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Screening and quantification of pesticide residues in ciders by liquid chromatography-high resolution mass spectrometry

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Abstract: The present study aimed to apply a multi-residue method for the screening and quantification of pesticide residues in ciders, low alcoholic beverages made by fermentation of apple juice. Twenty bottled craft cider samples purchased from the Czech market were analysed for pesticide residues. The residues of pesticides were extracted from samples using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedure in combination with additional solid-phase extraction (SPE) sample clean-up to achieve the lowest detection limit possible. In this study, targeted screening of pesticide residues in the samples was performed with the Q-Orbitrap mass spectrometry instrument. We identified 18 pesticides in cider samples analysed by screening method using an accurate-mass database of about 500 pesticide compounds, including their retention times, empirical formulas, and characteristic fragments. Additionally, liquid chromatography with high-resolution tandem mass spectrometer (LC-HRMS/MS) was used for re-analysis of positive findings of pesticides in samples and allowed to quantify compounds of interest at 0.2 $\mu g \cdot L^{-1}$ concentration level. The monitoring scheme was applied to the set of craft ciders, and the results revealed the presence of pesticide residues in most of the samples at trace levels ranging from 0.5 to 5 $\mu g \cdot L^{-1}$ and rarely at a level higher than 10 $\mu g \cdot L^{-1}$.

Keywords: low alcoholic beverages; determination; apples; pesticide residues; Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS)

An alcoholic beverage called cider in England and in the USA is made from fermented apple juice. The quality of apple ciders mainly depends on the apples used to produce apple juice.

Apples are known to be one of the commodities that are widely treated with various agrochemicals against several fungal diseases, such as Apple scab, Black rot, Cedar apple rust, Flyspeck, and Powdery mildew, and

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against an invasion of various insect orchard pests (Aphids, Apple maggot, Codling moth, Leafhoppers and Leafrollers). The most commonly used pesticides for the treatment of apples are insecticides, such as acetamiprid, chlorpyrifos-methyl, methoxyfenozide, pirimicarb, and fungicides, such as boscalid, carbendazim, cyprodinil, dithiocarbamate, fluopyram, and mandipropamid (Lozowicka 2015). They help to increase the efficiency, profitability, and quality of products. On the other hand, their application to farmland has increased rapidly worldwide, and it is important to control and monitor the uses of the pesticides used in crop production.

Chromatographic methods (GC – gas chromatography and LC – liquid chromatography) alone or in combination with different types of mass spectrometry (MS) techniques are mainly used for the determination or screening of pesticide residues in apples and apple products such as apple baby food, apple puree and various apple juices.

The authors used several types of extraction methods for the sample preparation. The most frequent extraction methods were Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) (Lozano et al. 2018), frequently in combination with dispersive solid-phase extraction (d-SPE) (Hercegová et al. 2006), matrix solid-phase extraction (MSPD) (Chu et al. 2005), dispersive liquid-liquid microextraction (DLLME) (Cunha et al. 2009), or extraction with ammonium acetate-acetic acid solution in methanol-water (Christensen et al. 2009).

The main objective of this study was to determine the concentration of pesticide residues, which are carried over from apples to ciders during brewing or from hops during dry hopping of ciders.

MATERIAL AND METHODS

Sample collection. In total, twenty samples, fifteen samples of craft apple cider from the Czech Republic, and five samples from foreign countries (two from Great Britain and three others from Slovakia, France, and Spain) have been analysed. Samples were purchased at local markets and obtained from local cider producers who have production throughout the Czech Republic. Five were made with hops (for bitter taste and aroma), two were organic.

Chemicals and materials. The pesticide analytical standards listed in Table 1 with > 99% purity were purchased from Sigma-Aldrich (St. Louis, USA) as well as the solvents (all in LC-MS grade), and formic acid, ammonium formate, sodium citrate tribasic dihydrate, and sodium hydrogencitrate sesquihydrate. Sodium chloride (anal. grade) and magnesium sulphate (anal. grade, > 98%) were obtained from Penta (Prague, Czech Republic) and Lach-Ner (Neratovice, Czech Republic), respectively. Ultrapure water with resistance > 18.2 $M\Omega \cdot cm^{-1}$ was obtained from a Milli-Q© purification system (Merck Millipore, Darmstadt, Germany). Standard stock solutions (1 $mg \cdot mL^{-1}$) of each compound of interest were prepared in acetonitrile, except for carbendazim, prepared in methanol. Multi-residue pesticide standard stock solution (0.1 $mg \cdot mL^{-1}$) was prepared by dissolving 1 mg of each pesticide standard in acetonitrile quantity sufficient to 10 mL in a volumetric flask. The stock solutions were stored generally at –20 °C.

Sample extraction and clean-up. Samples were prepared using the citrate-buffered QuEChERS method (EN 15662 2008). An aliquot of 10 mL of a degassed cider sample was pipetted into a 50 mL centrifugation tube, and 50 µL of triphenyl phosphate (internal standard at concentration 10 $mg \cdot L^{-1}$) was added to the tube. Then, 10 ml acetonitrile was added and vortexed for 30 s. A mixture of salts (4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate tribasic dehydrate, and 0.5 g sodium hydrogencitrate sesquihydrate) was added, shaken manually for 1 min and then centrifuged at 4500 rpm for 7 min. The supernatant (6 mL) from the tube was taken into a 15 mL centrifugation tube with 0.9 g $MgSO_4$, vortexed for 30 s, and the tubes were again centrifuged for 7 min at 4 500 rpm.

In the second step, the samples were purified using the SPE (solid-phase extraction) technique. An SPE column with PSA (primary-secondary amine) sorbent (200 mg, 3-mL tube; Supelco, Bellefonte, USA) was firstly conditioned with 2 mL of acetonitrile before the clean-up procedure. A portion of the QuEChERS extract (supernatant) was pipetted on the SPE column from a 15-mL centrifugation tube. The extract was eluted by gravity flow and collected into a 25 mL pear-shaped flask. Then, 2 mL of acetonitrile was pipetted on the column when the liquid level reached the top of the column bed, and in total, 10 mL of acetonitrile was used to wash out the sample of the cartridge. The washing eluate was collected into the same pear-shaped flask as the sample and then evaporated to dryness using a rotary evaporator (Hei-VAP ML; Heidolph, Germany) at 40 °C. The obtained residue was dissolved in 1 mL of 0.1% formic acid in a methanol- water mixture (50/50, *v*/*v*) to the final concentration equivalent to 2 mL of sample mL^{-1} and, finally, 5 µL of the extract was injected into LC-MS.

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Table 1. Chromatographic and mass spectrometric parameters for the selected compounds and internal standard

Pesticide	Molecular Formula	t_R (min)	Quantitation Ion (*m*/*z*)	Qualifying Ions (*m*/*z*)	ME (%)	Recovery (%)	RSD (%)	LOD (μg·L^{-1})	LOQ (μg·L^{-1})
Acetamiprid	$C_{10}H_{11}ClN_4$	4.7	223.0754	126.0099, 56.0500	–3	92	2	0.06	0.2
Boscalid	$C_{18}H_{12}Cl_2N_2O$	7.9	343.0399	307.0614, 139.9890	–10	103	4	0.10	0.3
Carbendazim	$C_9H_9N_3O_2$	3.1	192.0768	160.0496	–12	114	1	0.08	0.3
Cyprodinil	$C_{14}H_{15}N_3$	8.4	226.1339	93.0573, 108.0805	–11	89	7	0.06	0.2
Dimethoate	$C_5H_{12}NO_3PS_2$	4.7	230.0069	170.9700, 142.9928, 88.0222	–6	88	9	0.17	0.5
Fludioxonil	$C_{12}H_6F_2N_2O_2$	7.9	247.0325	180.0324, 126.0324	–22	95	3	0.06	0.2
Fluopyram	$C_{16}H_{11}ClF_6N_2O$	8.2	397.0527	208.0137, 173.0211	–8	95	2	0.06	0.2
Imazalil	$C_{14}H_{14}Cl_2N_2O$	6.2	297.0555	158.9762, 69.0454	–1	83	9	0.06	0.2
Imidacloprid	$C_9H_{10}ClN_5O_2$	4.2	256.0595	209.0576, 175.0968	–30	94	5	0.10	0.4
Mandipropamid	$C_{23}H_{22}ClNO$	7.9	412.1310	328.1096, 356.1044	–5	105	1	0.06	0.2
Methoxyfenozide	$C_{22}H_{28}N_2O_3$	8.1	369.2173	149.0598, 133.0649	–30	94	3	0.14	0.5
Myclobutanil	$C_{15}H_{17}ClN_4$	8.1	289.1215	70.0403, 125.0147	–27	90	4	0.14	0.5
Omethoate	$C_5H_{12}NO_4PS$	2.3	214.0297	182.9876, 142.9926, 154.9927	–6	84	11	0.15	0.5
Pirimicarb	$C_{11}H_{18}N_4O_2$	5.5	239.1215	72.0451, 182.1289	–4	79	4	0.07	0.2
Pirimicarb--desmethyl	$C_{10}H_{16}N_4O_2$	4.8	225.1346	72.0452, 168.1133	–8	85	9	0.06	0.2
Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$	8.9	388.1059	194.0800, 296.0566	–10	96	2	0.20	0.6
Pyrimethanil	$C_{12}H_{13}N_3$	7.5	200.1182	192.0561, 212.0666	–14	82	10	0.20	0.5
Tebuconazole	$C_{16}H_{22}ClN_3O$	8.8	308.1524	70.0403, 112.0761	–18	93	4	0.08	0.3
Thiacloprid	$C_{10}H_9ClN_4S$	5.2	253.0309	126.0107, 186.0140	–11	89	2	0.04	0.1
TPP – ISTD	**$C_{18}H_{15}O_4P$**	**8.8**	**327.0781**	**233.0348, 251.0453**	–	–	–	–	–

t_R – retention time (min); *m*/*z* – mass to charge ratio (exact mass of protonated molecular ion used for quantitation and the exact masses of confirmation fragment ions); bold – internal standard; LOD – limit of detection; LOQ – limit of quantification; ME – matrix effect (%); average recovery percentages (n = 6); RSD – relative standard deviation (%), obtained by extraction of sample spiked at the concentration level of 0.5 μg L^{-1}

Sample analysis. For the chromatography, a Dionex UltiMate 3000 UHPLC system (Thermo Scientific, Germering, Germany) was coupled to a Q-Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Scientific, Waltham, USA). The chromatographic separation was performed on a reversed-phase C18 Atlantis T3 column (2.1 × 100 mm, 3 μm) from Waters (Milford, USA) equipped with a C18 guard column (SecurityGuard ULTRA; Phenomenex, Aschaffenburg, Germany) with a flow rate of 0.34 mL·min^{-1}. The mobile phases used were *i*) 2 mM ammonium formate containing 0.1% (*v*/*v*) formic acid in the water and *ii*) methanol with the following gradient program: 0 min: 85% of solvent A + 15% of solvent B; 0.5 min: 85% A + 15% B; 9 min: 5% A + 95% B; 15 min: 95% A + 5% B. The column was kept at 40 °C, and the injection volume was 5 μL.

The mass spectrometer was operated in the positive and negative electrospray ionisation (ESI) mode. In the positive ESI mode, the ion spray voltage was set at 2.8 kV, the sheath gas flow was at 32 arbitrary units, the auxiliary gas flow rate was kept at 7 arbitrary units, the capillary temperature was set at 295 °C, and the auxiliary gas heater temperature at 295 °C. The ion spray voltage was set at –2.5 kV in the negative ESI mode. Nitrogen was used as a sheath and auxiliary gas. Data were acquired in data-dependent acquisition (DDA) mode. In the full MS scan, the mass resolution was set at 70.000 [FWHM, full width at half maximum, at *m*/*z* (mass to charge ratio) 200], the automatic gain control (AGC) target ion was set at 1e^6 for a maximum injection time of 200 ms, and the scan range from *m*/*z* 120 to 1 000. A data-dependent scans were triggered depending on the presence of the precursor ions defined in the inclusion list containing 565 and 73 pesticides in the positive and negative ionisation modes, respectively. The precursor ions with a signal

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threshold > $1.0e^4$ were fragmented in the HCD (higher energy collision-induced dissociation) collision cell at normalised collision energy (NCE = 30) to generate resulting data dependent (dd-MS/MS) fragmentation spectra. The DDA-triggered MS/MS scans were performed with the resolution 17 500 (FWHM at *m*/*z* 200), the AGC target ion set at $5e^4$ for a maximum injection time of 50 ms, isolation window at 1.4 *m*/*z*, apex trigger at 2 to 3 s, and dynamic exclusion time 2 s. The instrument was externally calibrated before each measurement using a mixture of mass calibrants.

Data processing. Data processing was performed using TraceFinder software version 4.1 (Thermo Scientific, Waltham, USA). For non-targeted screening and the confirmation of targeted residues, an in-house built accurate-mass database consisting of a compound name, summary formula, protonated molecular ion, and retention times for all compounds of interest was used. The defined criteria for confirmation were as follows: identical molecular ions (± 5 ppm), matching isotopic pattern (fit threshold 50%, allowed mass deviation 5 ppm, and allowed intensity deviation 15%), and retention time (± 0.2 min). Additionally, one to five MS/MS fragment ions were used to confirm within the 5-ppm wide mass error window.

Quantification of pesticide residues. A five-point solvent calibration curve was used for the pesticide concentration calculation prepared in the range of 0.2 to 10.0 $\mu g \cdot L^{-1}$. Internal standard triphenyl phosphate (TPP) was used to compensate for matrix suppression of target analytes and eliminate losses during the sample preparation. The peak area ratios of the target analytes and internal standard were used in the calibration process and calculated to determine unknowns in the sample.

RESULTS AND DISCUSSION

Pesticide residues were extracted from apple ciders by the QuEChERS method a combination with the additional clean-up on PSA SPE cartridges. Then, the clean-up procedure of QuEChERS extracts of apple ciders was optimised to achieve the quantification limit of at least 0.2 $\mu g \cdot L^{-1}$ for most of the target analytes. Therefore, firstly, the PSA sorbent was used for removing co-extracted compounds of the apple matrix, such as sugars, organic acids, and some polar pigments. The cartridge SPE operation under the gravity flow was used instead of the frequently used d-SPE (Anastassiades et al. 2003), which is less effective than cartridge SPE (c-SPE). The extract was used for LC analysis after a solvent change to methanol-water at a final concentration of 2 mL sample equivalent per one millilitre. The solvent change allowed performing 5-μL injection of the sample extract into the LC without any negative effects of the solvent on the peak shape of early eluting compounds. The limits of detection (LOD) and quantification (LOQ) based on the calibration curve slope were determined as 3.3 × (*SD*/*S*), and 10 × (*SD*/*S*), respectively, where *S* is the slope of the solvent calibration curve constructed in the range of 0.2 to 2.0 $\mu g \cdot L^{-1}$ and *SD* is the standard deviation of the response and the limit of quantification. The LOD and LOQ based on the calibration curve slope were determined as 3.3 × (*SD/S*) and 10 × (*SD/S*), respectively, where *S* is the slope of the solvent calibration curve constructed in the range of 0.2 to 2.0 $\mu g \cdot L^{-1}$ and *SD* is the standard deviation of the response. The LOD and LOQ values are shown in Table 2, ranging from 0.04 to 0.2 $\mu g \cdot L^{-1}$ and 0.1 to 0.5 $\mu g \cdot L^{-1}$, respectively. The methods' linearity (expressed as *r*) was evaluated in triplicate at five concentration levels, from 0.2 to 10 $\mu g \cdot L^{-1}$. In all cases, good linearity was achieved with $r > 0.99$. Matrix effects were determined using a 5-point matrix-matched calibration prepared in the range of 0.2 to 10 $\mu g \cdot L^{-1}$. When an appropriate volume of the pesticide working standard mixture and the internal standard solution was added to the extract prepared from organically grown apples. The matrix effects (ME) in percentage were calculated as ME (%) = [(slope matrix/slope solvent) − 1] × 100. As seen in Table 1, most of the analytes showed no or just a soft ME (% ME < ± 20%), and therefore an external solvent calibration curve could be adapted for accurate quantification.

Summary of the survey of the pesticide residues in ciders. For our experiment, twenty craft apple ciders were obtained in local stores. Fifteen samples originated from local cider producers, and five were produced outside the Czech Republic. It is a fact that pesticides are recurrently applied to control numerous pests and diseases in orchards, and therefore their residues are frequently found in apples. (Hercegová et al. 2007) as well as in processed apple products (Rasmussen 2003, Štěpán et al. 2005). The assumption was that apple ciders would contain some pesticide residues carried over, mainly from apple peels into beverages while making apple juice in a press. Five ciders involved in this study were dry hopped by adding hops during or after fermentation. These samples were also studied for carry-over residues typical for pesticide treatment on hops. The analytical results

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Table 2. Results of LC-MS analysis of pesticide residues (μg·L^{-1}) in twenty analysed ciders

	Samples																			
Sample no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Cider type	O/H	H	H	H	H	NS	NS	O	NS	NS	NS	NS	NS	NS	NS	NS	NS	O	NS	NS
Country of origin	CZ	CZ	CZ	CZ	SK	CZ	CZ	CZ	CZ	CZ	CZ	CZ	CZ	CZ	CZ	CZ	FR	GB	GB	ESP
	Pesticide residues (μg·L^{-1})																			
Acetamiprid	< LOD	1.2	< LOQ	< LOD	< LOD	4.5	1.2	< LOD	< LOQ	< LOD	< LOD	1.5	< LOD	0.4	1.1	< LOD	< LOD	< LOD	< LOQ	< LOQ
Boscalid	0.5	2.6	1.1	< LOQ	0.7	0.8	0.3	1.0	< LOQ	< LOD -	< LOD	3.5	14.1	0.2	1.7	< LOD	< LOD	< LOD	0.4	< LOD
Carbendazim	< LOD	< LOD	< LOD	< LOD	< LOQ	< LOD	0.5	< LOD	< LOD	< LOQ	< LOD	0.3	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOQ	< LOQ
Cyprodinil	< LOQ	< LOQ	< LOQ	< LOD	< LOD	< LOQ	< LOQ	< LOQ	< LOQ	< LOD	< LOD	0.4	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOQ	< LOQ
Dimethoate	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.5	< LOD	< LOD	< LOD	< LOD	–	< LOD	< LOD	< LOQ	< LOD	< LOD	< LOD	< LOQ	< LOD
Fludioxonil	< LOQ	< LOQ	< LOQ	< LOD	< LOD	< LOQ	< LOQ	< LOQ	< LOQ	< LOD	< LOD	0.4	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOQ	< LOQ
Fluopyram	< LOD	< LOQ	< LOQ	< LOD	< LOQ	< LOD	0.4	< LOD	0.5	< LOD	< LOD	< LOQ	3.7	< LOD	0.8	< LOD	< LOD	< LOD	< LOD	< LOD
Imazalil	< LOD	< LOD	< LOQ	< LOD	2.6	< LOD	< LOD	< LOD	< LOQ	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Imidacloprid	< LOD	< LOD	< LOD	< LOD	5.3	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Mandiprop-amid	0.3	2.1	< LOQ	< LOD	0.7	0.2	< LOD	0.8	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Methoxy-fenozide	< LOD	1.2	0.9	< LOD	< LOQ	0.9	0.3	< LOD	1.2	< LOD	< LOD	0.3	0.5	1.1	0.3	0.6	< LOD	< LOD	< LOQ	< LOD
Myclobutanil	< LOQ	< LOQ	< LOQ	< LOD	< LOD	< LOD	< LOQ	0.2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.2	< LOQ
Omethoate	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	4.2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.3	< LOD
Pirimicarb	< LOD	< LOQ	1.1	< LOQ	< LOQ	< LOQ	< LOQ	–	1.3	< LOQ	–	3.5	< LOQ	< LOQ	3.6	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Pirimicarb-desmethyl	< LOD	< LOQ	0.3	< LOD	0.2	< LOD	< LOD	< LOD	0.3	< LOD	< LOD	0.5	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Pyraclostrobin	< LOQ	< LOQ	< LOD	< LOD	< LOD	< LOQ	0.2	< LOQ	< LOD	< LOD	< LOD	< LOD	< LOQ	< LOD	< LOD	< LOD	< LOD	< LOD	< LOQ	< LOQ
Pyrimethanil	< LOD	< LOQ	< LOQ	< LOQ	0.2	< LOD	< LOD	< LOD	< LOQ	< LOQ	< LOQ	< LOD	4.3	< LOD	2.4	0.5	< LOD	< LOD	< LOD	< LOD
Tebuconazole	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.6	< LOD	0.3	< LOD	< LOD	< LOD	< LOD	< LOD
Thiacloprid	< LOD	< LOQ	< LOD	< LOD	0.5	0.2	0.3	< LOD	< LOQ	< LOD	< LOD	0.3	< LOQ	0.4	< LOD	< LOQ	< LOD	< LOD	< LOQ	< LOD

H – dry hopped cider; O – organic cider; NS – non-specified type of cider; < LOD – below the limit of detection; < LOQ – below the limit of quantification

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are summarised in Table 2. The study has proven that agrochemicals are transmitted from pesticide-treated apples to ciders.

In this study, eighteen different pesticide residues were found in twenty samples of apple ciders, and in twelve samples, pesticide residues were reliably quantified (Table 2). At least two pesticide residues were detected in each of the studied samples. The spectrum of pesticide residues was typical for pesticides used for apple treatment. The most commonly occurring pesticide was pirimicarb, an insecticide for controlling aphids on a wide range of crops. Pirimicarb was detected in thirteen samples, and in four samples, it was quantified at the highest concentration of 4 μg·L^{-1}. The second most occurring pesticide was boscalid, a fungicide active against a wide range of fungal pathogens. Boscalid was detected in fourteen samples and eight samples, it was quantified at the highest concentration of 14 μg·L^{-1} (sample 13). The frequently found pesticide residue was also methoxyfenozide, an insecticide used to control various insect moths. Methoxyfenozide was detected in eleven samples, and seven samples were quantified at the highest concentration of 1.2 μg·L^{-1} in apple cider with hops addition. Acetamiprid, an insecticide used to control aphids, was found frequently, and quantified in five samples at the highest concentration of 5 μg·L^{-1}. In the other five samples, acetamiprid was detected below the LOQ level. Fluopyram, a broad-spectrum fungicide used as a foliar application to control various diseases (grey mould, powdery mildew, or apple scab), was detected in eight samples at the highest concentration of 1 μg·L^{-1}. The remaining pesticide residues, such as carbendazim, myclobutanil, pyraclostrobin, and thiacloprid, were only detected below the LOQ levels.

Apart from the pesticides applied in orchards, imazalil residue was found in one sample. Imazalil is a fungicide solely used for post-harvest treatment to control citrus fruit, apples, and pears rotting during storage or long-distance transport caused by various fungal pathogens. The positive finding of imazalil in the sample of craft cider posed as a locally produced product could arouse a suspicion that at least part of the raw apples was not produced locally.

Another group of pesticide residues found in ciders is represented by pesticides carried over from hops added during or after fermentation. Mandipropamid, a fungicide used to control downy mildew in a range of vegetable crops, was found mainly in ciders with added hops at the highest concentration of 2.1 μg·L^{-1} in sample 2 (Table 2). Other fungicides and insecticides frequently applied for hops treatment, such as ametoctradin, azoxystrobin, dimethomorph, fenpyroximate, or spirotetramat, were not detected in any of dry hopped ciders.

Two cider samples declared as organically produced (samples 1 and 8) were also involved in this study. These organic apple ciders contained traces of pesticide residues at a maximal concentration of 1.0 μg·L^{-1}. Surprisingly, the lowest concentrations of pesticide residues were found in ciders that were not declared as organically produced. The data presented in Table 2 clearly show ciders brewed from raw apples grown in pesticide-treated orchards such as samples 5–9 and 12–13. On the other hand, pesticide residues could be considered proof that the pressed apple juice was used for brewing the cider. Thus, in this point of view, pesticide residue analysis in combination with the isotopic and elemental characterisation of ciders (Cristea 2019) could be a valuable tool for the authenticity confirmation and adulteration of especially craft ciders.

CONCLUSION

This study provides scientific evidence of residues of many pesticides found in craft apple ciders. A multiresidue HPLC-MS/MS method has been applied to determine pesticide residues at trace levels, which was used to analyse 20 samples of ciders. The results show that the majority of the analysed samples contained at least one pesticide, and fourteen of them contained residues of more than 4 pesticides. Nevertheless, residues were found in samples at trace levels ranging from 0.5 to 5 μg·L^{-1} and just rarely at a level higher than 10 μg·L^{-1}. In general, the results of this study could be evaluated from two points of view. Firstly, the presence of pesticide residues proves that the pressed apple juice was used for brewing the cider. Secondly, the profile of pesticides could give a clue about the geographical origin of apples in case the production only from locally grown apples is declared.

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