAN	**S**	0.056 ±0.0012[c]	0.011 ±0.001[a]	n.d	n.d	0.018 ±0.0015[ab]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.017 ±0.001[d]	n.d
	P	0.306 ±0.0243[f]	n.d	n.d	n.d	0.119 ±0.0015[d]	n.d	0.079 ±0.002[b]	n.d	0.013 ±0.002[a]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	E	0.009 ±0ab	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
INOBI	**S**	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	P	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.027 ±0.0006	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
PIXIE	**S**	0.014 ±0.001ab	0.019 ±0.0006b	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.022 ±0.001[f]	n.d
	P	n.d	n.d	n.d	n.d	n.d	n.d	0.356 ±0.0010[f]	n.d	0.042 ±0.001[b]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.014 ±0.001[b]	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CANDINE ESPANHA	**S**	n.d	n.d	0.103 ±0.002[c]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.012 ±0.001[a]	n.d
	P	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CANDINE FRANCA	**S**	n.d	n.d	0.021 ±0.001[b]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	P	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
EVELINA	**S**	0.543 ±0.0017[g]	n.d	n.d	n.d	0.172 ±0.0015[e]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	P	2.124 ±0.0074 h	n.d	n.d	n.d	0.563 ±0.0125[f]	0.011 ±0.001	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	E	0.08 5 ± 0[d]	n.d	n.d	n.d	0.012 ±0.001[a]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
F7E3	**S**	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.01 ±0.001a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	P	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
FENGAPI	**S**	n.d	n.d	n.d	n.d	n.d	n.d	0.042 ±0.001[a]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.019 ±0.001[e]	n.d
	P	n.d	n.d	n.d	n.d	n.d	n.d	0.32 ±0.0215[e]	n.d	n.d	n.d	n.d	n.d	n.d	0.012 ±0.001[b]	n.d	n.d	n.d	n.d	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.01 ±0.0006[a]	n.d	n.d	n.d	n.d	n.d
DALINETE	**S**	0.085 ±0.001[d]	n.d	n.d	n.d	0.025 ±0.0015b	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.016 ±0.001[c]	n.d
	P	0.192 ±0.0101[e]	n.d	n.d	n.d	0.069 ±0.006c	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	E	0.016 ±0.0006[b]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
ALPIGALA	**S**	n.d	n.d	n.d	n.d	n.d	n.d	0.229 ±0.0145[c]	0.01 ±0.0006[b]	n.d	n.d	n.d	n.d	n.d	0.022 ±0.0015[e]	n.d	n.d	n.d	0.038 ±0.002 h	n.d
	P	n.d	n.d	n.d	n.d	n.d	n.d	0.484 ±0.0065[g]	n.d	n.d	n.d	n.d	n.d	n.d	0.028 ±0.0006[f]	n.d	n.d	n.d	0.014 ±0.001[b]	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.02 ±0.001[d]	n.d	n.d	n.d	n.d	n.d
I3D7	**S**	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.014 ±0c	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	P	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

(*continued on next page*)

Table 5 (*continued*)

Cultivar		Boscalid	Carbendazim	Metribuzin	Pirimicarb-desmethyl	Pyraclostrobin	Spinosad D	Tebufenpyrad	Thiacloprid	Trifloxystrobin	Chlorantraniliprole	Kresoxim-methyl	Cyprodinil	Fenoxycarb	Pirimicarb	Hexythiazox	Pyriproxyfen	Tebuconazole	Difenoconazole	Indoxacarb
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
GALAVALE	S	n.d	n.d	n.d	0.012 ±0.0006[b]	n.d	n.d	0.268 ±0.0017[d]	n.d	n.d	n.d	n.d	n.d	n.d	0.044 ±0.0006[h]	n.d	n.d	n.d	0.022 ±0.001[f]	n.d
	P	n.d	n.d	n.d	0.01 ±0.001[a]	n.d	n.d	0.579 ±0.0045[i]	n.d	n.d	n.d	n.d	n.d	n.d	0.055 ±0.0006[i]	n.d	n.d	n.d	0.014 ±0.001[b]	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.01 ±0.001
BIGBUCKS	S	n.d	n.d	n.d	n.d	n.d	n.d	0.057 ±0.0035[a]	n.d	n.d	n.d	n.d	n.d	n.d	0.02 ±0.0015[d]	n.d	n.d	n.d	0.024 ±0.001[g]	n.d
	P	n.d	n.d	n.d	n.d	n.d	n.d	0.526 ±0.0068[h]	n.d	n.d	n.d	n.d	n.d	n.d	0.033 ±0.0015[g]	n.d	n.d	n.d	0.016 ±0.001[cd]	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.017 ±0.0006[c]	n.d	n.d	n.d	n.d	n.d
GAIA	S	n.d	n.d	0.012 ±0.0006[a]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	P	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

S-seeds; P- peels; E-Pulp; n.d.- not detected; Letters indicate significative difference within values of the same column ($p < 0.05$).

the highest concentration of pesticide residues, making up over 50% of the total pesticide burden. The seeds were the next most heavily contaminated, with contamination levels in the pulp falling far below 7% across all cultivars. The overall distribution pattern was fairly similar among the studied cultivars, however there was a minor variation in the proportional contributions of peel and seed. *Repinau* and *Noiva* had higher quantities of seeds, while *Pardo Lindo* had the largest proportion of peel (77%).

The residue profiles of each pesticide across cultivars were described through a compound-specific analysis. In all of the tissues that were tested, the concentration of cyprodinil did not exceed the limit of quantification. On 13 and 20 April, during the early stages of phenology (flower cluster separation and early flowering), this substance was applied twice. Considering the long interval between application and harvest, together with previously reported field residue occurrence and distribution profile data, its absence at harvest may be plausibly explained by degradation, dilution due to fruit growth, or other dissipation-related processes. However, as this study was not designed as a field dissipation experiment, no direct conclusions on cyprodinil degradation kinetics can be drawn. The reported half-lives of cyprodinil's field dissipation are quite matrix and context dependent. Marín et al. (2003) found that table grapes had a half-life of 4.68 days and lettuce a half-life of 2.46 days. (Marín et al., 2003). Newer research on grape crops has looked at edaphoclimatic parameter variations and found broader ranges: 9.6–20.8 days in fruit and 5.8–15.6 days in soil (Zhang et al., 2015). The rate of cyprodinil dissipation can be influenced by a variety of environmental conditions, according to study conducted by Zhang et al. (2015). These parameters include climate, sunshine, precipitation, and soil organic matter content. Fruit rot was less common in regions with moderate temperatures and less sunshine, according to their field research. Soil dissipation was shown to be accelerated in areas with higher organic matter levels and more rainfall (Zhang et al., 2015). The results show that cyprodinil residue behavior are very location-specific and influenced by environmental variables. Despite being sprayed early in the season, no detectable residues were detected in apple tissues during harvest due to its limited environmental persistence.

Fig. 1C shows that all regional apple cultivars, with the exception of *Pèro de Borbela*, contained detectable levels of difenoconazole residues in both the peel and the seeds. Concentrations as high as 0.029 mg/kg in the peel and 0.025 mg/kg in the seeds were recorded in the *Pèro Coimbra* cultivar, the most intense variety (Fig. 1B). *Noiva* and *Repinau* likewise had high seed concentrations, however the only part of *Pardo Lindo* where residues could be detected was in the peel (0.023 mg/kg). The pulp of every cultivar tested negative for contaminants.

The distribution of the substance in different tissues is in line with its physicochemical characteristics. The high lipophilicity of difenoconazole (log P = 4.36) makes it more likely to accumulate in lipophilic matrices like the cuticle and seed coat and less likely to penetrate the watery pulp (Craggs et al., 2020; Lewis et al., 2016). This preferred retention around the fruit surface is further exacerbated by its limited systemic movement. According to Shimshoni et al. (2019), pesticides like difenoconazole, which have log P values ≥ 4, have peel-to-pulp ratios higher than 15, and they remain on the fruit's surface even after being washed with water. This highlights their great attraction to cuticular waxes (Shimshoni et al., 2019).

Difenoconazole has a moderate persistence in terms of environmental behavior, with field dissipation half-life estimations averaging 91.8 days under standard agricultural circumstances. Its unusually long persistence explains its presence during harvest, particularly after many treatments. The current study used four difenoconazole administrations on 26 April, 5 May, 2 June, and 21 June to correspond with phenological stages. These treatments took place while the plants were in the early stages of flowering (F [61]) and beginning to ripen their fruits (J [74]). Even after a lengthy time has gone since the final application, residues still build during harvest, demonstrating the molecule's chemical

Table 6
Comparative levels of pesticide residues in commercial apple cultivars from the Experimental Station after the same phytosanitary treatment.

Cultivar		Chlorantraniliprole	Kresoxim-methyl	Cyprodinil	Fenoxycarb	Pirimicarb	Hexythiazox	Pyriproxyfen	Tebuconazole	Difenoconazole	Indoxacarb
Granny smith	**S**	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.015 ± 0.001^{c}	0.021 ± 0.0006^{fg}	0.032 ± 0.0006^{b}
	E	n.d	n.d	n.d	n.d	n.d	0.015 ± 0.0001^{e}	0.016 ± 0.0006^{cd}	0.029 ± 0.001^{h}	0.012 ± 0.0006^{a}	0.101 ± 0.0025^{jk}
	P	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Gala	**S**	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.017 ± 0.0006^{d}	0.025 ± 0.0006^{h}	0.043 ± 0.0006^{cd}
	P	n.d	n.d	n.d	n.d	n.d	0.011 ± 0.001^{b}	0.015 ± 0	0.019 ± 0.001^{e}	0.016 ± 0.001^{c}	0.166 ± 0.0006^{a}
	E	n.d	n.d	n.d	n.d	0.023 ± 0.0015^{d}	n.d	n.d	n.d	n.d	n.d
Fuji spur	**S**	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.01 ± 0.001^{a}	0.019 ± 0.0006^{a}
	P	n.d	n.d	n.d	n.d	n.d	0.014 ± 0.001^{d}	0.016 ± 0.0006^{cd}	n.d	0.011 ± 0.001^{a}	0.077 ± 0.0045^{h}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Fuji aztec	**S**	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.012 ± 0.0006^{b}	0.017 ± 0.0006^{cd}	0.018 ± 0.0006^{a}
	P	n.d	n.d	n.d	n.d	n.d	0.01 ± 0.0001^{a}	0.011 ± 0.0006^{a}	0.015 ± 0.001^{c}	0.014 ± 0.001^{b}	0.052 ± 0.0045^{e}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Redfu 20	**S**	n.d	0.011 ± 0.0014^{ab}	n.d	n.d	n.d	n.d	n.d	0.011 ± 0.001^{a}	0.022 ± 0.001^{g}	0.036 ± 0.0006^{bc}
	P	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.01 ± 0.0006^{a}	n.d	0.062 ± 0.002^{fg}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Challenger	**S**	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.014 ± 0.001^{c}	0.022 ± 0.001^{g}	0.044 ± 0.001^{d}
	P	n.d	n.d	n.d	n.d	n.d	0.01 ± 0.001^{a}	0.011 ± 0.001^{a}	0.021 ± 0.001^{f}	0.011 ± 0.001^{a}	0.067 ± 0.002^{g}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Rubelite	**S**	n.d	0.01 ± 0.0006^{a}	0.02 ± 0.0015^{a}	n.d	n.d	n.d	n.d	0.014 ± 0.001^{c}	0.018 ± 0.001^{de}	0.034 ± 0.001^{b}
	P	n.d	n.d	n.d	0.011 ± 0.0001^{a}	n.d	0.012 ± 0.001^{c}	0.016 ± 0.001^{d}	0.021 ± 0.0006^{f}	0.011 ± 0.001^{a}	0.1 ± 0.002^{ij}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Story	**S**	n.d	n.d	n.d	n.d	0.018 ± 0.001^{c}	0.016 ± 0.0006^{f}	n.d	0.028 ± 0.0006^{h}	0.037 ± 0.001^{j}	0.093 ± 0.002^{i}
	P	n.d	n.d	n.d	n.d	0.014 ± 0.0006^{b}	0.014 ± 0.0006^{d}	0.015 ± 0.0006^{cd}	0.028 ± 0.0006^{h}	0.024 ± 0.001^{h}	0.108 ± 0.006^{k}
	E	n.d	n.d	n.d	n.d	0.012 ± 0.0001^{a}	n.d	n.d	n.d	n.d	n.d
Fuji fubrax	**S**	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.012 ± 0.0006^{b}	0.029 ± 0.0015^{i}	0.048 ± 0.002^{de}
	P	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.01 ± 0.0006^{a}	n.d	0.068 ± 0.003^{g}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Bonita	**S**	n.d	0.012 ± 0.0006^{b}	n.d	n.d	n.d	n.d	n.d	0.014 ± 0.001^{c}	0.031 ± 0.001^{i}	0.059 ± 0.001^{f}
	P	n.d	n.d	n.d	0.019 ± 0.0006^{c}	n.d	n.d	0.012 ± 0.001^{b}	0.014 ± 0.001^{c}	n.d	0.108 ± 0.0025^{k}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ueb6581	**S**	n.d	n.d	0.022 ± 0.001^{b}	n.d	n.d	n.d	n.d	0.017 ± 0.001^{d}	0.041 ± 0.0012^{k}	0.033 ± 0.002^{b}
	P	0.012 ± 0.0006	n.d	n.d	0.018 ± 0.001^{b}	n.d	0.016 ± 0.001^{f}	0.021 ± 0.001^{e}	0.027 ± 0.001^{g}	0.019 ± 0.0006^{ef}	0.068 ± 0.001^{g}
	E	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

S-seeds; P- peels; E-Pulp; n.d.- not detected; Letters indicate significative difference within values of the same column ($p < 0.05$).

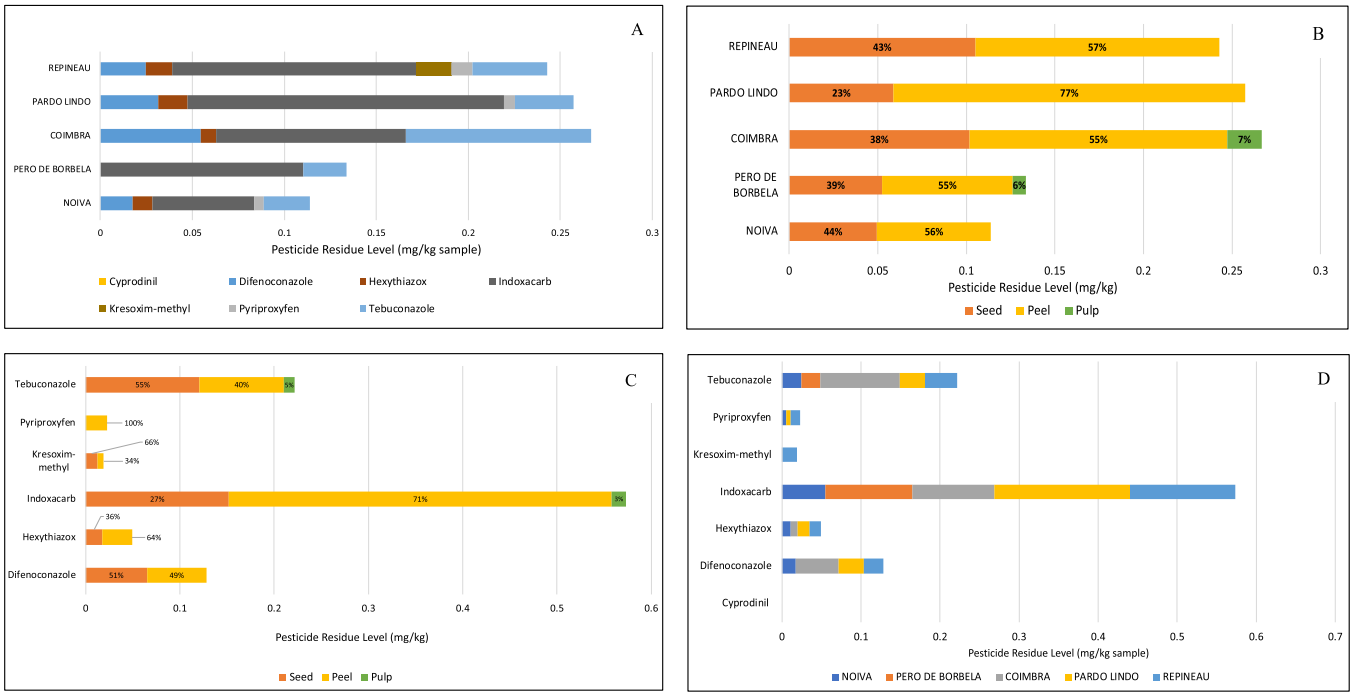

Fig. 1. Pesticide residue distribution in regional apple cultivars. (A) Contribution of individual pesticides to total residues. (B) Distribution of residues among apple tissues. (C) Tissue-specific distribution of pesticide residues by compound. (D) Contribution of each cultivar to pesticide residue levels. Results are expressed as mg/kg sample and percentage of total residues.

endurance and its great adherence to hydrophobic surfaces.

Additional insight into the dissipation of difenoconazole can be obtained from the study conducted by He et al. (2016). They discovered that after ten days of treatment with 78 g a.i./ha in controlled field conditions, residual levels in apples decreased to 0.01 mg/kg (He et al., 2016). Findings suggest a possible rapid decline in some usage scenarios; nevertheless, the elevated concentrations seen in *Pêro Coimbra* and other cultivars may be attributable to environmental influences, differences in cuticle structure, or metabolic capability for pesticide breakdown. Although difenoconazole levels in individual tissues (peel, pulp, seed) were below the MRL of 0.8 mg/kg for apples (Commision Regulation (EU), 2024/2612 amending Regulation (EC) No 396/2005) (Bellisai et al., 2024) the cumulative residue in the whole fruit did not exceed this limit, as shown in the Table 4.

Hexythiazox, applied twice (1 April and 2 June) during floral cluster emergence and early petal fall, was detected at low concentrations in both seeds (36%) and peel (64%) of all cultivars except *Pêro de Borbela*, and was consistently absent in the pulp (Figs. 1A and 1B). Detected levels ranged from 0.005 to 0.010 mg/kg in the seeds and approximately 0.010 mg/kg in the peel. The early application timing, combined with the very high lipophilicity of hexythiazox (log $P > 5$), its strong affinity for cuticular waxes, and its limited systemic mobility, likely promoted direct deposition and long-term retention on the surface of developing fruits. As the fruit developed, residues remained predominantly associated with the cuticular wax layer, resulting in their detection in peel tissues at harvest, while penetration into the aqueous pulp remained limited.

(Döker et al., 2021; PPDB, Pesticide Properties DataBase, 2023). All quantified values were below the MRL for apples (0.5 mg/kg), indicating compliance with regulatory limits (Table 3).

Indoxacarb, an oxadiazine insecticide widely used to control lepidopteran pests in fruit and vegetable crops (Cheng et al., 2010), was the most abundant pesticide detected across all regional cultivars and tissue types, as reflected by its predominance in the residue profiles shown in Figs. 1B and 1C. Residue levels ranged from 0.018 to 0.039 mg/kg in seeds (27%), 0.037–0.146 mg/kg in peel (71%), and up to 0.008 mg/kg in pulp (3%). The highest concentrations were detected in the peel, particularly in Pardo Lindo, Repinau, and Pêro Coimbra, while measurable residues in the pulp were only found in Pêro de Borbela and Pêro Coimbra.

From June 9 to August 3, the insecticide was used four times, covering important phenological stages from fruit set to fruit expansion (stages I [71] to J [74]). This repetitive application during later stages of development probably caused deposits in both the exterior and within of the fruit.This chiral insecticide is composed of two enantiomers, however only the S-enantiomer is biologically active. Its mode of action involves blocking sodium channels in insect neurons. Neurotoxic effects in mammals have been documented solely at elevated acute doses (e.g., LD50 179 mg/kg) (Lewis et al., 2016), resulting in neurological disorders including ataxia, tremors, and hemolysis in rats (Viswanathan et al., 2013).

It is probable that the translaminar mobility and moderate systemicity of indoxacarb, along with its high lipophilicity (log $P = 4.65$) (Lewis et al., 2016), allowed for measurable penetration into the pulp, thanks to the timing and frequency of administrations. Most of the other pesticides in this study accumulated in lipophilic matrices like the peel or seeds, in contrast to this interior distribution. To confirm these findings, in 2013, 2.7% of apple samples tested positive for indoxacarb, with maximum and mean amounts of 0.09 mg/kg and 0.013 mg/kg, respectively, according to the official EU pesticide monitoring program (EFSA, 2015).

Research conducted in Poland examined the effectiveness of indoxacarb in reducing populations of codling moth (*Cydia pomonella L.*) and leafrollers (Tortricidae) on apples intended for use in infant formula (Szpyrka et al., 2017). The first residues of indoxacarb were found to be 0.097 and 0.048 mg/kg, respectively, after spraying on two apple cultivars, Jonagold Decosta and Belle de Boskoop, according to the authors' study on residue dissipation. After seventy-four and forty-five days, the residues had fallen below the baby food MRL of 0.01 mg/kg, and they had declined by half in only twenty-four days.

According to research conducted by Hafez et al. (2021), indoxacarb residues were seen to persist on foliage for a full month following

spraying in cherry and apple orchards. Hafez et al. (2021) found that indoxacarb dissolves rather slowly in field circumstances, in contrast to other pesticide residues, which disappeared within seven days (Hafez et al., 2021).

The concentrations of indoxacarb are greater than the default legal threshold specified by Regulation (EU) 2024/376, which is 0.01 mg/kg, as shown in Table 4. Since this study was conducted before the implementation of Regulation (EU) 2015/845, the previous limit of 0.5 mg/kg was used. It is critical to reconsider the timing and frequency of indoxacarb treatments and to account for cultivar-dependent retention when assessing regulatory compliance and consumer safety. All five regional apple cultivars contained tebuconazole residues, mainly in seeds (55%) and peel (40%), with Pêro Coimbra showing the highest levels and being the only cultivar with detectable residues in the pulp (0.012 mg/kg). Additionally, unlike other pesticides, indoxacarb persisted on apple and cherry tree leaves throughout the monitoring period.

According to Hafez et al. (2021), this indicates that indoxacarb degrades at a slower rate in natural settings (Hafez et al., 2021).

Between 0.009 and 0.046 mg/kg, the levels were recorded. Outside of *Pêro Coimbra*, which had a value of 0.012 mg/kg, no cultivar's pulp contained any residues. Figs. 1A and 1C show that this cultivar had the highest residue concentrations in the seeds (0.043 mg/kg) and the peel (0.046 mg/kg), suggesting a distinct pattern of residue accumulation.

These results are in agreement with the physicochemical properties of the systemic fungicide tebuconazole. The triazole fungicide blocks the demethylation process. The body's production of ergosterol is halted, which is crucial for the function of fungal cell membranes (Deng et al., 2010). Due to its moderate systemicity and log P of 3.7, tebuconazole is less likely to be transported into the pulp and more likely to accumulate in lipophilic tissues such as the peel and seeds. Another possible explanation for its discovery during harvest is its longevity in the field (DT_{50} = 50–60 days), according to Cabrera and Pastor Luis Carrasco (2021).

Crucially, the fruitlets were only created on June 9, during the setting period of fruit growth, and tebuconazole was applied once. This early application likely contributed to the limited translocation into the inner tissues, as later applications tend to result in higher pulp concentrations due to ongoing systemic movement and reduced dilution by growth. The timing also coincides with the early differentiation of epidermal and seed tissues, which may have promoted retention in these compartments as the fruit matured. Further supporting the predominantly peel-associated accumulation of tebuconazole, similar findings were observed in the study by Kowalska et al. (2022). His research, which examined pesticide levels in apples subjected to basic culinary processing, showed that standard peeling removed all traces of tebuconazole from the fruit.

Pêro Coimbra may have different levels in all compartments since its cultivar has different degrees of metabolic residue occurrence, translocation efficiency, or cuticular permeability. The structural or biochemical properties of this kind may make tebuconazole more resistant to residue distribution or increase its retention. Aside from that, tebuconazole is chiral and usually used in a racemic mixture. Past research has shown that it degrades enantioselectively, which could be caused by enzymes that are particular to certain cultivars. *Pêro Coimbra's* peculiar residue profile may be explicable by the compound's stereoselective metabolism.

Specifically, in a study conducted by Chang et al. (2023) in a field environment, it was found that.

(-)-R-tebuconazole degraded at a faster rate than (+)-S-tebuconazole. This finding demonstrates that the tebuconazole enantiomers are dissipated stereoselectively in *Fuji* and *Hanfu* apples. It is worth noting that enantioselective residue behavior is cultivar-dependent, as it was not observed in *Huahong* apples. *Pêro Coimbra's* unique residue profile may be indicative of the compound's stereoselective metabolism, which is in agreement with recent findings regarding cultivar-dependent enantioselective action (Chang et al., 2023).

Table 4 shows that, in comparison to the 0.3 mg/kg MRL for apples (Reg. EU 2018/1514, 2018), the residues in the pulp of *Pêro Coimbra* remained compliant, while those in the peel and seeds did not exceed the legal limit. It is essential to consider application timing and varietal differences when evaluating residue behavior and consumer safety, as demonstrated in these research.

Pyripoxyfen is a popular insect growth regulator due to its low toxicity to mammals and high selectivity against pest insects. The environmental behavior becomes more complex when it breaks down into metabolites. As a result, there is cause for concern about the fate of these metabolites in various environmental compartments, particularly soils, because some of them are more dangerous and mobile than the parent molecule (Kumari et al., 2021). Plants, processed goods, and rotational crops are only a few of the matrices that have been extensively studied for pyriproxyfen residues and its transition products (Bellisai et al., 2022). Significant accumulation of apicultural commodities, including wax and honey, has also been discovered (Wu et al., 2021). Also, enantioselective degradation could affect environmental persistence and toxicological profiles because pyriproxyfen and some of its metabolites are chiral. Because pyriproxyfen and certain of its metabolites are chiral, Kumari et al. (2021) point out that enantioselective degradation could affect environmental persistence and toxicological profiles. Regulators regularly examine its MRLs to guarantee consumer health, although more evidence is needed for a comprehensive analysis (Carrasco Cabrera et al., 2024).

During the early stages of fruit growth, pyriproxyfen was administered twice in this study, between 1 April and 2 June. *Noiva* (0.005 mg/kg), *Pardo Lindo* (0.006 mg/kg), and *Repinau* (0.011 mg/kg) were the only cultivars whose peels were discovered to contain residues; none of the others had residues in their pulp or seeds. The early timing of application, in combination with the high lipophilicity of pyriproxyfen (log P > 5), its strong affinity for cuticular waxes and limited systemic mobility, likely favoured the direct deposition on developing fruits and longer retention within the fruit cuticle. This implies that residues found at harvest are more likely to be due to surface retention and slow dissipation from the cuticular wax layer rather than substantial translocation from leaves to fruit tissues.These results point to the fact that surface persistence and wash-off dynamics, and not translocation, are the main determinants of residue behavior. According to Table 3, the quantities of pyriproxyfen in all apple cultivars were below the current maximum residue limit (MRL) of 0.05 mg/kg (Reg. (EU) 2023/1753), suggesting that compliance may have been achieved. Additionally, significant data gaps concerning residue trials and analytical methodologies have been detected by the European Food Safety Authority (EFSA). Users should exercise caution while using pyriproxyfen, especially in cultivars that tend to have higher surface retention, because the present MRL's validity is being reviewed and could be changed if the needed information is not submitted by 12 September 2025.

Kresoxim-methyl is a strobilurin family fungicide that was found in trace amounts in the seed (66%) and peel (34%), exclusively in the cultivar Repinau. Despite three applications made during the crop cycle, on 5 May, 20 May, and 2 June, covering phenological stages from full flowering to early petal fall (F2 to G), no residues were detected in the pulp or any of the other cultivars that were analyzed. The compound's non-systemic character, fast breakdown, and cultivar-specific changes in absorption, surface retention, or degradation capacity are possible explanations for this restricted detection (Rahman et al., 2014).

Anke et al. (1977) identified strobilurin A, a metabolite of the fungus *Strobilurus tenacellus*, as the structural basis for the naturally occurring chemical kresoxim-methyl. Its action is limited to surface tissues due to its low systemicity, but it can impede mitochondrial respiration in target fungus, making it both a preventative and therapeutic fungicidal agent. Strong attachment to the lipid-rich cuticular layer and limited translocation into internal fruit tissues are favored by its physicochemical features, especially its fairly high lipophilicity (log P = 3.9) (Anke et al., 1977).

It should be noted that the amounts found in this investigation were

lower than the existing maximum residue limit (MRL) for apples, which is 0.2 mg/kg (Regulation (EU) 2020/856) (Table 3).

Varietal variations in cuticle permeability, wax composition, lipophilicity (log P), interact to determine the distribution of residues among peel, pulp, and seed tissues (Figs. 1B and 1C). According to Caboni et al. (2012) and EFSA (2015), indoxacarb and tebuconazole, which are highly lipophilic compounds, have a great affinity for cuticular waxes. Consequently, these substances mainly accumulate in the seeds and peel, with very rare instances of systemic translocation. However, the compounds cyprodinil and kresoxim-methyl, which have smaller effects and don't last as long, were either not found or found only in specific types of plants and tissues.

Low or undetectable levels of pesticide residue in pulp indicate that the pesticides were not systemic and highlight the importance of administering them at the correct time to protect consumers (More et al., 2021).

These features are consistent with the fact that Repinau's pulp contains zero residue and that its peel and seeds contain very low levels of residue. Following this line of thinking, Majumder et al. (2022) discovered that green chilli's kresoxim-methyl had short field dissipation half-lives of 6.3 days when administered at regular rate and 5.3 days when applied at double rate, indicating a tendency for rapid environmental deterioration (Majumder et al., 2022). Varietal variations in cuticle permeability, wax composition, or metabolic capability may impact the quantity of residue retention; hence, detection was confined to a single cultivar despite numerous treatments. According to Figs. 1B and 1C, the peel acted as the principal barrier to the penetration of pesticides, since its residue concentrations were often higher in it. A variety of physicochemical factors, including application timing/frequency, field dissipation half-life (DT_{50}), and lipophilicity (log P), interact to determine the distribution of residues among peel, pulp, and seed tissues.On the other hand, cyprodinil and kresoxim-methyl, which are substances with limited systemicity and shorter durability, were either not discovered or were limited to certain cultivars and tissues (Caboni et al., 2012; EFSA, 2015). It is crucial to apply pesticides at the correct times to ensure consumer safety, as the low or undetectable levels of pulp residue indicate that the pesticides used were not systemic (More et al., 2021).

Fig. 1D demonstrates that within this general pattern, there were discernible differences in residue accumulation. Herbicides such as difenoconazole, indoxacarb, and tebuconazole were regularly present in large numbers on *Pêro Coimbra*, with seed concentrations of 0.043 mg/kg and peel concentrations of 0.046 mg/kg. It may also have shown unusual pesticide absorption, transport, or metabolic breakdown tendencies since it was the only cultivar with detectable tebuconazole levels in its pulp. The timing of agricultural harvests is a key component that seems to account for these variations. Late in August, the *Pêro Coimbra* variety is brought in for gathering.

While most compounds in *Pêro de Borbela* remained below the limit of quantification in all tissues, the residual levels were modest. Improved metabolic degradation, decreased cuticular permeability, or other structural features that restrict pesticide retention could explain this pattern.

Cuticle composition, stomatal density, tissue lipophilicity, and cultivar-specific activity of xenobiotic-metabolizing enzymes are among the fundamental biological and physicochemical parameters that impact these varietal differences (Lara et al., 2014; Martin et al., 2014).

Moreover, for chiral compounds such as tebuconazole, enantioselective degradation may further contribute to variability, as enantiomers can be differentially absorbed, metabolized, or translocated depending on the cultivar (Chang et al., 2023). It is important to mention that the *Pêro de Borboleta* cultivar is one of the final ones to be harvested, along with the *Noiva* cultivar, towards the end of September or the start of October. As a result, since these late-harvesting types go through a

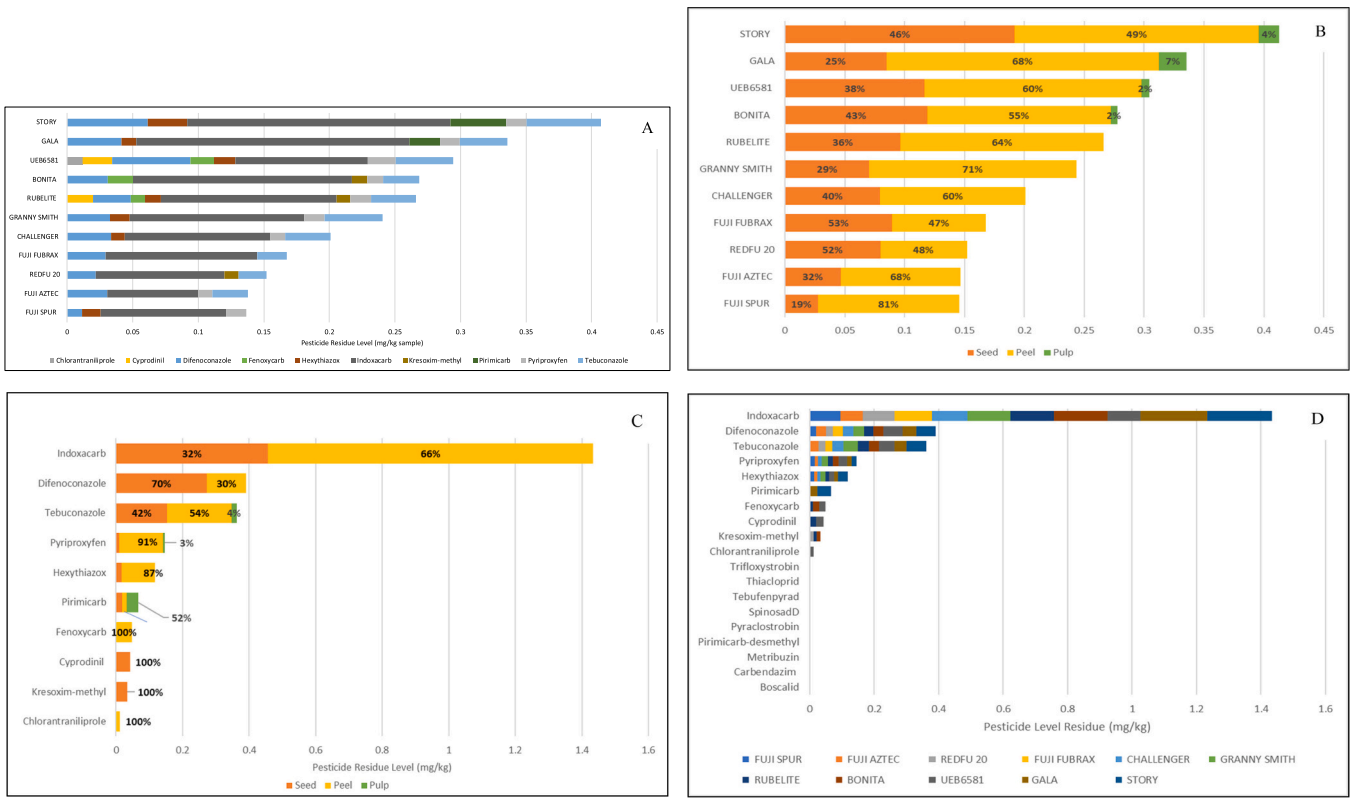

Fig. 2. Pesticide residue distribution in commercial apple cultivars grown at the Experimental Station under known phytosanitary treatments. (A) Contribution of individual pesticides to total residues. (B) Distribution of residues among apple tissues. (C) Tissue-specific distribution of pesticide residues by compound. (D) Contribution of each cultivar to pesticide residue levels. Results are expressed as mg/kg sample and percentage of total residues.

longer period between their last treatment and the picking time, they are likely to show reduced residue amounts when compared to those that are harvested earlier. Residues of hexythiazox and pyriproxyfen detected at harvest are more likely associated with early direct deposition on young fruits and strong retention in the cuticular wax layer rather than significant transfer from leaves to fruits.

3.2.2. Pesticide residues in commercial apples

As with the analysis conducted for the regional apple cultivars, the commercial samples were evaluated for pesticide residues separately in peel, pulp, and seed tissues. This assessment includes twenty-nine cultivars: eleven grown under controlled settings at the experimental research station (ENFVN - known treatment cultivars) (Table 6) and the remaining cultivars produced in different regions across Western Portugal under non-controlled field conditions - unknown treatment cultivars (Table 5). All seven active substances applied during the production cycle were detected in at least one tissue of the commercial cultivars. Additionally, residues of twelve other pesticides not listed in the official treatment schedule were also detected. Fig. 2 consolidate the findings and give a comprehensive picture of the different residue levels in the various cultivars and fruit tissues. *Evelina* had the greatest total residues, at around 3 mg/kg, mostly from difenoconazole, compared to the other cultivars. *Bigbucks*, *Galaval*, and *Alpigala* had somewhat higher amounts, mostly from kresoxim-methyl. The peel was the main location for the majority of compounds (up to 70%), whereas the seeds contained smaller amounts and the pulp had very low levels (<5%) (Fig. 2B). *Pixie* and *Fuji San* had the highest percentage of peel at over 88%, whereas *Bonita* and *Fuji Fubrax* showed more even distributions. The peel was filled with remains, just like *Evelina*'s (Fig. 2B).

All of the compounds, along with their distribution across various cultivars and tissues, are examined in detail in the section that follows.

3.2.3. Evaluation of commercial apple cultivars and their treatments at the experimental station

Results from the study's application of procedures at the Experimental Station (ENFVN) are as follows. The majority of the tissues and cultivars tested showed cyanodinil residues below the limit of quantification (LOQ, 0.01 mg/kg). However, two cultivars, *UEB6581* and *Rubelite*, did show trace residues in their seeds, although at low amounts (0.0198 and 0.0224 mg/kg, respectively). The peel and pulp did not contain any detectable contaminants.

Cyprodinil was given twice at early phenological phases (April 13 and 20), coinciding with the separation of floral clusters and early flowering, and this residue pattern aligns with its known dissipation tendency. The dissipation half-lives in fruit can vary from 2.5 to 20 days, depending on environmental conditions, and the fact that there are no or very little leftovers after harvest indicates that it degrades moderately to rapidly in the field (Marín et al., 2003; Zhang et al., 2015). Conditions including temperature, sunshine, rainfall, and soil organic matter content greatly affect how quickly it breaks down. In cooler and less sunny weather, dissipation is slower.

Although the measured residues in seeds were higher than the limit of quantification, they were significantly lower than the current EU maximum residue level (MRL) for cyprodinil in apples (2.0 mg/kg; Reg. (EU) 2023/1069), hence there was no cause for worry regarding safety with the treatment schedule in place (Table 5). These results are in line with what other research have shown and support the idea that using cyprodinil early in the season leaves very little residue at harvest.

Difenoconazole residues were found mostly in the seeds (70%) and peel (30%) of several types of commercial apples. There were no residues above the limit of quantification (<LOQ) in the pulp of any sample. In UEB6581, the seeds had a concentration of 0.041 mg/kg. In the peel, residues were usually lower, ranging from 0.010 mg/kg in Rubelite to 0.024 mg/kg in Story. Most cultivars had values close to the detection limit.

The tissue-specific distribution observed in the commercial cultivars, with residues concentrated in the seeds and peel but undetectable in the pulp, is consistent with the physicochemical properties of difenoconazole and the mechanisms previously discussed for the regional cultivars (Craggs et al., 2020; Lewis et al., 2016; Shimshoni et al., 2019).

All of the values found in the commercial cultivars were well below the MRL of 0.5 mg/kg set for hexythiazox in apples (Commision Regulation (EU) 2024/2612), which means they followed the rules (Table 3).

There have been reports of rapid dissipation under certain field conditions (He et al., 2016), but the residue levels seen here, especially in *UEB6581* and *Alpigala*, are likely due to the cumulative effects of repeated treatments and cultivar-specific factors like differences in cuticle structure, seed coat permeability, or residue distribution.

Hexythiazox residues were detected mainly in the peel (87%) of certain commercial apple cultivars, at concentrations ranging from 0.010 to 0.016 mg/kg. A measurable residue was also found in the seeds of Story (0.016 mg/kg; 13%), while all pulp samples remained below the LOQ. This isolated detection in seeds suggests limited penetration or contamination rather than significant systemic translocation.Indoxacarb residues were detected in nearly half of the twenty-nine apple cultivars, predominantly in the peel (68%) and seeds (32%), while no measurable residues were found in the pulp. Residue levels ranged from 0.0178 to 0.0927 mg/kg in seeds and from 0.0516 to 0.166 mg/kg in peel, with the highest concentrations observed in Story, Bonita, and Gala cultivars.

However, when considering the cumulative residue load in the whole fruit, certain commercial cultivars exceeded the MRL (Table 3). The persistence of residues at harvest is consistent with previous reports of slow field degradation of this compound. The relatively high residue levels compared with the 2013 EU monitoring programme, where maximum and mean indoxacarb concentrations in apples were 0.09 and 0.013 mg/kg, respectively ("EFSA," 2015) may reflect differences in application timing and frequency, as indoxacarb was applied during late phenological stages in the present study, favoring residue retention at harvest.

Tebuconazole residues were detected mainly in the peel (54%) and seeds (42%) of several commercial apple cultivars, while all pulp samples remained below the LOQ. Residue concentrations ranged from 0.0108 to 0.0283 mg/kg in seeds and from 0.0101 to 0.0284 mg/kg in peel, with the highest levels observed in Story, followed by Granny Smith and UEB6581 (Fig. 2A,B,C).

(Deng et al., 2010; EFSA, 2020) It is important to note that all of the residues found in the marketed cultivars were substantially below the EU MRL of 0.3 mg/kg for apples (Reg. EU 2018/1514, 2018) (Table 3). The differences between cultivars, including the greater amounts seen in *Story*, *Granny Smith* and *UEB6581*, could be due to differences in the structure of the cuticle, how well the plant moves nutrients around, or how well it can use energy, as was suggested for the regional cultivars. According to Chang et al. (2023), enantioselective degradation may also play a role in these variances, although more research is needed to establish this in the commercial cultivars studied (Chang et al., 2023).

Pyriproxyfen residues were detected exclusively in the peels of some commercial apple cultivars, while no quantifiable levels were observed in either the pulp or seeds of any sample. The highest concentration was found in UEB6581 (0.021 mg/kg), followed by Rubelite, Fuji Spur, and Granny Smith (0.016 mg/kg), Gala and Story (0.015 mg/kg), Bonita (0.012 mg/kg), and Challenger and Fuji Aztec (0.011 mg/kg). All detected concentrations remained below the maximum residue limit (MRL) established for apples (0.05 mg/kg) under Regulation (EU) 2023/1753 (2023) (Table 3).

Only the seeds of three commercial cultivars (*Redfu*, *Rubelite*, and *Bonita*) had low levels of Kresoxim-methyl residues, ranging from 0.010 to 0.012 mg/kg. In all other cultivars and tissues tested, the levels were below the <LOQ. There were no measurable residues in the peel or pulp of any sample.

The low incidence and concentrations observed for kresoxim-methyl in the present study align with the relatively short environmental

Fig. 3. Pesticide residue distribution in commercial apple cultivars grown under unknown phytosanitary treatments. (A) Contribution of individual pesticides to total residues. (B) Distribution of residues among apple tissues. (C) Tissue-specific distribution of pesticide residues by compound. (D) Contribution of each cultivar to pesticide residue levels. (E) Comparison of total pesticide residues in regional and commercial apple cultivars. Results are expressed as mg/kg sample and percentage of total residues.

persistence of kresoxim-methyl, as reported by Majumder et al. (2022).

All detected residues were well below the current MRL of 0.2 mg/kg for apples (Regulation (EU) 2020/856), confirming that use of kresoxim-methyl under the applied conditions poses no risk to consumer safety.

In general, the patterns of residue distribution in commercial cultivars were significantly affected by the lipophilicity, systemicity, timing of application, and cultivar-specific characteristics of the pesticides employed. Residues in pulp typically remained below LOQ, suggesting little translocation into edible tissues, while highly lipophilic compounds tended to accumulate in peel and seed tissues.

For cultivars with records indicating that certain pesticides had not been applied, subsequent analyses revealed that these compounds originated from applications to adjacent rows of pear trees. As previously noted, these cultivars were situated in a test field intended for evaluating their adaptability to the region, and no measures were taken to minimize cross-contamination.

3.2.4. *Commercial apple cultivars with unknown treatment history*

Boscalid, a carboxamide fungicide, showed the highest total residue levels among all detected pesticides in the commercial apple cultivars (Fig. 3A, B), particularly in Evelina, with the highest concentrations found in peel (77%), followed by seeds (20%) and pulp(3%). When considering the whole fruit, residue levels exceeded the MRL of 2.0 mg/kg established for apples.

The distribution pattern, with higher residues in the peel and seeds and minimal levels in the pulp, reflects its moderate lipophilicity (log P = 2.96) and limited systemicity, which favors accumulation in lipophilic outer tissues. The occasional detection in pulp of some cultivars suggests possible varietal differences in permeability or metabolism. This pattern, already observed by Sdeek et al. (2023), who reported a peel-to-pulp distribution ratio < 0.8 for boscalid in apples, further supports the conclusion that boscalid largely remains confined to the lipophilic outer tissues (Sdeek et al., 2023).

Pyraclostrobin residues were found in the seeds (22%), peel (77%), and sometimes the pulp (1%) of three types of apples (Fuji San, Evelina, and Dalinette) at levels between 0.012 and 0.56 mg/kg. The peel of Evelina had the most, which was more above the maximum residue limit of 0.5 mg/kg for apples (Regulation (EU) 2024/342).

Pyraclostrobin is a strobilurin fungicide that has a high lipophilicity (log P = 3.99) and stays in the environment for different amounts of time depending on the soil and weather (Lewis et al., 2016). This unpredictability is due to the fact that it tends to stick to organic materials and not break down in soils that aren't very biologically active.

Tebufenpyrad residues were found in the peel (80%) and seeds (20%) of seven out of the eighteen cultivars that were studied (24%). According to the current maximum residue limit (MRL) for apples, which is 0.3 mg/kg (Regulation (EU) 2024/2600), the greatest concentration was detected in *Apple Galaval* peel (0.58 mg/kg) (Table 5).

No detectable residues were observed in pulp tissues. This distribution pattern is consistent with the high lipophilicity of tebufenpyrad (log P > 4.9), favoring retention in outer fruit tissues rather than translocation into edible compartments.

A small percentage of the seeds (33%), peel (49%), and pulp (17%) of six different cultivars contained pyrimicarb residues, ranging from 0.011 to 0.054 mg/kg. Based on its lower lipophilicity (log P = 1.7) and enhanced mobility within plant tissues, pirimicarb likely exhibited a larger tissue distribution compared to most compounds, which is expected given their more compartmentalized distribution. All levels found were lower than the current maximum residue level (MRL) for apples, which is 0.5 mg/kg according to Regulation (EU) 2016/71.

Carbendazim, metribuzin, and thiacloprid were only found in seeds, at amounts ranging from 0.010 to 0.10 mg/kg, while remaining below the LOQ in peel and pulp tissues. Their reduced presence in reproductive tissues may imply limited mobility and partitioning behavior that is cultivar dependent. When looking at the entire fruit, only thiacloprid (Table 5) exceeded the MRL among these chemicals.

Low amounts of chlorantraniliprole, fenoxycarb, spinosad D, and trifloxystrobin (0.011–0.042 mg/kg) were identified only in peel tissues; in seeds and pulp, these compounds were below LOQ. They have a moderate to high lipophilicity and limited translocation within the fruit, which explains why they preferentially accumulate in exterior tissues. When whole-fruit concentrations were examined, only fenoxycarb surpassed the MRL (see Table 5).

Several active compounds that were not part of the approved crop protection calendar were also detected, which is an additional important result. Similar phenomena have been previously observed in orchard systems (Picó et al., 2018; Zaller et al., 2023) and may be connected with spray drift, runoff from nearby plots, environmental persistence, or carry-over from prior growing seasons.

The residue profiles of commercial cultivars showed a great deal of variation. Cultivars such Candine France, I3D7, Gaia, and F7E3 had relatively low residue levels, in contrast to Evelina, which had the highest total residue burden primarily from boscalid and pyraclostrobin. In general, the peel tissues and seeds had the highest concentrations of residues, whereas the pulp always had the lowest amounts of contamination. Results indicate that cultivar characteristics, properties of pesticides and agronomic approaches influence the retention and distribution characteristics of residues in apples (Lewis et al., 2016; Picó et al., 2018; Zaller et al., 2023; Kowalska et al., 2022).

3.2.5. Pesticide residues in apples grown at the experimental station (with known treatment)

Pesticide residues in apples showed a consistent pattern of distribution throughout different parts of the fruit when analyzed in both commercial and regional varieties. Residues were found mostly in the outer tissues, especially the peel, and only in small amounts in the seeds in both varieties; the pulp showed almost little residue at all (Table 6). This distribution is a result of the fact that the majority of the active compounds that are administered have a high lipophilicity and a restricted systemicity, favoring retention in outer fruit tissues and restricting translocation into the aqueous mesocarp. Similar findings have been reported in apples from other production systems, including Poland, China, and Turkey (Łozowicka et al., 2025; Odabas et al., 2024; Zhao et al., 2023).

Apart from this common pattern of distribution, there was a discernible variation in the overall residue load between the two varieties of crops. Fig. 3E shows that commercial cultivars routinely accumulate greater levels of total pesticide residues, typically exceeding 0.5 milligrams per kilogram, whereas regional cultivars accumulate substantially lower levels, with median and mean levels around 0.24–0.25 milligrams per kilogram. Differences in pesticide residue buildup and dissipation under identical climatic and agronomic settings point to the importance of intrinsic varietal characteristics (Khazaal et al., 2022; Picó et al., 2018).

The same sample of apples showed multiple pesticide residues, whether they were from a commercial or regional orchard. There were two to six residues in regional apples, but as many as eight in commercial apples. This confirms what other studies have found: that even apples grown in environmentally friendly ways often have a cocktail of pesticides in them when picked. Similar findings have been reported in apples from other production systems, including Poland, China, and Turkey (Łozowicka et al., 2025). The presence of numerous substances at once emphasizes the significance of taking cumulative exposure and combination effects into account when evaluating consumer risk, even though individual residues were often below regulatory limits (El Hawari et al., 2019; Odabas et al., 2024; Toptanci et al., 2021;Zhao et al., 2023).

Commercial cultivars appear to have a higher residue load than regional cultivars, which could be attributed to variations in treatment intensity, agronomic management, or cultivar-specific retention behavior. However, it should be noted that none of the cultivars examined in this study were grown in experimental orchards that were actively maintained using residue-minimization or zero-residue production techniques (Toptanci et al., 2021).

4. Conclusion

It is vital to remember that the apples utilized in this study were grown purely for scientific purposes. This investigation's experimental scope was the only intended use of the materials, and they were not intended for commercial dissemination.

This study demonstrates that the dynamics of pesticide residues throughout different fruit tissues can be accurately monitored by integrating comprehensive phytosanitary records with controlled management of regional apple cultivars. Since the pulp is the main part of the fruit that people eat, the fact that residues are mostly detected in the peel and seeds and are hard to find in the pulp (mesocarp) shows that the food is safe to eat.

There are noticeable differences in residue accumulation amongst cultivars, which shows that characteristics like developmental time and cuticle composition affect pesticide uptake and persistence. In order to optimize management techniques and ensure consumer safety, these results emphasize the importance of cultivar-specific residue assessments.

The identification of only applied substances and the lack of unauthorized residues verify the robustness and traceability of the experimental design. All things considered, the results provide support to the notion that regional cultivars grown in controlled conditions might be great models for residue behavior research. Research like these can open the way for better agronomic advice, safer pesticide use in traditional fruit varieties, and better regulatory monitoring.These results show how important it is to take varietal traits into account when determining consumer safety and regulatory compliance with residue levels, when planning fair and efficient pesticide applications, and when choosing apple by-products to be further valued.

CRediT authorship contribution statement

Sanches-Silva Ana: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Sílvia Barros:** Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Duarte Torres:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Miguel Leão de Sousa:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis, Conceptualization. **Claudia Sánchez:** Writing – review & editing, Supervision, Resources, Investigation, Formal analysis. **Nicola Nicolini:** Writing – review & editing, Validation, Resources, Investigation, Formal analysis, Data curation. **Monica Rosa Loizzo:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Data curation. **Ana Rita Mateus:** Writing – original draft, Software, Resources, Investigation, Formal analysis. **Martina Vitelli:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Patricia Cazón:** Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation. **João David Teixeira:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2026.109372.

Data Availability

The data that has been used is confidential.

References

Anastassiades, M., Lehotay, S.J., Štajnbaher, D., Schenck, F.J., 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. J. AOAC Int. 86 (2), 412–431.

Andrés, v., Villanueva, M.J., Tenorio, M.D., 2016. The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. Food Chem. 192, 328–335. https://doi.org/10.1016/j.foodchem.2015.07.031.

Anke, T., Oberwinkler, F., Steglich, W., Schramm, G., 1977. The strobilurins–new antifungal antibiotics from the basidiomycete *Strobilurus tenacellus*. J. Antibiot. 30 (10), 806–810. https://doi.org/10.7164/antibiotics.30.806.

Barchanska, H., Danek, M., Sajdak, M., Turek, M., 2018. Review of sample preparation techniques for the analysis of selected classes of pesticides in plant matrices. Crit. Rev. Anal. Chem. 48, 467–491. https://doi.org/10.1080/10408347.2018.1451297.

Bellisai, G., Bernasconi, G., Brancato, A., Cabrera, L.C., Castellan, I., Ferreira, L., Giner, G., Greco, L., Jarrah, S., Leuschner, R., Magrans, J.O., Miron, I., Nave, S., Pedersen, R., Reich, H., Robinson, T., Ruocco, S., Santos, M., Scarlato, A.P., Theobald, A., Verani, A., 2022. Review of the existing maximum residue levels for pyriproxyfen according to Article 12 of Regulation (EC) No 396/2005. EFSA J. 20 (11). https://doi.org/10.2903/j.efsa.2022.7617.

Bellisai, G., Bernasconi, G., Carrasco Cabrera, L., Castellan, I., del Aguila, M., Ferreira, L., Santonja, G.G., Greco, L., Jarrah, S., Leuschner, R., Mioč, A., Nave, S., Pedersen, R., Reich, H., Ruocco, S., Scarlato, A.P., Szot, M., Theobald, A., Tiramani, M., Verani, A., 2024. Modification of the existing maximum residue level for clopyralid in honey. EFSA J. 22 (1). https://doi.org/10.2903/j.efsa.2024.8546.

Caboni, P., Ntalli, N.G., Aissani, N., Cavoski, I., Angioni, A., 2012. Nematicidal activity of (*E*, *E*)-2,4-decadienal and (*E*)-2-decenal from Ailanthus altissima against Meloidogyne javanica. J. Agric. Food Chem. 60 (4), 1146–1151. https://doi.org/10.1021/jf2044586.

Cabrera, Luis Carrasco Pastor, P.M., 2021. The 2019 European Union report on pesticide residues in food. EFSA J. 19 (4), 6491. https://www.efsa.europa.eu/en/efsajournal/pub/6491. Accessed: July 6, 2026.

Campos-Mañas, M.C., Plaza-Bolaños, P., Martínez-Piernas, A.B., Sánchez-Pérez, J.A., Agüera, A., 2019. Determination of pesticide levels in wastewater from an agro-food industry: Target, suspect and transformation product analysis, 232, 152–163. https://doi.org/10.1016/j.chemosphere.2019.05.147.

Chang, W., Liu, M., Ren, Q., Shi, Z., Wang, W., Nie, J., Cai, Z., 2023. Enantioselective behavior and bioactivity of chiral tebuconazole in apples. Food Control. 153, 109941. https://doi.org/10.1016/j.foodcont.2023.109941.

Cheng, L., Dong, F.S., Liu, X., Chen, W., Li, Y., Zheng, Y., Qin, D., Gong, Y., 2010. Determination of indoxacarb enantiomer residues in vegetables, fruits, and soil by high-performance liquid chromatography. J. AOAC Int. 93 (3), 1007–1012. https://academic.oup.com/jaoac/article-abstract/93/3/1007/5655662.

Commission Regulation (EU) 2024/2612 of 7 October 2024 amending Annexes II, III and IV to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for chitosan, clopyralid, difenoconazole, fat distillation residues, flonicamid, hydrolysed proteins, and lavandulyl senecioate in or on certain products. Official Journal of the European Union, L series, 1-32, 8.10.2024, Available at: http://data.europa.eu/eli/reg/2024/2612/oj Accessed: July 6, 2026.

Craggs, M., Gibson, G.R., Whalley, P., Collins, C.D., 2020. Bioaccessibility of difenoconazole in rice following industry standard processing and preparation procedures. J. Agric. Food Chem. 68 (37), 10167–10173. https://doi.org/10.1021/acs.jafc.0c02648.

van den Berg, H., Gu, B., Grenier, B., Kohlschmid, E., Al-Eryani, S., da Silva Bezerra, H.S., Nagpal, B.N., Chanda, E., Gasimov, E., Velayudhan, R., Yadav, R.S., 2020. Pesticide lifecycle management in agriculture and public health: where are the gaps? Sci. Total. Environ. 742. https://doi.org/10.1016/j.scitotenv.2020.140598.

Deng, Z., Hu, J., Qin, D., Li, H., 2010. Simultaneous analysis of hexaconazole, myclobutanil, and tebuconazole residues in apples and soil by SPE clean-up and GC with nitrogen-phosphorus detection. Chromatographia 71 (7–8), 679–684. https://doi.org/10.1365/s10337-010-1505-1.

DGADR. Direção-Geral de Agricultura e Desenvolvimento Rural, 2011. Normas Técnicas para a Produção Integrada de Pomóideas. Volumes I and II [Technical Standards for Integrated Production of Pome Fruits. Volumes I and II]. Direção-Geral de Agricultura e Desenvolvimento Rural, Lisboa, Portugal. Available at: https://www.dgadr.gov.pt/images/docs/prod_sust/normas_pi/i012008.pdf Accessed: July 6, 2026.

Döker, İ., Kazak, C., Ay, R., 2021. Resistance status and detoxification enzyme activity in ten populations of Panonychus citri (Acari: Tetranychidae) from Turkey. Crop. Prot. 141. https://doi.org/10.1016/j.cropro.2020.105488.

EC, 2005. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Off. J. Eur. Union. L 70, 1–16. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R0396. Accessed: July 6, 2026.

EFSA, 2015. The 2013 European Union report on pesticide residues in food. EFSA J. 13 (3), 4038. https://doi.org/10.2903/j.efsa.2015.4038.

EFSA, 2020. EFSA Consolidated Annual Activity Report 2020. https://doi.org/10.2805/817257.

EFSA, Carrasco Cabrera, L., Di Piazza, G., Dujardin, B., Marchese, E., Medina Pastor, P., 2024. The 2022 European Union report on pesticide residues in food. EFSA J. 22 (4), e8753. https://doi.org/10.2903/j.efsa.2024.8753.

El Hawari, K., Mokh, S., Al Iskandarani, M., Halloum, W., Jaber, F., 2019. Pesticide residues in Lebanese apples and health risk assessment. Food Addit. Contam. Part. B Surveill. 12 (2), 81–89. https://doi.org/10.1080/19393210.2018.1564370.

EN 15662:2018, 2018. Foods of plant origin — Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE — Modular QuEChERS-method. CEN, Brussels.

FAO, 2024. FAOSTAT: Crops and livestock products. Food and Agriculture Organization of the United Nations. https://www.fao.org/faostat/en/#data/QCL (Accessed October 1, 2025).

Feng, S., Yi, J., Li, X., Wu, X., Zhao, Y., Ma, Y., Bi, J., 2021. Systematic review of phenolic compounds in apple fruits: compositions, distribution, absorption, metabolism, and processing stability. J. Agric. Food Chem. 69 (1), 7–27. https://doi.org/10.1021/acs.jafc.0c05481.

Hafez, A.M., Mota-Sanchez, D., Vandervoort, C., Wise, J.C., 2021. Resistance affects the field performance of insecticides used for control of choristoneura rosaceana in michigan apples and cherries. Insects 12 (9), 846. https://doi.org/10.3390/insects12090846.

Harris, J., van Zonneveld, M., Achigan-Dako, E.G., Bajwa, B., Brouwer, I.D., Choudhury, D., de Jager, I., de Steenhuijsen Piters, B., Dulloo, M.E., Guarino, L., Kindt, R., Mayes, S., McMullin, S., Quintero, M., Schreinemachers, P., 2022. Fruit and vegetable biodiversity for nutritionally diverse diets: challenges, opportunities, and knowledge gaps. Glob. Food Secur. 33, 100618. https://doi.org/10.1016/j.gfs.2022.100618.

He, M., Jia, C., Zhao, E., Chen, L., Yu, P., Jing, J., Zheng, Y., 2016. Concentrations and dissipation of difenoconazole and fluxapyroxad residues in apples and soil, determined by ultrahigh-performance liquid chromatography electrospray ionization tandem mass spectrometry. Environ. Sci. Pollut. Res. 23 (6), 5618–5626. https://doi.org/10.1007/s11356-015-5750-6.

Jakobek, L., Matić, P., 2024. Phenolic compounds from apples: from natural fruits to the beneficial effects in the digestive system. Molecules 29, 568. https://doi.org/10.3390/molecules29030568.

Khazaal, S., El Darra, N., Kobeissi, A., Jammoul, R., Jammoul, A., 2022. Risk assessment of pesticide residues from foods of plant origin in Lebanon. Food Chem. 374, 131676. https://doi.org/10.1016/j.foodchem.2021.131676.

Kowalska, G., Pankiewicz, U., Kowalski, R., 2022. Assessment of pesticide content in apples and selected citrus fruits subjected to simple culinary processing. Appl. Sci. (Switz.) 12 (3), 1417. https://doi.org/10.3390/app12031417.

Kumari, P., Duhan, A., Rani, N., Tomar, D., 2021. Ultimate fate and toxicological consequences of insecticide pyriproxyfen and its metabolites in soil ecosystem. Environ. Adv. 4, 100040. https://doi.org/10.1016/j.envadv.2021.100040.

Lara, I., Belge, B., Goulao, L.F., 2014. The fruit cuticle as a modulator of postharvest quality. Postharvest Biol. Technol. 87, 103–112. https://doi.org/10.1016/j.postharvbio.2013.08.012.

Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. Human. Ecol. Risk Assess. 22 (4), 1050–1064. https://doi.org/10.1080/10807039.2015.1133242.

Lončarić, A., Matanović, K., Ferrer, P., Kovač, T., Šarkanj, B., Babojelić, M.S., Lores, M., 2020. Peel of traditional apple varieties as a great source of bioactive compounds: extraction by micro-matrix solid-phase dispersion. Foods 9 (1), 80. https://doi.org/10.3390/foods9010080.

Łozowicka, B., Kaczyński, P., Wołejko, E., Jankowska, M., Iwaniuk, P., Hrynko, I., Rutkowska, E., Łuniewski, S., Ilyasova, G., Jabłońska-Trypuć, A., Wydro, U., Pietruszyńska, M., 2025. Comprehensive toxicological multi-year study on pesticides in apples: control, trends and dietary risk assessment. Food Chem. 464 (Pt 3), 141897. https://doi.org/10.1016/j.foodchem.2024.141897.

Majumder, S., Verma, C.K., Rani, V., Rani, A.T., Pandey, K.K., Singh, J., 2022. Residue dynamics and food safety evaluation of fungicide kresoxim-methyl in green chilli (Capsicum annum L. Int. J. Environ. Anal. Chem. 102 (19), 7433–7443. https://doi.org/10.1080/03067319.2020.1830986.

Marchese, E.M.P.P., 2024. National summary reports on pesticide residue analyses performed in 2022. EFSA Support. Publ. 21 (4). https://doi.org/10.2903/sp.efsa.2024.EN-8751.

Marín, A., Oliva, J., Garcia, C., Navarro, S., Barba, A., 2003. Dissipation rates of cyprodinil and fludioxonil in lettuce and table grape in the field and under cold storage conditions. J. Agric. Food Chem. 51 (16), 4708–4711. https://doi.org/10.1021/jf021222e.

Martin, L.B.B., Rose, J.K.C., 2014. There's more than one way to skin a fruit: formation and functions of fruit cuticles. J. Exp. Bot. 65, 4639–4651. https://doi.org/10.1093/jxb/eru301.

Mignard, P., Beguería, S., Reig, G., Font i Forcada, C., Moreno, M.A., 2021. Genetic origin and climate determine fruit quality and antioxidant traits on apple (Malus x domestica Borkh). Sci. Hortic. 285, 110142. https://doi.org/10.1016/j.scienta.2021.110142.

More, S.J., Bampidis, V., Benford, D., Bragard, C., Hernandez-Jerez, A., Bennekou, S.H., Halldorsson, T.I., Koutsoumanis, K.P., Lambré, C., Machera, K., Naegeli, H., Nielsen, S.S., Schlatter, J.R., Schrenk, D., Silano, V., Turck, D., Younes, M., Benfenati, E., Crépet, A., Hogstrand, C., 2021. Guidance Document on Scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals. EFSA J. 19 (12). https://doi.org/10.2903/j.efsa.2021.7033.

Odabas, E., Keklik, M., Golge, O., González-Curbelo, M.Á., Kabak, B., 2024. Monitoring and risk assessment of multi-pesticide residues in apples: a focus on consumer safety. Foods 13 (19), 3186. https://doi.org/10.3390/foods13193186.

Peng, F., Ren, X., Du, B., Niu, K., Yu, Z., Yang, Y., 2022. Insoluble dietary fiber of pear fruit pomace (Pyrus ussuriensis Maxim) consumption ameliorates alterations of the obesity-related features and gut microbiota caused by high-fat diet. J. Funct. Foods 99, 105354. https://doi.org/10.1016/j.jff.2022.105354.

Picó, Y., El-Sheikh, M.A., Alfarhan, A.H., Barceló, D., 2018. Target vs non-target analysis to determine pesticide residues in fruits from Saudi Arabia and influence in potential risk associated with exposure. Food Chem. Toxicol. 111, 53–63. https://doi.org/10.1016/j.fct.2017.10.060.

Pihlström, T., Fernández-Alba, A.R., Ferrer Amate, C., Erecius Poulsen, M., Lippold, R., Carrasco Cabrera, L., Pelosi, P., Valverde, A., Unterluggauer, H., Mol, H., Jezussek, M., Malato, O., Štěpán, R., & Lambert, M. (2021). Analytical quality control and method validation procedures for pesticide residues analysis in food and feed SANTE 11312/2021 v2.

PPDB, Pesticide Properties DataBase (2023). University of Hertfordshire,. Available at: https://sitem.herts.ac.uk/aeru/ppdb/. Accessed: July 6, 2026.

Rahman, M.M., Jang, J., Park, J.H., Abd El-Aty, A.M., Ko, A.Y., Choi, J.H., Yang, A., Park, K.H., Shim, J.H., 2014. Determination of kresoxim-methyl and its thermolabile metabolites in pear utilizing pepper leaf matrix as a protectant using gas chromatography. J. Adv. Res. 5 (3), 329–335. https://doi.org/10.1016/j.jare.2013.05.003.

SANTE, 2024. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed. Available at: https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE-11312_2021-V2.pdf.

Sdeek, F.A., Seloma, A.S.O., Ismail, I.I., Ramadan, M.M., Ali, A.A.I., 2023. Dissipation behavior and dietary risk assessment of abamectin, pyraclostrobin and boscalid in apple fruit. Egypt. J. Chem. 66 (13), 1977–1986. https://doi.org/10.21608/EJCHEM.2023.222580.8248.

Shimshoni, J.A., Bommuraj, V., Chena, Y., Sperling, R., Barel, S., Kaye, Y., Fallik, E., 2019. Residual distribution kinetics of pesticides in cherry tomato peel, pulp, and fruit as a function of irrigation water salinity, household rinsing, and storage regimen. Agronomy 9 (12). https://doi.org/10.3390/agronomy9120800.

Szpyrka, E., Matyaszek, A., Słowik-Borowiec, M., 2017. Dissipation of chlorantraniliprole, chlorpyrifos-methyl and indoxacarb—insecticides used to control codling moth (Cydia Pomonella L.) and leafrollers (Tortricidae) in apples for production of baby food. Environ. Sci. Pollut. Res. 24 (13), 12128–12135. https://doi.org/10.1007/s11356-017-8821-z.

Tao, H., Sun, H., Wang, Y., Wang, X., Guo, Y., 2023. Effects of water stress on quality and sugar metabolism in 'Gala' apple fruit. Hortic. Plant. J. 9 (1), 60–72. https://doi.org/10.1016/j.hpj.2022.03.008.

Teixeira, J.D., Soares Mateus, A.R., Sanchez, C., Parpot, P., Almeida, C., Sanches Silva, A., 2023. Antioxidant capacity and phenolics profile of portuguese traditional cultivars of apples and pears and their by-products: on the way to newer applications. Foods 12, 1537. https://doi.org/10.3390/foods12071537.

Tirado, M.C., Clarke, R., Jaykus, L.A., McQuatters-Gollop, A., Frank, J.M., 2010. Climate change and food safety: a review. Food Res. Int. 43 (7), 1745–1765. https://doi.org/10.1016/j.foodres.2010.07.003.

Toptanci, İ., Kiralan, M., Ramadan, M.F., 2021. Levels of pesticide residues in fruits and vegetables in the Turkish domestic markets. Environ. Sci. Pollut. Res. 28 (29), 39451–39457. https://doi.org/10.1007/S11356-021-13538-W.

Tu, F.Q., Yang, M., 2022. Determination of pesticides in apples by high-performance liquid chromatography–mass spectrometry (HPLC–MS) with high-resolution multiple reaction monitoring. Anal. Lett. 55 (3), 438–448. https://doi.org/10.1080/00032719.2021.1938594.

Viswanathan, S., Kumar, S., Kandan, B., 2013. İndoksakarb ilişkili ARDS, nörotoksisite ve turuncu idrar. Eurasia J. Med. 45 (2), 135–137. https://doi.org/10.5152/eajm.2013.27.

Wu, C., Liu, X., He, M., Dong, F., Wu, X., Xu, J., Zheng, Y., 2021. Quantitative determination of pyriproxyfen and its metabolite residues in bee products of China using a modified QuEChERS approach with UPLC-MS/MS. Ecotoxicol. Environ. Saf. 220, 112388. https://doi.org/10.1016/j.ecoenv.2021.112388.

Zaller, J.G., Oswald, A., Wildenberg, M., Burtscher-Schaden, H., Nadeem, I., Formayer, H., Paredes, D., 2023. Potential to reduce pesticides in intensive apple production through management practices could be challenged by climatic extremes. Sci. Total. Environ. 872, 162237. https://doi.org/10.1016/j.scitotenv.2023.162237.

Zhang, W., Chen, H., Han, X., Yang, Z., Tang, M., Zhang, J., Zeng, S., Hu, D., Zhang, K., 2015. Determination and analysis of the dissipation and residue of cyprodinil and fludioxonil in grape and soil using a modified QuEChERS method. Environ. Monit. Assess. 187 (7), 445. https://doi.org/10.1007/s10661-015-4661-9.

Zhao, H., Li, R., Hu, J., 2023. Frequently used pesticides and their metabolites residues in apple and apple juice from markets across China: occurrence and health risk assessment. LWT 178. https://doi.org/10.1016/j.lwt.2023.114610.